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Citrus Research International, Nelspruit

2012/13

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1 INTRODUCTION

Tim G Grout (Manager: Research & Technical)

The year 2013 will be remembered as the year when the European Union ultimately imposed a ban on South African citrus due to citrus black spot (CBS) interceptions. However, the report period 2012/3 once again also justified the citrus industry's decision taken almost 60 years ago to have its own dedicated research staff who could focus on and challenge the scientific claims being made by the EU regarding the threat of CBS. Apart from being CRI's CEO, Vaughan Hattingh also coordinates the technical aspects of market access issues and due to CBS taking up most of his time I am writing this introduction this year in order to get this report out.

Once again, CRI received most of its funding from the levy administered by the Citrus Growers Association on fresh citrus exports and a relatively small amount of funding from the Postharvest Innovation Programme. CRI researchers seconded to universities within the CRI Group Alliance also benefited from THRIP funding, as did some university researchers that were funded by the citrus industry.

Apart from CBS, increasing incursions of the fruit fly *Bactrocera invadens* and its eventual establishment in the Vhembe district of Limpopo Province, also required additional attention from the CEO and our fruit fly entomologist Aruna Manrakhan. CRI worked closely with DAFF on both these issues as well as surveys for greening disease. CRI also presented a practical course to train DAFF inspectors on the identification of various phytosanitary organisms. Technical inputs were also provided by CRI on requests to import citrus from Brazil and Egypt.

Within the Integrated Pest Management (IPM) research portfolio, most of the research funds were spent on false codling moth (FCM), which is likely to be the next major market access challenge after CBS, followed by fruit flies. A total of 17 projects are underway on FCM and many of these involve Rhodes University which has been awarded a chair in "Insects in sustainable agricultural ecosystems" that will result in more future funding for research on this citrus pest. Six of the projects have a postharvest approach while most of the others involve biological control strategies that could be implemented early in the season such as the use of entomopathogenic nematodes in the soil under the trees. In fruit fly research there were equal numbers of projects addressing *B. invadens* and *Ceratitis* spp. and the results of a modelling project showed that the distribution of *B. invadens* would be mainly limited by relative humidity rather than temperature.

Within the Disease Management research portfolio, research on CBS resulted in the publication of three important refereed papers that could be cited in deliberations with the EU. Researchers were also involved in a collaborative project with USA, Brazilian and Argentinian researchers to develop a probabilistic model to quantitatively predict the risk of fruit as a pathway for CBS. However, other issues that have more of an impact on citrus production than CBS also continued to receive attention. One of these was the search for alternative nematicides to methyl bromide and aldicarb and another was the continual search for better cross-protection strains for Citrus Tristeza Virus that will prolong tree lifespan. The CRI plant pathologists also provide essential services for the Citrus Improvement Scheme through re-indexing of mother block trees, pathogen elimination and pre-immunisation of new entries.

Horticultural research is suffering from a lack of capacity because few horticultural students choose a research career. However, the small team that we have at CRI is making breakthroughs in understanding Peteca spot, rind pitting and chilling injury and how these postharvest issues can be minimized, as well as determining the water and nutritional needs of citrus of different ages in different soil conditions and climates. With the resignation of a cultivar evaluator we took the opportunity to appoint a replacement in the Western Cape to better serve that area and the Northern Cape. Research funds are also being used by the ARC at the ITSC to distinguish between *Citrus* species and varieties using molecular techniques. It is hoped that this will allow for the identification of non-bearing plants.

The CIS continues to be operated by CRI with advice from the CIS Advisory Committee. The number of buds supplied by the Citrus Foundation Block (CFB) continues to increase and Eureka lemon was the most popular variety. The extreme demand for Eureka lemon resulted in unusually high amounts of buds having to be supplied by authorised certified nurseries. Similar challenges were experienced with some privately managed late navels and mandarin hybrids. The virus-free gene source at CRI-Nelspruit now comprises 270 citrus cultivars and selections. Biological indexing is done in Nelspruit to establish whether graft transmissible disease agents are present in CFB trees and molecular diagnostic techniques are now also being used to supplement the biological indexing results.

The Seventh CRI Citrus Research Symposium was held in the Drakensberg in 2012 with a total of 55 oral presentations and 30 posters. The meeting was extremely successful thanks to good financial support from

many different companies. The five regional CRI Postharvest Workshops were also equally successful and 2012 saw the roll-out of this regional meeting strategy for similar workshops in autumn and spring. CRI Extension still operates the CRI Postharvest Technical Forum which is the new name given to the Citrus Cold Chain Forum. This is coordinated by Dawid Groenewald who has contributed enormously towards the improvement of carton standards. Apart from the many other means of technology transfer being utilised by CRI researchers such as the SA Fruit Journal, Cutting Edge and CRInet, many researchers were given the opportunity to present scientific papers at the International Citrus Congress in Spain in November 2012 and network with citrus researchers from all over the world. It was rewarding to see that despite the many challenges thrown at the South African citrus industry, the research and technical support that its dedicated staff provide allows it to stand head and shoulders above other better-funded industries and institutes. In the words of Winston Churchill, "Never was so much owed by so many to so few".

INLEIDING

Tim G Grout (Bestuurder: Navorsing & Tegnieis)

Die jaar 2013 sal onthou word as die jaar toe die Europese Unie (EU) uiteindelik 'n verbod geplaas het op Suid-Afrikaanse sitrus as gevolg van sitrus swartvlek (SSV) onderskeppings. Desnieteenstaande het die 2012/13 verslagperiode weereens die besluit van die sitrus bedryf wat amper 60 jaar gelede geneem is regverdig. Dit was om sy eie toegewyde navorsingspersoneel te hê wat nou kon fokus en krities kommentaar lewer op die Europese Unie se wetenskaplike stellings aangaande die bedreiging van SSV. Behalwe dat Vaughan Hattingh die hoofuitvoerende beamppte van CRI is, koördineer hy ook die tegniese aspekte rondom marktoegang en a.g.v. SSV wat meeste van sy tyd in beslag neem, skryf ek hierdie inleiding hierdie jaar ten einde die verslag te finaliseer.

CRI het weereens meeste van sy befondsing ontvang van die heffing, geadministreer deur die Sitrus Kwekers Vereniging (SKV), op vars sitrus uitvoere; asook 'n relatiewe klein hoeveelheid befondsing van die Na-oes Innovasie Program. CRI navorsers wat aan universiteite binne die CRI Groep Alliansie gesekondeer is, is ook bevoordeel deur THRIP befondsing, net soos sommige universiteitsnavorsers wat deur die sitrus bedryf befonds is.

Behalwe vir SSV het toenemende deteksies van die *Bactrocera invadens* vrugtevlug, en die eindlike vestiging daarvan in die Vhembe distrik in die Limpopo Provinsie, ook addisionele aandag van die hoofuitvoerende beamppte en ons vrugtevlug entomoloog, Aruna Manrakhan, geveerg. CRI het nou met die Dept. van Landbou, Bosbou en Visserye ("DAFF") saamgewerk aangaande beide hierdie kwessies, asook met vergroening opnames. CRI het ook 'n praktiese kursus aangebied om "DAFF" inspekteurs op te lei in die identifikasie van 'n verskeidenheid fitosanitêre organismes. Tegnieiese insette is ook deur CRI verskaf aangaande versoeke om sitrus van Brasilië en Egipte af in te voer.

Binne die Geïntegreerde Pes Bestuur ("IPM") navorsingsportefolio is meeste van die navorsingsfondse op vals kodling mot ("FCM") spandeer wat waarskynlik die volgende groot marktoegang uitdaging gaan wees na SSV, gevolg deur vrugtevlug. Op "FCM" is 'n total van 17 projekte onderweg met baie van hierdie wat Rhodes Universiteit insluit aan wie 'n navorsingstoel toegeken is vir "Insects in sustainable agricultural ecosystems" wat in die toekoms sal lei tot meer navorsingsbefondsing vir hierdie sitrus pes. Ses van hierdie projekte het 'n na-oes aanslag terwyl meeste van die ander biologiese beheer strategieë wat vroeg in die seisoen toegepas kan word, bv. entomopatogeniese nematodes in die grond onder bome, omvat. Ten opsigte van vrugtevlug navorsing is daar 'n gelyke aantal projekte wat *B. invadens* en *Ceratitis* spp. aanspreek en die resultate van 'n modelleringsprojek het aangedui dat die verspreiding van *B. invadens* hoofsaaklik beperk sal word deur relatiewe humiditeit en nie temperatuur nie.

Binne die Siektebestuur navorsingsportefolio het navorsing op SSV gelei tot die publikasie van drie belangrike wetenskaplike artikels wat kon aangehaal word tydens onderhandelings met die EU. Navorsers was ook betrokke met 'n samewerkingsprojek met navorsers van die VSA, Brasilië en Argentinië vir die ontwikkeling van 'n waarskynlikheidsmodel wat kwantitatief die risiko van vrugte wat kan dien as draers van SSV, kan voorspel. Ander kwessies wat meer 'n impak het op sitrus produksie as SSV het ook steeds aandag ontvang. Een hiervan was die soeke na alternatiewe nematisiedes wat metielbromied en aldicarb kan vervang, terwyl 'n ander was die voortdurende soeke vir isolate van Sitrus Trsteza Virus wat beter kruisbeskerming bied en dus boomleefyd sal verleng. CRI se plantpatoloë verskaf ook noodsaaklike dienste aan die Sitrus Verbeteringskema (SVS) d.m.v. reindeksering van moederblok bome, patogeen eliminasië en pre-immunisasië van nuwe toevoegings.

Hortologie navorsing gaan gebuk onder 'n gebrek aan kapasiteit weens die feit dat min hortologie studente navorsing as 'n loopbaan kies. Ten spite van dit maak die klein span by CRI deurbroke in die verstaan van Peteca vlek, skil verpitting en koue skade en hoe hierdie na-oes probleme verminder kan word; asook met

die bepaling van die water en voedingsbehoefte van sitrus op verskillende ouderdomme in verskillende toestande en klimaat. Met die bedanking van 'n kultivar evalueerder het ons van die geleentheid gebruik gemaak om 'n plaasvervanger in die Wes-Kaap aan te stel om daardie area en die Noord-Kaap beter te bedien. Navorsingsfondse word ook deur die LNR ITSG gebruik om tussen *Citrus* spesies en kultivars te onderskei d.m.v. molekuleêre tegnieke. Dit word gehoop dat hierdie werk die identifikasie van nie-draende plante sal toelaat.

CRI gaan voort om die SVS te bestuur met advies van die SVS Advieskomitee. Die getal ogies wat deur die Sitrus Grondvesblok (SGB) verskaf word hou aan om toe te neem en Eureka suurlemoen was die mees populêre kultivar. Die uitermatige vraag na Eureka suurlemoen het gelei tot ongewone hoë getalle ogies wat verskaf moes word deur goedgekeurde, gesertifiseerde kwekerie. Soortgelyke uitdagings is ondervind met sommige privaat bestuurde laat nawels en mandaryn hibriede. Die virusvrye bron by CRI-Nelspruit bestaan nou uit 270 sitrus kultivars en seleksies. Biologiese indeksering word in Nelspruit gedoen te einde vas te stel of entoordraagbare siektes teenwoordig is in SGB bome met molekuleêre tegnieke wat ook nou ingespan word om die biologiese indekseringsresultate aan te vul.

Die Sewende CRI Sitrus Navorsingsposium is in 2012 in die Drakensberge aangebied met 'n totaal van 55 mondelinge aanbiedings en 30 plakaat aanbiedings. Die samekoms was uiters suksesvol te danke aan goeie finansiële ondersteuning deur verskillende maatskappye. Die CRI Na-oes Werkswinkels in die vyf streke was ook baie suksesvol en in 2012 is daar ook afgeskop met soortgelyke werkswinkels in die verskillende streke tydens herfs en lente. CRI Voorligting bestuur steeds die CRI Na-oes Tegnieke Forum wat die nuwe naam is vir die Sitrus Koueketting Forum. Hierdie word deur Dawid Groenewald gekoördineer wat 'n enorme bydrae gemaak het tot die verbetering van karton standaarde. Apart van die verskeie mediums vir tegnologie oordrag wat deur CRI navorsers gebruik word soos die SA Vrugtejoernaal, Snykant en CRI-net, het verskeie navorsers ook die geleentheid gekry om in November 2012 wetenskaplike aanbiedings te maak tydens die Internasionale Sitrus Kongres in Spanje. Hier kon hulle skouers skuur en kontakte opbou met sitrus navorsers van reg oor die wêreld. Dit was belonend om te sien dat ten spyte van verskeie uitdagings wat die Suid-Afrikaanse sitrus bedryf in die gesig staar, die navorsing en tegnieke ondersteuning wat die toegewyde personeel verskaf, dit toelaat om kop en skouers bo ander beter befondste industrieë en institute uit te staan. In die woorde van Winston Churchill: "Never...was so much owed by so many to so few."

2 MARKET ACCESS TECHNICAL COORDINATION

Coordinator: Vaughan Hattingh and Elma Carstens (CRI)

2.1 SUMMARY

The market access issues dominating this report period were the CBS issue in the EU and the incursions of the fruit fly *Bactrocera invadens*. The EU CBS issue remained unresolved. Citrus fruit was successfully exported from South Africa to Thailand. Market access issues pertaining to the following markets received attention: EU, Japan, USA, China, South Korea, Thailand, The Philippines, Cambodia, Kazakhstan, Syria, Australia and Lebanon. Technical inputs were made on the conditions for import of citrus fruit and propagation material in general and also specifically citrus fruit from Egypt and Brazil. Phytosanitary concerns pertaining to the export of citrus seed to China received attention. The following biosecurity issues also received attention: *B. invadens*, CBS surveys and greening disease surveys.

OPSOMMING

Die CBS-kwessie in die EU en die verskeie opsporings van die vrugtevlug, *Bactrocera invadens*, was die marktoegangsaspekte wat hierdie verslagperiode gedomineer het. Die EU CBS-kwessie bly steeds onopgelos. Sitrusvrugte is suksesvol vanaf Suid-Afrika na Thailand uitgevoer. Marktoegangsaspekte rakende die volgende markte het aandag geniet: EU, Japan, VSA, China, Suid-Korea, Thailand, Phillipyne, Kambodja, Kazakhstan, Sirië en Lebanon. Tegnieke insette is gelewer oor vereistes vir die invoer van sitrusvrugte en voortplantingsmateriaal oor die algemeen, en ook spesifiek vir die invoer van sitrusvrugte van Egipte en Brasilië. Fitosanitiêre kommer rakende die uitvoer van sitrusaad na China, het ook aandag geniet. Die volgende aspekte rakende biosekuriteit het ook aandag geniet: *B. invadens*, CBS- en sitrusvergroeningsopnames.

2.2 EUROPE (EU)

The EU notified SA in October 2012 that SA fresh citrus exports may be banned if the threshold of five interceptions for CBS is exceeded in the 2013 export season since the current EU Phytosanitary regulation (Directive 2000/29/EC) requires that CBS-infected fruit are not allowed to enter the EU.

As this notification is not only a threat to the industry (market closure) but to all involved in the supply chain several national and international meetings were held. The SA CBS Steering Committee, convened by SA-DAFF with representation from CGA, FPEF, CRI and Government Departments was restarted and several notices were sent out to the industry to keep everybody updated on the issue. A CBS Disaster Management Committee was formed by the CGA.

Not much progress was made within the International Plant Protection Convention (IPPC) with the dispute lodged between South Africa and the European Union regarding the EU's CBS citrus import regulation until February 2013. In February 2013, an IPPC facilitated bilateral meeting between SA and EU was held in Rome. SA proposed proceeding to the expert panel step of the process, but the EU did not consent, invoking a revision of their PRA on CBS as justification. They undertook to submit a draft of the PRA to public comment before finalising. The industry requested that a Trade Concern be lodged at the WTO in March 2013, but SA-DAFF elected to rather read a statement at the meeting. SA-DAFF did proceed with lodging a formal Trade Concern at the June 2013 meeting.

Amendments to the current CBS-RMS included compulsory orchard inspections, on line PPECB inspections in the packhouses, phytosanitary inspections on fruit that are still in storage after 14 days since the initial PPECB inspections in the packhouses and cooling of fruit under 10⁰C within 4 days of PPECB inspection.

On international level several meetings were held with key parties in the EU as well as other countries (USA, Brazil, Argentina and Australia) to mobilise support for the SA opinion that the current EU regulation pertaining to CBS is scientifically unjustified.

Documents in support of recognising the Orange River area in the Northern Cape as CBS free were sent to the EU by SA-DAFF in 2012. Specific concerns were raised by the EU in 2012 and further surveys were requested. The follow up surveys were conducted in February 2013, 37 field samples were analysed by SA-DAFF's laboratory in Stellenbosch and all were negative for CBS, thereby confirming the CBS-free status of the region.

2.3 JAPAN

The ongoing issues for access to Japan were (1) the broadening of access for soft citrus cultivars to include all other SA mandarins except Satsumas and (2) the adoption of a revised cold treatment condition for the export of all citrus types. Regarding the second point, experimental work to address concerns raised by Japan-MAFF was completed. The report together with the data package will be compiled and submitted to SA-DAFF within the next reporting period.

2.4 USA

There were four outstanding issues pertaining to this market at the beginning of the reporting period: (1) reversion of the FCM cold treatment period from 24d to 22d (long outstanding), (2) equivalence between USA domestic CBS regulations and USA import regulations (access to USA for all production areas), (3) expansion of CBS pest free areas to include the whole of the W Cape in the work plan and (4) adoption of CBS pest free places of production (N Limpopo region).

After a long delay, DAFF responded to APHIS in August 2012 on the counter proposal from USDA-APHIS regarding the 22/24 FCM cold treatment. A response on the equivalence between USA domestic CBS regulations and USA import regulations, expansion of CBS pest free areas and adoption of CBS pest free places of production was only provided to USDA-APHIS in December 2012. SA-DAFF was requested to send the latest Citrus pest list to USDA-APHIS to amend the current incorrect SA pest list.

Feedback with regard to the 22/24 FCM cold treatment was received from USDA-APHIS but no response was received from USDA-APHIS about the three issues pertaining to CBS. After discussions with DAFF it was decided that a technical bilateral meeting between experts in South Africa will be the only way to address this long outstanding issue.

A meeting was held in March 2013 with the Western Cape Producers. The purpose of the meeting was to discuss three new issues that are problematic to this export programme namely, the division of the Western Cape province into two areas, each with its own running average, SA citrus fruit be allowed to enter the USA through ports in Texas, New Orleans and Miami and the termination of the obligatory cutting of SA citrus fruit used to confirm successful cold treatment of SA citrus fruit. The meeting was attended by Minister Vangili Titi and other senior people from the USA. The 22/24 day cold treatment and the other outstanding issues were

also discussed and the meeting also agreed that a technical bilateral meeting between experts in South Africa will be the only way to address this long outstanding issue of the 22/24 day cold treatment. The information wrt the new requests and the request for a technical bilateral meeting was submitted to SA-DAFF.

2.5 CHINA

The non-containerised bulk shipping and a system approach for FCM to address the quality problems encountered with shipments of citrus fruit was discussed at a SPS-meeting that was held in June 2012 between NPPOs of SA and China. The Chinese authorities indicated that the system approach for FCM control did not meet their phytosanitary requirements and also that they are of the opinion that temperature control is more efficient and controlled in containers than in reefer ships and therefore China is not prepared to accept shipment of citrus fruit in bulk reefers. At the Market Access Working group it was decided that further information exchange wrt to the system approach will not solve the problem and that a meeting between the NPPOs in SA is needed. Information on the temperature control in containers and bulk reefers will be prepared and submitted to SA-DAFF.

2.6 SOUTH KOREA

In the previous reporting period it was announced that a notice was received from the Animal, Plant and Fisheries Quarantine and Inspection Services of the Republic of South Korea that the importation of fresh lemons and grapefruit from South Africa are allowed into South Korea. Although the current export protocol for sweet oranges was amended to allow for the importation of lemons and grapefruit, problems were encountered with the interpretation of the new protocol during the 2012 export season. In order to solve the problems, inputs were obtained from role players and guidelines were drafted to clarify the interpretation of the protocol for submission to South Korea before the start of the 2013 export season. These guidelines were submitted to SA-DAFF in December. In March feedback was received from the South Korean authorities but the wording was again confusing. Inputs were again obtained from all role players and a counter-proposal was submitted for communication to the South Korean authorities. By the end of this reporting period no response had been received from the South Korean authorities.

2.7 THAILAND

As reported in the previous period, SA-DAFF has received confirmation from the Department of Agriculture of Thailand that SA can commence with the export of fresh citrus fruit as from 30th May 2012. This protocol was finally signed after negotiations started prior to 2000. No phytosanitary problems were encountered during the first export season.

2.8 REUNION

Revised import conditions from Reunion stipulated that consignments must be free from all Fruit Flies, except *Bactrocera cucurbitae*, *Bactrocera zonata*, *Carpomya vesuviana*, *Ceratitidis capitata*, *Ceratitidis cattoirii*, *Ceratitidis rosa*, *Dacus ciliatus*, *Dacus demmerezi*, *Pardalaspis cyanescens*. Two options are available to comply with this requirement namely fruit should be from pest free areas (PFA) or consignments should be subjected to a cold treatment.

Problems were encountered when SA-DAFF requested a mandatory cold treatment for all consignments since there is no PFA for fruit flies in SA. A SA Citrus pest list was provided to SA-DAFF which indicates that apart from *Bactrocera invadens*, the only other fruit flies that are present in SA and that are important to citrus are *C. capitata* and *C. rosa*, which are present in Reunion. SA-DAFF changed the working instructions and mandatory cold treatment is now only applicable to consignments from areas where *Bactrocera invadens* is present.

2.9 NEW MARKETS

2.9.1 The Philippines

In 2009, SA-DAFF submitted a Pest Information Package to The Philippine Authorities to gain access for all fresh citrus fruit types from South Africa. In 2010 feedback from the authorities indicated that the PRA process is at the Pest Categorization Stage and additional information will be requested from SA when needed. In feedback received in October 2102, the Philippine Authorities requested more information on specific pests and diseases. The information was compiled by CRI and submitted to SA-DAFF in January 2013. In February a meeting was held with SA-DAFF to clarify concerns raised and to provide support to

draft the letter to the Philippine authorities. By the end of this reporting period DAFF had not yet sent the letter to the Philippine authorities.

2.9.2 Cambodia, Kazakhstan and Syria

During this reporting period a decision was taken at the Market Access Working Group that SA-DAFF will determine which commodities can be exported to Cambodia and Kazakhstan as this is a long outstanding point on the agenda. It was determined that fresh Citrus fruit can be exported to these two countries and that the completion of Pest Information Packages is no longer needed. A decision was also taken by the Fresh Producers Exporters Forum (FPEF) that there is no need to further investigate the opportunity to gain access to Syria for all fresh Citrus fruit types due to the unstable political environment in the country. The decision will be reconsidered if there are changes in the political arena.

2.9.3 Australia and Lebanon

No feedback was received from these countries during this reporting period, despite several follow up requests by SA-DAFF.

2.10 IMPORTS

2.10.1 Brazil

A request was received from SA-DAFF to provide inputs on import conditions for fresh fruit of *Citrus latifolia* from Brazil. Many pests and diseases of quarantine importance to SA are present in Brazil. Inputs were submitted to SA-DAFF and were included in the final import conditions.

2.10.2 Egypt

A consignment of fresh Citrus fruit from Egypt was intercepted in SA for the presence of the exotic mite *Brevipalpus lewisi*. All imports from Egypt were put on hold. CRI was requested to provide inputs to amend import conditions for fresh Citrus fruit from Egypt. Egypt was requested to provide data on the occurrence of *Brevipalpus lewisi* and upon feedback, the import conditions were amended to allow certified consignments from places of production that are free from *Brevipalpus lewisi*.

In order to safeguard SA against the exotic fruit fly *Bactrocera zonata*, Egypt was requested to provide documentation on the cold treatment for disinfestation of consignments. It was indicated that a USDA accepted treatment protocol would also be accepted by SA. By the end of this reporting period no documentation on the cold treatment had been received from Egypt and therefore all imports were still on hold.

2.10.3 Import conditions

In order to safeguard the industry, CRI held meetings with SA-DAFF to revise the import conditions for fresh Citrus fruit and vegetative propagation material. Special attention was given to the handling of imported vegetative propagation material. The amended requirements for fresh fruit were implemented by SA-DAFF, but by the end of the reporting period the requirements for vegetative propagation material were still pending.

2.11 BIOSECURITY AND REGULATIONS

During this reporting period further incursions of the exotic fruit fly, *Bactrocera invadens* were detected in SA. The pest was detected in 2010 and 2011 in seven areas of SA but was successfully eradicated from all of these areas.

In May 2012 a pest report was sent to the IPPC to report on the detection of *B. invadens* in South Africa in the Vhembe district in the northern part of Limpopo and Ehlanzeni district (Burgershall area) in Mpumalanga Province. A further report was sent to the IPPC in June 2012 to report on detection in the Vhembe, Mopani and Bohlabela districts in the Limpopo province. The affected areas were Levubu (Vhembe district), Deerpark (Mopani districts) and Hoedspruit (Bohlabela district). In August 2012 and September 2012 SA reported to the IPPC that incursions had been detected in the Vhembe (Dzanani-Makhado), the Waterberg (Limburg and Baltimore) and Mopani (Tzaneen) districts of the Limpopo province and in Ehlanzeni district municipality (Komatipoort) of the Mpumalanga Province respectively. In November 2012 a further report was sent to the IPPC on incursions that had been detected in the Witvlag and Tshidzini areas (Vhembe district municipality) of the Limpopo province. At that stage it was reported with each of the

notifications that quarantine and eradication measures were implemented and the status of the incursions are “transient - under eradication”.

In March 2013 SA reported to the IPPC that the status of *Bactrocera invadens* in SA has changed from “transient, actionable and under eradication” to “present, only in some areas and under official control”. Specimens of *B. invadens* were detected in January 2013 and February 2013 in several areas in Limpopo, Mpumalanga and North West Provinces in South Africa. Various communications, endorsed by the *B. invadens* Steering Committee, were distributed to keep the industry informed.

During the 2011-12 reporting period a CBS survey was conducted in the remaining magisterial districts of the Western Cape province (in parts with no commercial citrus plantings). The official laboratory report was provided in 2013 and declared that the whole of the Western Cape province had been surveyed and found to be free from CBS.

A new Plant Health (Phytosanitary) Bill was drafted by the Directorate Plant Health of the Department of Agriculture, Forestry and Fisheries. This Bill is a revision of the Agricultural Pests Act, 1983 (Act No.36 of 1983) and the draft was gazetted and inputs were submitted on request. All documents on route to the Minister for approval such as Regulations R110 and R1013 of the Agricultural Pests Act and the establishing of buffer zones with specific phytosanitary regulations to maintain the greening free status of the Eastern Cape were not finalised because of this pending Bill.

The workgroup that was formed to address the detection of Citrus Greening within the greening free Eastern Cape Province met in Port Alfred and Umtata. At the meeting in Port Alfred the affected farmers were invited to make them aware of the disease. Further surveys were conducted and more positive sites were found. Although destruction orders were issued by SA-DAFF not all the infected trees were removed by the end of this reporting period. Detection surveys were also conducted in magisterial districts of the Western Cape to monitor the spread of the disease within this province. In order to safeguard the country against Asiatic greening, detection surveys were conducted in the hot spot areas. An official report confirmed absence of the disease in South Africa.

During 2012 a notification was received from the Chinese authorities that a consignment of SA Citrus seed had been intercepted due to the presence of *Xanthomonas axonopodis* (Citrus canker). This pathogen is not present in SA and also not seed transmissible. To address this issue seeds were tested in SA and a survey was conducted in the nursery from which the seeds originated. Results from both the PCR tests and the survey were negative for this pathogen. During the SPS meeting in China in 2012, the Chinese authorities gave assurance that import conditions for seed from SA will not be amended and the interception will not be reported to other countries. By the end of this reporting period the official report from SA-DAFF on the seed tests and survey conducted had not been submitted to the Chinese authorities.

3 PORTFOLIO: INTEGRATED PEST MANAGEMENT

3.1 PORTFOLIO SUMMARY

By Sean D Moore (Portfolio Manager: IPM)

During the 2012/13 year, the climate in the citrus industry was dominated by three potentially industry-changing happenings. These were the greater stringency of the European Union on citrus black spot (CBS) from South Africa and particularly interceptions of CBS; the Pest Risk Assessment on false codling moth (FCM) conducted by the European Plant Protection Organisation (EPPO); and the change of status of the Invasive fruit fly, *Bactrocera invadens*, from absent or transient to “present in specified areas”. The last two of these are entomological issues and have been addressed as aggressively as possible within the Integrated Pest Management (IPM) Portfolio. Consequently, these two pests – FCM and invasive fruit fly – have dominated the focus within the portfolio.

CRI continues to build and benefit from research associations with a number of other entities, particularly universities (Stellenbosch, Rhodes, Nelson Mandela Metropolitan and Free State). CRI's association with Rhodes University has been a catalyst in the prestigious awarding of a chair in “Insects in sustainable agricultural ecosystems” to the university. This chair, which comes with substantial funding and a probable life-span of fifteen years, will most certainly further increase and improve the entomological research capacity within the citrus industry. With any university association comes students and consequently several of the projects reported within the IPM Portfolio, include post-graduate students, which bodes well for entomological research succession in our industry. Additionally, a number of research associations with a range of other private entities from far and wide complete the landscape within the IPM Portfolio. Examples are e-nema (Germany), BioBee (Israel), Du Roi IPM, Villa and Xsit.

Unsurprisingly, the programme which has received the most funding and which has the highest number of registered projects and researchers involved is the FCM Programme. Seventeen research projects are reported, which is a 13% growth on the previous year. Six of these focused on post-harvest issues, recognising that even excellent suppression of FCM pre-harvest is not sufficient for a phytosanitary pest. Two of these post-harvest projects investigated detection – one through the use X-ray and the other through detection of volatiles associated with infested fruit. Both studies produced promising results. However, it is clear that there remains a long road between positive research results and practical implementation. The remaining four post-harvest projects focussed on treatment of FCM in fruit. This included ionizing radiation, a combination of sub-lethal ionizing radiation and cold-treatment, carbon dioxide fumigation for amelioration of cold-treatment and cold-treatment alone. Irradiation of 100 Gy provided probit 9 disinfestation of fruit. The remaining three disinfestation projects are still in progress but are all showing potential as either complete quarantine treatments or at least for dramatic risk mitigation. Not only are export markets placing tremendous pressure on South Africa to control phytosanitary pests, such as FCM, but pressure is also being asserted to reduce chemical pesticide usage and residues on fruit. Consequently, only one project within the FCM Programme investigated chemical control of FCM. This was not a novel study, but did rank the ability of the various available products to deal with a high level of FCM activity shortly before harvest. Six projects investigated biological control of FCM or related aspects. This included the use of entomopathogenic nematodes (EPN), entomopathogenic fungi (EPF), granulovirus and egg parasitoids. EPN research has progressed to the stage of large scale field trials, indicating significant potential for suppression of FCM in a vacant niche i.e. in the soil in winter. It appears that this may be an option which is commercially available to growers for the 2013/14 season. The final four projects within the programme investigated mating disruption, including an “overkill” treatment to test the full potential of the technology; the relative susceptibility of various Navel orange varieties to FCM; FCM infestation of lemons in the field; and a taxonomic study to develop an identification key for a range of tortricids that could infest fruit. Finally, although not reported as a research item, a Systems Approach for management of FCM, as an alternative to cold-sterilisation, was devised. This is in preparation for presentation to the EU once the outcome of the PRA is known.

Fruit flies remain pests of phytosanitary concern to the citrus industry. Increased detections of the invasive fruit fly in the northern parts of South Africa have also raised enormous concerns amongst the grower community. The increased importance of *B. invadens* in South Africa is reflected in the number of projects addressing this pest under the Fruit fly Programme. In the 2012-2013 year, the number of projects addressing *B. invadens* equalled those addressing the local *Ceratitidis* fruit flies for the first time. There was a total of 9 fruit fly projects, one of which looked at both *Ceratitidis* species and *B. invadens*. The four *B. invadens* projects focussed on national surveillance, field control and modelling to determine potential global distribution. It was demonstrated that a combination of the male annihilation technique (MAT) using methyl eugenol and protein baiting could adequately control the pest. The modelling study indicated that moisture, rather than temperature would be the main factor limiting the dispersal of *B. invadens*. In the project which looked at all species of fruit fly in South Africa, a molecular means for identifying fruit fly larvae was developed. It has been recognised that the *B. invadens* threat is not only to the citrus industry but all fruit industries in South Africa. There is therefore a coordinated effort between industries to consolidate projects for greater affordability and to seek an alternative source of funding. Hopefully this will bear fruit in the near future. Research focussed on *Ceratitidis* fruit flies involved development of a new attract and kill system and two post-harvest treatment projects. These investigated cold-disinfestation for the Japanese market and fumigation with a GRAS product for not only fruit fly, but also FCM and grain chinch bug.

Three of the seven mealybug species known to occur on citrus in South Africa are considered as phytosanitary threats for certain export markets. Three of the four projects reported within this programme addressed different aspects of mealybug management. These were post-harvest gamma irradiation of mealybug as a potential phytosanitary treatment; inundative augmentation of the parasitoid, *Anagyrus* sp. nr. *pseudococci*, for biological control of mealybug; and laboratory examination of entomopathogenic fungi for control of mealybug. All three approaches demonstrated significant potential. However, only *Anagyrus* is ready for commercial roll out and is expected to be available to farmers as a new control option in the coming season. The final project in this programme looked at the morphology and ecology of carob moth on citrus, with the ultimate aim being to assess the threat status of carob moth on citrus and other hosts associated with citrus.

The 2011/2 season was unusual in that no research was conducted on citrus thrips due to most research being focussed on market access pests and the need to delay some planned research on thripicides until new isolates of entomopathogenic fungi are available. However, 7 years of research on the development of an original ant bait that is effective against the two most important ant species in citrus orchards was completed and the bait is now ready to be commercialised. The other area of research in the programme on key non-phytosanitary pests demonstrated that sublethal levels of imidacloprid in fruit could increase reproductivity of citrus moth pests, particularly FCM.

Pests may be considered minor because they only occur sporadically, only affect a low percentage of citrus production areas or cause little economic damage. However, when they do occur they are capable of causing serious damage, such as is the case with woolly whitefly (WWF), which is now present in many citrus production regions in South Africa. Research focussed on chemical control of WWF and surveys for biological control agents and their levels. Finally, a contract trial on a new snail bait was conducted for Villa Crop Protection.

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. This was particularly apparent at the International Citriculture Congress in Valencia, Spain, and our own biennial Citrus Research Symposium. 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.

PORTEFEULJEOPSOMMING

Gedurende die 2012/13 jaar was die klimaat in die sitrusbedryf gedomineer deur drie gebeurtenisse wat die vermoë het om die bedryf dramaties te verander. Hierdie is die verhoogte strengheid van die Europese Unie teenoor swartvlek van Suid-Afrika veral wat onderskeppings van swartvlek betref; die Plaag Risiko Raming (PRR) op valskodlingmot (VKM) wat deur die Europese Plantbeskerdings Organisasie (EPPO) uitgevoer word; en die verandering in status van die Indringervlieg, *Bactrocera invadens*, van afwesig of verbygaande na “teenwoordig in sekere gespesifiseerde gebiede”. Die laaste twee is entomologies van geaardheid en is so aggresief as moontlik binne die IPM Portefeulje aangepak. Gevolglik het hierdie twee plaë – VKM en die Indringervlieg – die fokus in die portefeulje gedomineer.

CRI bou aanhoudend verhoudings met ‘n verskeidenheid ander navorsingsinstansies waaruit daar voordeel getrek kan word. Dit sluit veral universiteite soos Stellenbosch, Rhodes, Nelson Mandela Metropolitaan en Vrystaat in. CRI se verhouding met Rhodes Universiteit het gelei tot gesogte toekenning van ‘n stoel in “Insekte in onderhoubare landbou ekosisteme” aan die universiteit. Die stoel kom met beduidende bevondsing en ‘n waarskynlike lewensduur van 15 jaar en sal hoogswaarskynlik die entomologiese navorsingskapasiteit van die sitrusbedryf selfs verder verbeter. Enige universiteitsvenootskap sluit studente in en gevolglik is daar heelwat nagraadse studentebetrokkenheid in baie van die projekte in die IPM Portefeulje. Hierdie is uiters positief vir opvolgingsbeplanning vir entomologiese navorsing in ons bedryf. Derhalwe bestaan daar ‘n klomp navorsingsgenootskappe met ander private instansies, selfs internasionaal, om die prentjie binne die IPM Portefeulje te voltooi. Voorbeelde is e-nema (Duitsland), BioBee (Israel), Du Roi IPM, Villa en Xsit.

Dit is geen verassing dat die program wat die meeste bevondsing ontvang het, die meeste geregistreerde projekte het en waar die meeste navorsers betrokke was, die VKM Program is nie. Sewentien navorsingsprojekte word gerapporteer, wat ‘n 13% groei van die vorige jaar beteken. Ses van hierdie het op na-oes faktore gefokus, wat ‘n aanduiding is van die erkenning dat selfs uitstekende vooroesonderdrukking van VKM nie voldoende vir ‘n fitosanitêre plaag is nie. Twee van hierdie na-oes projekte het opsporing ondersoek – een deur gebruik van X-straal en die ander deur opsporing van vlugtigestowwe wat met besmette vrugte geassosieer is. Albei studies het tot dusver belowende resultate getoon. Nietemin is dit duidelik dat daar nog ‘n lang pad is tussen positiewe navorsingsresultate en praktiese implementasie. Die ander vier na-oes projekte het gefokus op behandeling van VKM in vrugte. Hierdie het bestraling, ‘n kombinasie van subletale bestraling en kouebehandeling, koolstofdiksiedberoking vir vermindering van kouebehandeling en kouebehandeling op sy eie ingesluit. Bestraling met 100 Gy het probit 9 ontsmetting van vrugte veroorsaak. Die oorbylwende drie disinfestasië projekte is nog ver van voltooi maar lyk alreeds belowend as volle kwarantyn behandelings of minstens metodes om risiko dramaties te verminder. Daar is nie net beduidende druk van uitvoermarkte op Suid-Afrika wat fitosanitêre plaë (soos VKM) betref nie, maar daar bestaan ook druk om gebruik van chemiese plaagdoders en residue daarvan op vrugte te verminder. Gevolglik is daar net een projek in die VKM Program wat chemiese beheer van VKM ondersoek het. Hierdie was nie ‘n oorspronklike studie nie maar het die vermoë van verskeie beskikbare produkte vergelyk om hoë VKM druk kort voor oes te hanteer. Ses projekte het biologiese beheer van VKM of verwante faktore ondersoek. Hierdie het die gebruik van entomopatogeniese nematodes (EPN), entomopatogeniese swamme (EPF), granulovirus en eierparasiete ingesluit. EPN navorsing het gevorder tot die stadium waar grootskaalse veldproewe nou uitgevoer word wat opwindende potensiaal aandui vir onderdrukking van VKM in ‘n vakante nis d.w.s in die grond gedurende winter. Hierdie sal heel waarskynlik ‘n opsie wees wat in die 2013/14 seisoen kommersieel beskikbaar vir produsente sal wees. Die finale vier projekte in die program

het die volgende ondersoek: paringsontwrigting (insluitend 'n oordosis behandeling om die volle vermoë van die tegnologie te toets); die relatiewe vatbaarheid van verskeie Nawellemoen varieteite vir VKM; VKM besmetting van suurlemoene in die veld en 'n taksonomiese studie om 'n identifikasie sleutel te ontwikkel vir 'n reeks tortrisiede wat vrugte kan besmet. Ten slotte, alhoewel dit nie 'n navorsingsitem is nie, is daar 'n Stelselsbenadering vir bestuur van VKM ontwikkel as 'n alternatief vir koue sterilisasie. Hierdie is voorberei om aangebied te word wanneer die uitkoms van die PRR bekend gemaak word.

Vrugtevlieë bly 'n plaag van fitosanitêre belang vir die sitrusbedryf. Die verhoogde opname in opsporings van die Indringervlieg in die noordelike dele van Suid-Afrika het ook groot kommernis onder die produsentegemeenskap ontlok. Die verhoogde belangrikheid van *B. invadens* in Suid-Afrika word weerspieël in die hoeveelheid projekte op dié plaag in die Vrugtevlieg Program. In die 2012-2013 jaar was daar vir die eerste keer dieselfde hoeveelheid projekte op *B. invadens* as op die plaaslike *Ceratitis* vrugtevlieë. In totaal was daar 9 vrugtevlieg projekte, insluitend een wat albei *Ceratitis* spesies en *B. invadens* gedek het. Die vier *B. invadens* projekte het gefokus op nasionale waarneming, vooroesbeheer en ontwikkeling van 'n model om potensiele globale verspreiding te bepaal. Dit is bewys dat 'n kombinasie van die mannetjie uitwissingstegniek (MUT), deur gebruik van metiel-eugenol en proteïene lokaasbespuitings of lokstasies, die plaag voldoende kon beheer. Die CLIMEX-model studie het aangedui dat vog die hoof beperkende faktor in die verspreiding van *B. invadens* sal wees en nie temperatuur nie. In die projek wat na alle vrugtevlieg spesies in Suid-Afrika gekyk het, is 'n molekulêre metode ontwikkel om vrugtevliegglarwes te identifiseer. Daar word erken dat die *B. invadens* bedreiging nie net vir die sitrusbedryf is nie maar alle vrugtebedrywe in Suid-Afrika. Daarom bestaan daar 'n gesamentlike poging tussen bedrywe om navorsingsprojekte te konsolideer vir groter bekostigbaarheid en om 'n alternatiewe bron van bevondsing te werf. Hoopelik sal hierdie poging in die nabye toekoms vrugte begin dra. Navorsing wat op *Ceratitis* vrugtevlieë gefokus het het ontwikkeling van 'n nuwe lok-en-vrek stelsel en twee na-oes behandelingsprojekte ingesluit. Hierdie het kouebehandeling vir die Japanese mark ondersoek en beroking met 'n GRAS produk – nie net vir vrugtevlieg nie maar ook VKM en graanstinkbesie.

Drie van die sewe witluis spesies wat op sitrus in Suid-Afrika voorkom word beskou as fitosanitêre bedreigings vir sekere uitvoermarkte. Drie van die vier projekte in hierdie program het gefokus op verskillende aspekte van witluis bestuur. Hierdie is na-oes gammabestraling van witluis as 'n moontlike fitosanitêre behandeling; aanvullende loslating van die parasiet *Anagyrus* sp. nr. *pseudococci* vir biologiese beheer van witluis; en 'n laboratoriumondersoek aan entomopatogeniese swamme vir beheer van witluis. Al drie benaderings het belowend gelyk, maar net *Anagyrus* is gereed om beskikbaar gemaak te word vir produsente as 'n beheeropsie in die komende seisoen. Die finale projek in hierdie program het die morfologie en ekologie van karobmot bestudeer, met die uiteindelige doel om die bedreigingsstatus van karobmot op sitrus en ander verwante gashere te evalueer.

Die 2011/2 seisoen was ongewoon omdat geen navorsing op sitrus blaaspootjies gedoen is nie aangesien die meeste navorsing wat op marktoegangsplae gefokus het en van die beplande navorsing op blaaspootjiedoders uitgestel moes word totdat nuwe isolate van entomopatogeniese swamme beskikbaar was. Nietemin is 7 jaar se navorsing voltooi in die ontwikkeling van 'n nuwe mierlokmiddel wat doeltreffend teen die twee mees belangrike miere in sitrusboorde is, en dit is nou gereed vir kommersialisering. Die ander navorsingsarea in die program op sleutel nie-fitosanitêre plae het gewys dat subletale vlakke van imidacloprid in vrugte aantelingsvermoë van sitrus motplae kon verhoog, insluitend VKM.

Plae kan as minder belangrik beskou word wanneer hul slegs sporadies voorkom, slegs 'n lae persentasie van sitrus produksie-areas affekteer of min ekonomiese skade veroorsaak. Wanneer hulle egter voorkom kan hulle ernstige skade veroorsaak soos in die geval van wollerigewitvlieg (WWV) wat nou in meeste sitrus produksie streke in Suid-Afrika teenwoordig is. Navorsings het op chemiese beheer van WWV gefokus en opnames vir natuurlike vyande en hul vlakke. Laastens is 'n kontrak proef op 'n nuwe slaklokaas vir Villa Crop Protection uitgevoer.

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. Hierdie was veral duidelik by die Internasionale Sitrus Kongres in Valencia, Spanje, en ook by ons eie tweejaarlikse Sitrusposium. 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 oordra van belangrike informasie wat uit hulle navorsing gekom het aan die produsente gemeenskap. Hierdie is veral deur produsentestudiegroepe en Snykant publikasies gedoen.

3.2 PROGRAMME: FALSE CODLING MOTH

Programme coordinator: Sean D Moore (CRI)

3.2.1 Programme summary

With the knowledge that an official Pest Risk Assessment (PRA) of FCM for Europe is being conducted by the European Plant Protection Organisation (EPPO), research within this programme has become even more important than ever before and is being conducted with a greater sense of urgency than ever before. Preempting possible restrictive export regulations emanating from the PRA proposals, a systems approach for management of FCM from the start of the growing season to the shipping of the packed fruit, has been devised, has been presented to growers and packhouses and is being implemented by certain role players on a trial basis.

Within the research programme, management of FCM is being tackled from all possible angles, with a growing focus on post-harvest detection and control. Out of 17 research projects reported here, six focused on post-harvest issues. Two of these investigated detection – one through the use X-ray (3.2.6) and the other through detection of volatiles associated with infested fruit (3.2.11). Using X-ray radiography, FCM infestation could not be detected two days after infestation of Delta Valencias, as many of the larvae were still in the albedo. However, microfoc radiography detected 60% of infestation after 10 days, 77% after 7 days, and 40% 4 days after infestation. Micro focus tomography detected 100% of infestation. Five major volatile compounds of interest were detected from FCM-infested oranges. These were D-limonene, 3,7-dimethyl-1,3,6-octatriene, (E)-4,8-dimethyl-1,3,7-nonatriene, caryophyllene and naphthalene. Naphthalene would be a good indicator of FCM infestation as higher levels than healthy fruit were emitted throughout the infestation period.

The four post-harvest treatment projects looked at ionizing radiation (3.2.2), a combination of sub-lethal ionizing radiation and cold-treatment (3.2.10), carbon dioxide fumigation for amelioration of cold-treatment (3.2.5) and cold-treatment alone (3.2.14). Irradiation of 100 Gy was shown to provide probit 9 disinfestation of fruit, through larval mortality (85%) and an inability by the surviving adults to reproduce. Both irradiation and cold treatment requirements for disinfestation could be reduced if the treatments are combined. A combination of 2.5°C for 14 to 18 days combined with 60 Gy ionizing radiation is recommended for assessment at probit-9 level. High carbon dioxide may be a viable method for reducing post-harvest cold sterilization time and obtaining the same mortality levels as in longer treatments. Additionally, a high temperature pre-treatment may be considered prior to cold sterilization, as there does not appear to be an overlap in mechanisms of high and low temperature tolerance in FCM. Combinations of 12 h fumigation with 60% CO₂ 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%. It is therefore clear that a 24 h break after the CO₂ stress improved survival in the following cold treatment. Using cold-treatment alone, there was no survival of first instar larvae at 2°C for 14 days, and no fifth instar larval survival after 18 days at 2°C in diet. In naturally infested fruit there was no first or second instar survival after 18 days at 2°C, 2% third instars survived, 8% fourth instars and 12% fifth instars.

Three projects examined some aspect of entomopathogenic nematodes (EPNs) with a view to pre-harvest control of FCM. The first of these projects (3.2.3) was initiated in 2005 and has eventually drawn to a close, producing a considerable body of fundamental and partially applied work on the potential of EPNs for control of FCM. Most recently it was determined that imported formulated *Heterorhabditis bacteriophora* induced similar mortality of FCM larvae to *in vivo* produced nematodes of a local isolate of the same species. Imported recycled *Steinernema feltiae* was found to be more effective at cold temperature (14°C for 48 h) than *H. bacteriophora*. The second EPN study (3.2.7) was also completed, as a PhD. This examined *in vitro* mass culture techniques for two EPN species. It was determined that *H. zealandica* and *S. yirgalemense* and their associated bacterial symbionts, could be successfully cultured in a liquid medium. For *S. yirgalemense*, 77 000 IJs were produced per ml of medium and for *H. zealandica*, 41 000 IJs/ml. The final EPN study, examined control of FCM and fruit fly in large scale field trials in citrus orchards (3.2.16). Survival of EPNs was variable but generally for a longer duration than expected (up to 5 months after application). In four out of five trials, FCM trap catches were lower in the treatments than in the untreated control. The success rate measured by fruit infestation was similar, with up to 81% reduction in one case. Only one trial showed notable reduction in the fruit fly population relative to the untreated control.

A further two projects looked at other forms of microbial control (or related aspects) of FCM. One of them investigated the potential of entomopathogenic fungi (3.2.12). The three most promising isolates (two *Metarhizium anisopliae* and one *Beauveria bassiana*) were subjected to dose and exposure time response assays. At 1 x 10⁷ conidia/mL, it was estimated that late fifth instar larvae are required to be exposed to the fungal isolates for a period of nine days to ensure 90% mortality. Results also indicated the better

performance of these isolates over commercial isolates and their ability to viably persist for six months in soil in a citrus orchard. The final microbial project was somewhat more fundamental in nature, investigating gene expression of FCM when challenged with different granulovirus isolates (3.2.17), with the ultimate objective being to identify genes responsible for resistance development to these viruses.

Another three trials investigated additional unrelated techniques for controlling FCM in the field. *Trichogrammatoidea cryptophlebiae* egg parasitoids released at 100 000 per hectare during January to April, failed to make a difference (3.2.9). At three out of four trial sites, FCM levels were too low to obtain meaningful results. At the fourth site, FCM levels were very high. However, so too was natural parasitism, thus obscuring any impact which the released parasitoids might have had. A field trial was conducted to compare various mating disruption and attract and kill products (3.2.4). Mating disruption "overkill", a combination of Isomate and Checkmate (the latter applied fortnightly), was the most effective treatment in reducing both moth catches and fruit infestation. The final field control project involved an ad hoc trial to compare the efficacy of all FCM-registered spray options applied a few weeks before harvest (3.2.18). Runner and Cryptogran were the only two products to significantly reduce FCM infestation.

The final three projects addressed susceptibility of fruit to FCM and a means to correctly identify larvae found in fruit. Over a period of a month during winter in three lemon orchards, no infested marble-sized fruit and hanging yellow fruit were recorded (3.2.8). However, 1.25% of fallen mature fruit were infested with FCM. Another project compared the preference of FCM for a range of early, mid-season and later maturing varieties of Navel orange and the susceptibility of these to FCM larval penetration (3.2.15). As a result of these laboratory trials, it is recommended that farmers increase cultivation of Fischer, Cambria and Glenora Late Navels and reduce Palmer Navel production. In the final project within the FCM programme (3.2.13), a taxonomic key was developed to enable morphological differentiation between all of the major tortricid pests on fruit crops, particularly at the larval stage, which is notoriously difficult to identify. This included FCM.

Programopsomming

Met die kennis dat 'n amptelike Plaaig Risiko Raming (PRR) van VKM vir Europa tans deur die Europese Plantbeskermings Organisasie (EPPO) uitgevoer word, het navorsing binne in hierdie program selfs meer belangrik geword as ooit te vore, so ook die dringendheid waarmee die navorsing aangepak word. Dit is bekend dat hierdie PRR tot inperkende uitvoer regulasies kan lei en dus is 'n stelsels benadering ontwikkel vir die bestuur van VKM van die begin van die seisoen tot verskeping van gepakte vrugte. Dit is al aan produsente en pakhuis voorgestel en word tans deur sekere rolspelers op 'n proefbasis geïmplementeer.

Binne die navorsingsprogram word bestuur van VKM van alle moontlike kante aangepak, met 'n groeiende fokus op na-oes opsporing en bestryding. Uit 17 navorsingsprojekte wat hier gemeld word, fokus ses op na-oes faktore. Twee van hierdie het opsporing ondersoek – een deur gebruik van X-straal (3.2.6) en die ander deur opsporing van vlugtige stowwe wat met besmette vrugte geassosieer is (3.2.11). Met die gebruik van X-straal radiografie, was dit nie moontlik om VKM besmetting in Delta Valencias twee dae na besmetting plaasgevind het op te spoor nie, omdat baie van die larwes nog in die albedo was. Nietemin het mikrofokus radiografie 60% van besmetting na 10 dae opgespoor, 77% na 7 dae en 40% na 4 dae. Mikrofokus tomografie het 100% van besmetting opgespoor. Vyf belangrike vlugtige stowwe van VKM besmette lemoene is ontdek. Hierdie is D-limonien, 3,7-dimetiel-1,3,6-oktarien, (E)-4,8-dimetiel-1,3,7-nonatrien, kariofileen and naftaleen. Naftaleen sal moontlik 'n goeie aanduiding van VKM besmetting wees omdat hoër vlakke as in gesonde vrugte deur die hele besmettings tydperk opgespoor is.

Die vier na-oes behandelingsprojekte het gekyk na gammabestraling (3.2.2), 'n kombinasie van subletale gammabestraling en kouebehandeling (3.2.10), koolstofdioksied vergassing vir verbetering van kouebehandeling (3.2.5) en kouebehandeling op sy eie (3.2.14). Bestraling met 100 Gy het probit 9 disinfestasië van vrugte gegee, deur mortaliteit van larwes (85%) en die onvermoë van oorlewendes om aan te teel. Albei bestraling en kouebehandeling behoeftes vir disinfestasië kon verminder word as die twee behandelings gekombineer was. 'n Kombinasie van 2.5°C vir 14 tot 18 dae en 60 Gy bestraling behoort getoets te word as 'n ontsmettingsbehandeling teen probit-9 vlak. Hoër koolstofdioksied kan moontlik 'n metode wees om tydsduur van na-oes kouesterilisasie te verminder met dieselfde mortaliteits vlakke as langer koue blootstelling. So ook kan 'n hoër temperatuur behandeling voor kouesterilisasie oorweeg word, omdat daar heel waarskynlik geen oorlewing van mekanismes vir hoër en laer temperatuur toleransie in VKM is nie. Kombinasies van 12 h beroking met 60% CO₂ gevolg deur 'n onmiddellike waks en 'n 5-dag koue behandeling het ongeveer 90% mortaliteit tot gevolg gehad, maar as die koue behandeling met 24h ná waks vertraag is, het die mortaliteit met omtrent 25% verminder. Dit is dus duidelik dat 'n 24 h pouse ná die CO₂ stres, die oorlewing in die koue behandeling wat gevolg het, verbeter het. Met kouebehandeling op sy eie was daar geen oorlewing van eerste instar larwes teen 2°C vir 14 dae, en geen vyfde instar larwe oorlewing na 18 dae teen 2°C in kunsmatige dieet. In natuurlik besmette vrugte is daar

geen eerste of tweede instar oorlewing na 18 dae teen 2°C, 2% derde instars het oorleef, 8% vierde instars en 12% vyfde instars.

Drie projekte het verskillende aspekte van entomopatogeniese nematodes (EPNs) bestudeer met die oog op vooroes beheer van VKM. Die eerste van hierdie projekte (3.2.3) het in 2005 begin en is uiteindelik voltooi, met die gevolg dat daar 'n aanmerklike massa fundamentele en gedeeltelik toegepaste werk op die moontlike gebruik van EPNs vir beheer van VKM bestaan. Mees onlangs is dit bepaal dat ingevoerde geformuleerde *Heterorhabditis bacteriophora* vergelykbare mortaliteit van VKM larwes veroorsaak het as *in vivo* geproduseerde nematodes van 'n plaaslike isolaat van dieselfde spesies. Ingevoerde hergesirkuleerde *Steinernema feltiae* is meer doeltreffend as *H. bacteriophora* teen koue temperature (14°C vir 48 h). Die tweede EPN studie is ook voltooi, as 'n PhD (3.2.7). Hierdie het *in vitro* massa teel tegnieke vir twee EPN spesies ondersoek. Dit is bepaal dat *H. zealandica* en *S. yirgalemense* en hulle simbiotiese bakteria suksesvol in vloeistof medium massa geproduseer kon word. Vir *S. yirgalemense* is 77 000 IJ/ml medium geproduseer en vir *H. zealandica*, 41 000 IJ/ml. Die finale EPN projek het beheer van VKM en vrugtevlug in grootskaalse veldproewe in sitrusboorde ondersoek (3.2.16). Oorlewing van EPNs was wisselvallig maar was oor die algemeen langer as wat verwag is (tot 5 maande na toediening). In vier uit vyf proewe is VKM lokval vangstes laer in die behandelings as in die onbehandelde kontrole. Doeltreffendheid gemeet deur vrugbesmetting is ewe oortuigend, met tot 81% vermindering in een geval. Net een proef het 'n beduidende vermindering in die vrugtevlug populasie getoon in vergelyking met die onbehandelde kontrole.

'n Verdere twee projekte het ander soorte mikrobiiese beheer (of verwante aspekte) van VKM ondersoek. Een van hulle het die potensiaal van entomopatogeniese swamme ondersoek (3.2.12). Die drie mees belowende isolate (twee *Metarhizium anisopliae* en een *Beauveria bassiana*) is aan dosis en blootstelling tydsduur respons bio-toetse blootgestel. Teen 1×10^7 konidia/mL, is dit geskat dat vyfde instar larwes vir nege dae blootgestel sal moet word om 90% mortaliteit te bereik. Resultate het ook die beter vertoning van hierdie isolate teenoor kommersieel isolate aangedui, so ook hulle vermoë om in sitrusboord gronde vir 'n tydperk van ses maande lewensvatbaar voort te duur. Die finale mikrobiiese projek was meer fundamenteel van geaardheid. Gene uitdrukking van VKM wanneer hulle met verskillende granulovirus isolate getoets is, is ondersoek (3.2.17). Die uiteindelige doel is om genes verantwoordelik vir ontwikkeling van weerstandbiedendheid teen hierdie viruse te identifiseer.

Nog drie proewe het verskillende nieverwante tegnieke vir beheer van VKM in die veld ondersoek. *Trichogrammatoidea cryptophlebiae* eier parasiete wat teen 100 000 per hektaar losgelaat is tussen Januarie tot April, het nie 'n verskil gemaak nie (3.2.9). By drie uit vier proefpersele is VKM vlakke te laag om betekenisvolle resultate te kry. By die vierde perseel is VKM vlakke baie hoog, maar natuurlike parasitisme was egter ook hoog gewees en het dus enige impak wat die losgelate parasiete dalk gehad het verduister. 'n Veldproef is uitgevoer om verskeie paringsontwrigting en lok-en-vrek produkte te vergelyk (3.2.4). Paringsontwrigting "oordosis", 'n kombinasie van Isomate en Checkmate (laasgenoemde elke twee weke toegedien), is die mees doeltreffende behandeling, albei wat vermindering in mot vangstes en vrugbesmetting betref. Die finale vooroes bestrydings projek was 'n ad hoc proef om die werking van alle VKM geregistreerde spuit opsies, 'n paar weke voor oes toegedien, te vergelyk (3.2.18). Runner en Cryptogran is die enigste twee produkte wat VKM besmetting betekenisvol verminder het.

Die finale drie projekte het die vatbaarheid van vrugte vir VKM ondersoek en 'n metode om larwes wat vrugte kan besmet korrek te identifiseer. Oor 'n tydperk van 'n maand gedurende winter in drie suurlemoen boorde is geen besmette albaster-grote vrugte en geen besmette geel vrugte in die bome gekry nie (3.2.8). Egter is 1.25% van volwasse vrugte op die grond met VKM besmet. Nog 'n projek het voorkeur van VKM vir 'n reeks vroë, mid-seisoen en laat rypwordende Nawellemoen varieteite vergelyk en ook die vatbaarheid van hierdie varieteite vir larwe penetrasie (3.2.15). As gevolg van hierdie laboratorium proewe word dit aanbeveel dat produsente hulle aankweking van Fischer, Cambria en Glenora Late Nawels vermeerder en hulle Palmer Nawel produksie verminder. In die finale projek binne die VKM program (3.2.13), is 'n taksonomiese sleutel ontwikkel om morfologiese onderskeiding tussen al die belangrike tortrisied mot plae op vruggewasse moontlik te maak, insluitend VKM. Veral belangrik is identifikasie van larwes, wat bekend is as moeilik om te herken.

3.2.2 **FINAL REPORT: Assessment of Ionizing Radiation for the Phytosanitary Disinfestation of False Codling Moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) on Citrus in South Africa: Validation of a Post-harvest Treatment**
Project 719 (2003-2013) by J.H. & M. Hofmeyr (Ibbsbüren Navorsing)

Summary

Problems inherent to current post-harvest control procedures for false codling moth in citrus are discussed. The effects of ionizing radiation treatment to false codling moth larvae in previous studies are summarized to demonstrate the increased tolerance of eggs and larvae with increasing age and developmental stage. An experiment was consequently conducted to validate the use of ionizing radiation as a post-harvest mitigation treatment for false codling moth. At 100 Gy a population of more than 124 000 treated 5th instar larvae was reduced by 85%. Moths developing from the surviving larvae were unable to fly, they consisted mainly of males and mating was reduced by 80%. Not a single egg was deposited in a mass egg-laying test. The procedure is regarded as suitable for the maintenance of quarantine security against false codling moth larvae in export citrus.

Opsomming

Probleme inherent met huidige na-oes beheerprosedures vir valskodlingmot in sitrus word bespreek. Die uitwerking van ioniserende stralingsbehandeling op valskodlingmot in vorige studies word opgesom om die toenemende vatbaarheid van eiers en larwes met toenemende ouderdom én ontwikkelings stadium te illustreer. 'n Proef is derhalwe uitgevoer om die gebruik van ioniserende straling as 'n na-oes beheermaatreel vir valskodlingmot te bekragtig. Teen 100 Gy is 'n bevolking van meer as 124 000 5^{de} instar larwes met 85% verminder. Motte wat uit die oorlewende larwes ontwikkel het, kon nie vlieg nie, het meesal uit mannetjies bestaan en paring is met 85% verminder. Nie 'n enkele eier is in 'n massa-eierlêtoets gelê nie. Die tegniek word as geskik vir die instandhouding van kwarantynsekuriteit teen valskodlingmotlarwes in uitvoersitrus beskou.

Introduction

The pre- and post-harvest risks presented by false codling moth (FCM), *Thaumatotibia leucotreta*, have been well documented (Bloem *et al.*, 2003). Afforded the opportunity, the insect can cause fruit losses of commercial concern in orchards. It can also pose a real threat to the quarantine security of countries possessing climates suitable for the distribution and establishment of the pest insect. For many years the southern African citrus export market consequently had to contend with control measures aimed at reducing the overall occurrence of FCM in export citrus – sometimes to the point of zero tolerance (Hattingh, pers. com.). The development of a cold disinfestation mitigation treatment dates back 5 decades when Myburgh (1963) conducted a series of studies to investigate the ability of various cold treatments to kill FCM larvae in oranges. This ground-breaking research eventually culminated in a disinfestation treatment involving the storage of citrus fruit at –0.6°C for 22 days, originally developed for the Republic of Korea (Hofmeyr, 1998). The procedure was subsequently approved for use by other countries such as the U.S.A. and the People's Republic of China.

The cold disinfestation treatment made it possible to export citrus to markets that would otherwise have been either impossible or may have caused the enforcement of a zero tolerance policy that would have crippled the citrus industry. Although proved biologically effective since its inception the treatment was variably detrimental to fruit quality and a protocol that could mitigate cold damage while maintaining quarantine security was required. An investigation into the potential of ionizing radiation (IoR) to suppress FCM and reduce the risk to fruit quality was consequently initiated in 2002.

Doses of IoR in the range 200 Gy to 400 Gy were initially assessed on FCM eggs and larvae. A concurrent investigation to study the relative tolerance of oranges, mandarins and lemons to IoR was conducted using a commercial 500 kCi Cobalt-60 irradiator. Dose uniformity ratios (DUR) of 3.5:1 to 4:1 were encountered in pallets fully stacked with the different cultivars indicating that the maximum effective dose would have to be restricted to 160 Gy to accommodate sensitive cultivars (Barry *et al.*, 2007). Due to the high DUR experimental doses had to be reduced considerably in attempts to develop the Lowest Effective IoR Dose (LERD) that could be utilized as a phytosanitary measure and still be tolerated by citrus fruit, viz. 40-50 Gy. The most relevant results were as follows (Hofmeyr & Hofmeyr, 2013a,b in press):

- Fully developed eggs and larvae were more tolerant to IoR than the respective younger stages.
- Larvae were more radiotolerant than eggs.
- Larvae reared in artificial diet were at least as radiotolerant as larvae that developed in naturally infested oranges.

- The LERD that was probably adequate for phytosanitary application exceeded the 40-50 Gy maximum as predetermined by fruit quality restraints.

The horticultural data was re-evaluated in 2012 by Hattingh and Cronje (pers. com.). The original conclusions were revised and the maximum acceptable dose was increased to 500 Gy, allowing experimental doses to be increased substantially to 125 Gy within the restraints imposed by the DUR. Taking into consideration all direct and indirect effects from the various doses, it was decided to use a dose of 100 Gy for treatment validation.

Objective

This study is aimed at the validation of 100 Gy IoR as a post-harvest mitigating treatment for the elimination of false codling moth larvae in citrus fruit destined for export markets at a level acceptable for phytosanitary purposes.

Materials and methods

1 Test material

The required number of FCM larvae was reared in the laboratories of Citrus Research International (Pty) Ltd in Citrusdal, Western Cape, South Africa. Eggs for this purpose were supplied by the sterile insect rearing facility, Xsit (Pty) Ltd, in Citrusdal. The gene pool of the insects reared by this facility was replenished on a seasonal basis by introducing feral FCM into the culture. FCM were reared on a diet consisting predominantly of corn flour (Moore, 2002) in 500 ml wide-necked glass rearing jars with membrane-fitted screw-on metal lids. Five hundred to 800 24-h old eggs on wax paper were inoculated into each jar, incubated at 26°C and 5th (final) instar larvae were obtained approximately 14 days later. The larvae pupated in rolled-up strips of corrugated cardboard, 30 mm wide, which replaced the metal lids just before pupation commenced. The cardboard strips were pulled apart to collect the pupae or when required, were put into emergence containers for moth eclosion.

2 Dosimetry

- **Dosimetry:** All dosimetry conducted for this project were conducted by Prof. Kobus Slabbert (Head: Department of Radiation Biophysics) at iThemba LABS, Somerset-West, South Africa:

- **Irradiator design:** A Cobalt-60 point source, panoramic irradiator at the FCM rearing facility of Xsit (Pty) Ltd in Citrusdal, Western Cape, South Africa was used. The targets were located on a primary turntable, 1 370 mm in diameter with 8 secondary turntables, each 300 mm in diameter. The primary turntable rotated at 2 r.p.m. and the secondaries at 15 r.p.m..
- **Irradiation container:** Artificially reared larvae were treated in the glass rearing jars as the container sides provided sufficient build-up. The set-up for dosimetry and treatment applications consisted of a stack of 3 jars with larvae placed on each of the secondary turntables.
- **Initial calibration:** The initial calibration was done using Fricke dosimeters with standard G-value for $\text{Fe}^{3+} = 15.5/100$ eV radiation energy absorbed. The Molar extinction coefficient for a HP diode array spectrophotometer was determined at 304 nanometre using spectrosol grade Fe^{3+} solutions (value 2081 l/Mole/cm). This was to convert optical density readings made using a quartz glass flow cell – optical path 10 mm and reflecting $[\text{Fe}^{3+}]$ concentrations into J/kg (Gray) values. The dosimeter response was checked in a Cobalt-60 radiation field – set-up 6 mm build-up and 50 mm backscatter in a 300 mm x 300 mm collimated field. The output factor was determined for the above using a Farmer tissue equivalent ionization chamber calibrated in a standard field of National Metrology Laboratory (C.S.I.R). Routine consistency checks were conducted on newly prepared Fricke solutions using 6 MV X-rays in a 300 mm x 300 mm field of a clinical photon therapy linear accelerator. Calibration factors relative to the National Standard as above applied.
- **Dose mapping:** Dose mapping was conducted at the top and bottom of the rearing jar stack. The dose uniformity from top to bottom was 17%. No reference position was used; the dosimetry was conducted for various positions in the irradiation set-up and the mean was calculated. The dose rate was updated before every irradiation procedure using the decay half-life of Cobalt-60 at 63.26 months.
- **Uncertainties:** Repeated readings were taken at each position with differences less than 0.5%. Each radiation set-up was calibrated using a range of doses that covered the radiation levels used in the application. Uncertainties in the jar stack from repeated dose rate readings were less than 4%.
- **IoR dose:** The IoR treatment in the validation study consisted of one dose only, viz. 100 Gy. Due to a dose uniformity variation of 17% in the treated jar stack, this dose had to be regarded as a mean, varying from 91.5 Gy to 108.5 Gy.

3 Treatment assessment

Probit-9 required the treatment of a total of 94 000 insects. From previous experience it was expected that a mean of 400-500 test insects would be reared per rearing jar. It was not possible to determine the actual number of larvae used in the study as they could not be removed from the diet. Instead, all pupae were counted in a subsample [section (b)(i)] and their numbers were used to calculate the minimum total number of larvae treated. Three replicates of 75 rearing jars each were prepared consecutively at weekly intervals. Each replicate was allocated as follows:

a) **Instar determination:** On the day of treatment one representative rearing jar per replicate was removed at random for larval instar verification. The diet was removed from the jar and 50 larvae were collected at random. Head capsule measurements (Daiber, 1979) were conducted with the aid of an electronic calliper.

b) **Untreated control:** Eight untreated jars (a 'subsample') were removed at random from each replicate and used to study:

(i) **Pupal production:** The pupae from each jar were counted. *These numbers were used to calculate the number of test insects involved in the study.*

(ii) **Reproductive potential:** The pupae from each subsample were combined into one batch. One hundred and sixty pupae were removed 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 the number of dead pupae was recorded. The first 10 pairs of moths to eclose were used to assess reproductive potential: A newly eclosed female and male were placed into each of 10 x 100 ml plastic oviposition cages. Only inbreeding was assessed, *i.e.* untreated ♀ x untreated ♂ ($U_{\text{♀}} \times U_{\text{♂}}$). Oviposition was allowed for 5 days. The females were then collected, dissected and examined for the presence of spermatophores in the *bursae copulatrix* to confirm mating status. The eggs were incubated and all hatched and dead eggs per oviposition cage were recorded 7-d later.

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

(iv) **Fecundity and fertility:** All pupae from the subsamples except the 160 removed for the purposes mentioned in (i)-(iii) above, were placed into an emergence box for moth eclosion. The moths were collected and placed into oviposition containers in the Xsit rearing facility. Two A4-sized wax paper sheets on which the females deposited their eggs were removed every second day and incubated at 26°C, for a total of 6 egg sheets per replicate. Fecundity and fertility of the moths were assessed by counting the number of hatched and dead eggs in each of 5 sectors, each 25 mm x 25 mm, randomly demarcated on each egg sheet.

c) **Treated control:** It was impractical to conduct the type of supportive studies discussed in section (b)(i-iv) above using test material from the mass experiment. An additional 8 rearing jars were therefore removed at random from each replicate directly after treatment. They were incubated separately for comparison with the untreated control subsamples. The pupae from each jar were counted and then combined into one batch. A sample of 160 pupae was removed per subsample and evaluated similarly to the untreated control. Inbreeding only, *i.e.* treated ♀ x treated ♂ ($T_{\text{♀}} \times T_{\text{♂}}$) moth combinations, was assessed. To prevent the recording of unhatched eggs caused by possible delayed hatching due to larval treatment, eggs were incubated for 14-d, *i.e.* 7-d longer than control eggs. All collected pupae from each subsample excluding the 160, were added to the emergence box of the main treatment [see (d) below].

d) **Main treatment:** Ten rearing jars had to be discarded post-treatment in replicate 1 because of poor production due to a diet problem and only 56 jars, including the 8 jar subsample, were used. Sixty six jars, including the treated 8 jar subsample mentioned in (c), were used in each of replicates 2 and 3. All treated jars were fitted with cardboard tops as described in section 1 (Test material). When pupation was completed, the stoppers with cocoons from each replicate were removed and suspended in an emergence box for moth eclosion. A shallow tray with sawdust was placed into each emergence box as pupation substrate for larvae leaving their cocoons.

All moths were hand-collected and placed on wax paper sheets under flour strainers, each 160 mm in diameter, for oviposition. Oviposition was allowed for 5 days when the (mostly dead) moths were removed and discarded. Six egg sheets were collected for each replicate and the entire oviposition surface of all

sheets was examined for the presence of eggs with stereo-microscopes. Each egg sheet was assessed on 2 occasions 3-d apart by 2 researchers working independently.

Results and discussion

1 Developmental stage

Respectively 98%, 98% and 100% of the larvae in the 3 replicates consisted of 5th instars. These larvae have been shown to be the most radiotolerant larval stage (Hofmeyr & Hofmeyr, 2013b in press). The remainder were 4th instar larvae.

2 Numbers of test insects

The larvae treated in the study were not counted as they could not be removed from the diet. Dried-out larval cadavers were noticed when the cardboard rolls in the untreated and treated control subsamples were pulled apart to remove the pupae. They were not counted, but there were noticeably more cadavers in the treated than in the untreated controls. Overall the mortality was too small to be considered as a useful treatment side-effect. Nonetheless, from a comparison of the numbers of pupae collected from the untreated and treated controls it was clear that many treated larvae must also have died in the diet undetected (Table 3.2.2.1).

Table 3.2.2.1. Apparent mortality of larvae treated with 100 Gy ionizing radiation.

Replicate	Mean no. of pupae per rearing jar subsample collected from		% reduction in pupae from treated larvae
	untreated larvae	treated larvae	
1	532.9	308.7	42.1
2	673.6	318.4	52.7
3	760.5	368.9	51.5

It was consequently considered justifiable to use the numbers of pupae recorded in the untreated controls to calculate the numbers of treated larvae initially present in the 3 treatment replicates. Based on this premise, more than 124 000 test insects were treated (Table 3.2.2.2).

Table 3.2.2.2. Numbers of false codling moth larvae used in the validation study.

	Replicate 1	Replicate 2	Replicate 3
Mean number of pupae per jar in untreated control	532.9	673.6	760.5
Number of jars treated per replicate	56	66	66
Potential number of pupae per treatment replicate	29 842	44 458	50 193
Minimum number of FCM larvae treated	124 493		

3 Reduction in pupal and moth numbers

The IoR treatment reduced the mean numbers of pupae and moths by 49.4% and 85.6% respectively relative to the controls. Although not of significance as a stand-alone effect that would meet phytosanitary standards, it would certainly contribute to reducing the over-all level of an FCM infestation in packed fruit.

4 Gender ratio

The ratio of females to males in the untreated controls was 1:0.8. This is normal as the ratio often varies from 0.1 to 0.3 in favour of either gender on both sides of the 1:1 level. The gender ratio in the IoR treatment was 1:2.4, reflecting the lower radiotolerance of the females and consequently, higher mortality in their larval and pupal stages (Bloem *et al.* 2003; Hofmeyr & Hofmeyr 2013b, in press).

5 Flight capability

In the flight tests 95.8% of the moths in the untreated controls flew normally. There was a high percentage of malformed moths in the IoR treatment. These moths, including those showing slight or no aberrations, were clearly debilitated and lethargic. No flight capable moths were observed in the IoR treatment.

The many thousands of moths in the ionizing treatment were almost invariably too weak to enter the collection jars attached to the emergence boxes. A close-fitting shallow tray was placed on the bottom of each emergence box as an alternative pupation site for larvae sometimes leaving their cocoons. These trays unintentionally but fortuitously served as collection containers for the moths. All eclosed moths dropped into the trays and only a very few succeeded in temporarily climbing up the sides of the emergence boxes from where they either dropped back again or were flicked back into the trays during collection. The trays were removed to an open laboratory bench where the moths were hand-collected – not a single moth managed to fly away.

Collectively, these unequivocal cases of flight inability is essential supportive evidence of the potential merit of the ionizing treatment to achieve set phytosanitary standards.

6 Fecundity and fertility

In the oviposition experiments all 30 females from the untreated control jars had mated, in contrast to 5 from 30 females only in the treated control jars. No eggs were produced by moths in the latter treatment, while egg production and hatching was normal in the untreated control. Large numbers of viable eggs were also deposited in the untreated control of the mass oviposition study, while no eggs were produced in the IoR treatment (Table 3.2.2.3).

Table 3.2.2.3. Fecundity and fertility of false codling moths treated as 5th instar larvae with 100 Gy of ionizing radiation.

Treatment	Replicate 1		Replicate 2		Replicate 3	
	Su bsa mpl e	Ma ss ovi pos itio n	Su bsa mpl e	Ma ss ovi pos itio n	Su bsa mpl e	Ma ss ovi pos itio n
Fecundity: No. of eggs/replicate						
Untreated control	4 643	7 497	4 356	5 146	5 122	5 351
100 Gy ionizing radiation	0	0	0	0	0	0
Fertility: Mean % egg hatch						
Untreated control	85. 4	77. 7	79. 5	81. 0	82. 9	77. 9
100 Gy ionizing radiation	No eggs					

Conclusion

In the inclusive research preceding this validation study, the effect of IoR doses ranging from 40 Gy to 400 Gy were studied on eggs and larvae of *T. leucotreta*. A treatment dose of 100 Gy of ionizing radiation prevented mature eggs and the most radiotolerant larval stage, 5th instar, from producing viable off-spring. It was also demonstrated that insectary reared larvae were at least as radiotolerant as feral larvae developing naturally in oranges.

The current study was conducted on a scale 33% larger than required for probit-9. The results indicated that 100 Gy of ionizing radiation would reduce the number of larvae able to develop into moths by more than 85%. The survivors would consist largely of males. Any moth emerging from a consignment of fruit would be incapable of flying and therefore unable to disperse and locate a mate. Mating would be reduced by at

least 80% and no eggs would be produced by mated or unmated females. The IoR treatment for FCM is therefore regarded as adequate at 100 Gy to maintain phytosanitary security in oranges.

Future research

No further research is deemed necessary as the IoR treatment as tested afforded excellent control of FCM larvae post-harvest at a treatment dose safe for the most important export citrus cultivars.

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3.2.3 FINAL REPORT: The use of entomopathogenic nematodes to control codling moth Project 793 (April 2006 - March 2012) by A.P. Malan (SU) and S.D. Moore (CRI)

Summary

During a survey to determine the occurrence and distribution of entomopathogenic nematodes (EPNs) in citrus orchards, three *Steinernema* spp. and three *Heterorhabditis* spp. were isolated. The species were identified using morphological, morphometric and molecular techniques. *Steinernema citrae* and *H. noenieputensis* were described as new species. During laboratory screening using 24-well bioassay plates, *Steinernema yirgalemense* gave the best performance as a biocide against false codling moth (FCM), *Thaumatotibia leucotreta*. Nematodes were also shown to be able to infect emerging moths and to aid, as such, in their long-distance transport. Sand and soil bioassays in the laboratory had shown nematodes to work exceptionally well in locating and infecting FCM larvae and pupae. In horizontal and vertical soil columns, *Heterorhabditis bacteriophora* were able to infect FCM larvae over a distance of 15 cm within 4 h, with main differences in movement and penetration capabilities between different species over time, as well as in different soil types. In semi field trails with different concentrations of *Heterorhabditis bacteriophora*,

good control was obtained with 20 infective juveniles (IJs)/cm² cm, with persistence of up to 6 months. In another field trial with three different species, *H. bacteriophora* performed better than did *H. zealandica* and *S. khoisanae*. In a concentration trial with *H. zealandica*, good control was obtained using a concentration of 5 IJs/cm³. Infection of FCM larvae was obtained, with nematodes moving horizontally and vertically through soil columns after 4 h. When imported formulated *H. bacteriophora* were compared with local *in vivo* produced nematodes, no significant difference in mortality was found at optimum conditions. At a temperature of 14°C after 48 h, no mortality was found for *H. bacteriophora*, while, for recycled *S. feltiae*, good control was obtained. Despite FCM larvae not being killed at 48 h at 14°C using *H. bacteriophora*, they were infected and killed after a further 2 days at optimum conditions.

Opsomming

Drie spesies van *Steinernema* en drie van *Heterorhabditis* is isoleer gedurende 'n opname om die voorkoms en verspreiding van entomopatogeniese nematodes in sitrusboorde te bepaal. Die spesies is bepaal deur gebruik te maak van morfologiese, morfometriese en molekulêre tegnieke. Biotoetse gedoen met 24-putjie plate het aangetoon dat *Steinernema yirgalemense* het die beste presteer as beheer agent teen valse kodling mot (FCM), *Thaumatotibia leucotreta*. Daar is ook aangetoon dat nematodes die ontpoppende mot van FCM in die grond kan infekteer en sodoende bydra tot die lang termyn verspreiding van nematodes. Deur gebruik te maak biotoetse met sand en grond in die laboratorium, is aangedui dat nematodes besonder goed vaar in die opsporing en infeksie van FCM larwes en papies. Horisontaal en vertikaal georiënteerde sandbuis toon aan dat *Heterorhabditis bacteriophora* FCM larwes oor 'n afstand van 15 cm kan infekteer binne 'n periode van 4 h, met verskille in spoed van beweging en die penetrasie vermoë tussen verskillende nematode spesies. Deur gebruik te maak van verskillende konsentrasies van *Heterorhabditis bacteriophora* in semi-veldproewe is goeie beheer gekry met 20 infektiewe larwes (IJs)/cm², met 'n nawerking van tot 6 maande. 'n Verdere veldproef het met drie verskillende spesies is aangetoon dat *H. bacteriophora* beter resultate oplewer as *H. zealandica* wanneer gebruik word teen 'n konsentrasie van 5 IJs/cm³. Wanneer ingevoerde geformuleerde *H. bacteriophora* vergelyk is met lokale *in vivo* produseerde nematodes, is daar geen beduidende verskil in mortaliteit gevind onder optimale toetstande nie. By 'n temperatuur van 14°C na 48 h is geen mortaliteit gevind vir *H. bacteriophora* nie, terwyl in die geval van *in vivo* produseerde nematodes goeie beheer gekry is. Alhoewel geen mortaliteit verkry vir *H. bacteriophora* by 14°C na 48 uur nie, was die FCM nog steeds infekteer en gedood na 2 dae onder optimale toetstande.

Introduction

The false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), occurs naturally in South Africa and also in sub-Saharan Africa and the Indian Ocean islands (CABI/EPPO, 1976). It is a serious pest of citrus in South Africa, and can cause major economic losses (Moore *et al.*, 2004). It is also a pest of phytosanitary concern, with restrictive import regulations having been imposed by importing countries (Bloem *et al.*, 2003) such as the USA. During 2008, a total of 58 102 ha of citrus were cultivated in all provinces of South Africa, except for the Free State. The most hectares planted are in Limpopo, the Eastern Cape, Mpumalanga and the Western Cape provinces (CGA, 2010). In South Africa, citrus is a major export-based industry and represents a huge investment in both foreign exchange earnings and human resources.

The life cycle of FCM is 25-60 days and up to six generations per year has been recorded in South Africa (Daiber, 1980; Georgala, 1969; Stofberg, 1954). The moths lay their eggs on the fruit. The hatched larvae penetrate the fruit, causing premature fruit drop (Daiber, 1989). The last-instar FCM larvae drop with a silken thread onto the soil, in which they bury themselves under a few millimetres, and spin tightly woven cocoons in which soil is incorporated (Daiber, 1979a). After a period of approximately two to three days, the prepupa in the cocoon changes into a pupa, and the adult moth emerges after a further 12 to 16 days at 25°C, with longer intervals at lower temperatures (Daiber, 1979b).

Entomopathogenic nematodes (EPN) of the families Steinernematidae and Heterorhabditidae, with their associated symbiotic bacteria, are widely distributed in soils throughout the world (Hominick (Adams *et al.*, 2006; Hominick, 2002; Hominick *et al.*, 1996). These nematodes are parasites of insects, killing them within 48 hours with the aid of their associated bacterial symbiont. Since the late 1970s, these nematodes have gained status as one of the best non-chemical alternatives for the control of insect pests, mainly due to their ability to reach insects in cryptic habitats, their high reproductive ability, the ease of mass producing them, and their safety to humans and other vertebrates (Gaugler, 2002).

In South Africa, the citrus industry currently employs a combination of cultural, chemical and microbial control techniques to suppress FCM. The methods employed include orchard sanitation (Moore & Kirkman, 2008), mating disruption (Hofmeyer *et al.*, 2005), and biological control, using a *Cryptophlebia leucotreta* granulovirus, as well as the sterile insect technique (Hofmeyer *et al.*, 2005). However, none of the control measures named target the soil-borne stages of FCM. As soil is the natural habitat of EPN, the last-instar FCM larvae which fall onto the soil, as well as the pre-pupae, pupae and emerging moths, offer a window of opportunity for the use of nematodes as biological control agents. Nematodes can also fill an important niche in early spring, summer, autumn, and after harvest, when, traditionally, no control measures are implemented.

Stated objectives

- Survey of local EPNs in citrus orchards;
- Screening local species and isolates of EPNs for mortality against both FCM larvae and pupae;
- Sand and soil bioassay was used for selected EPN isolates in a more natural environment;
- Different concentrations and persistence of *Heterorhabditis bacteriophora*, in a semi-field trial;
- Effective evaluation method and the immediate and long term control potential of the three nematode species in a field trial;
- Different concentrations and persistence of *Heterorhabditis zealandica*, in a semi-field trial;
- The virulence of endemic entomopathogenic nematodes and imported formulated *Heterorhabditis bacteriophora* at 25°C against FCM;
- The virulence of local *H. bacteriophora* and imported *Steinernema feltiae* at different temperatures (25°C and 14°C) against FCM;
- The lethal concentrations of *S. feltiae* and *H. bacteriophora* at 14°C against FCM.

Materials and methods

1. Soil sampling, trapping and maintenance of nematodes during survey

Soil samples were collected from citrus orchards in the Western Cape, the Eastern Cape and Mpumalanga. Subsamples were taken from a depth of up to 20 cm, by using a hand spade, from underneath the canopy of four trees, situated in each quadrant of an orchard, and the 16 subsamples from the orchard were combined to form one composite sample of approximately 1 kg.

Nematodes were recovered from the soil samples by using the insect-baiting technique (Bedding & Akhurst, 1975). All the soil from each sample was split up into 250-ml plastic containers. Five larvae, of either the greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Phylalidae) and/or the mealworm, *Tenebrio molitor* (L.) (Coleoptera: Tenebrionidae) were placed on the soil surface in each of the containers, which were then closed. The two trapping-hosts were added together or the one followed by the other, depending on the availability of the hosts. During a seven-day period for wax moth and a fourteen-day period for mealworm, the samples were periodically checked for the presence of dead insects. Possible EPN-infected cadavers were placed on modified White traps (Kaya & Stock, 1997). Nematodes were harvested within the first week of emergence and used to inoculate wax moth larvae, in order to confirm Koch's postulates for pathogenicity (Steyn & Cloete, 1989). Nematode isolates were maintained at Stellenbosch University in 150 ml of filtered tap water in vented culture flasks, which were kept horizontally at 14°C and shaken weekly. Infective juveniles (IJs) were maintained by recycling through wax moth larvae every three months for heterorhabditids, and every six months for steinernematids (Dutky *et al.*, 1964; Nguyen, 1988).

2. Molecular characterisation of nematodes

For DNA extraction, the technique described by Nguyen (2007) was used. One first generation female for steinernematids or one hermaphrodite for heterorhabditids, was placed in 30 ml lyses buffer (16mM $[\text{NH}_4]_2\text{SO}_4$, 67 mM Tris-HCl pH 8.8, 0.1% Tween-20) containing 60 µg/ml Proteinase K on the side of a 0.5 µl Eppendorf tube. The nematode was cut into pieces with the sharp side of a syringe needle and immediately put on ice and frozen overnight at -80°C. The Eppendorf tubes were put in a thermocycler at 65°C for 1 hour followed by 95°C for 10 min and then centrifuged for 2 min at 12 000 rpm. The top 20 µl was transferred to a clean 0.5 µl Eppendorf tube and kept at -20°C.

The 18S and 26S primers (Whitehead Scientific), suggested by Vrain *et al.* (1992), were used for amplification of the ITS region. If a good sequence could not be obtained, the primers TW81 and AB28 (Hominick *et al.*, 1997) were used. The technique of Nguyen (2007) for PCR amplification was followed. Purified DNA was sequenced at the Analytical Centre of the Department of Genetics at Stellenbosch University, using the BigDye 3.1 chemistry (PE Applied Biosystems). The base-pair calls of the sequences were verified and edited, using the software CLC DNA Workbench, version 6.

To indicate the phylogenetic position of the nematode isolates found during the survey, sequences of *Heterorhabditis* and *Steinernema* isolates were compared to sequences from Genbank. Phylogenetic and molecular analyses were conducted based on maximum parsimony (MP) of the ITS region using the software ClustalX ver. 1.83 (Thompson *et al.*, 1997) and PAUP* ver. 4.08b (Swofford, 2002), 2002) or maximum likelihood (ML) using Mega5 (Tamura *et al.*, 2011).

3. Morphometric and morphological characterization

For morphometrics, 25 IJs and 20 first generation males for *Steinernema* and second generation males for *Heterorhabditis* of an isolate of each species were randomly selected from different infected larvae. Nematodes were killed with hot (85°C) triethanolamine-formalin (TAF) (Courtney *et al.*, 1955) fixative, and processed to anhydrous glycerine for mounting ((Seinhorst, 1959). Measurements were taken, using a Leica DM2000 research microscope equipped with a camera, drawing tube and digital image software Leica Application Suite (LAS), version 3.5.0.

Morphological observations were made following the taxonomic criteria suggested by Stock and Kaya (1996) (Stock & Kaya, 1996) and Hominick *et al.* (1997). Additionally, to confirm the identity of the different isolates, live specimens and biological observations were used to confirm key diagnostic features of specific species.

4. Source of nematodes and insects used for inoculum

For each bioassay of endemic nematodes, the inoculum used was freshly prepared, using *G. mellonella* (L.) larvae. Nematodes were harvested within the first week of emergence from modified White traps (White, 1927), and used within one month of storage. Concentrations were calculated according to the technique of (Navon & Ascher, 2000).

Heterorhabditis zealandica (SF41), *H. bacteriophora* (SF351) and *S. yirgalemense* (157-C) are endemic nematode species that have previously being tested in laboratory bioassays for their infectivity against last-instar FCM (Malan *et al.*, 2011). *Heterorhabditis bacteriophora* (Hb-e-nema) and *S. feltiae* (Sfel-e-nema) were imported from Germany as commercially formulated products (Table 3.2.3.1).

Table 3.2.3.1. *Heterorhabditis* and *Steinernema* species, strain, habitat and locality.

Species	Ref.	Habitat	Locality/Origin
<i>H. zealandica</i>	SF41	Natural vegetation	Patensie, Eastern Cape Province
<i>H. bacteriophora</i>	SF351	Agricultural soil	Wellington, Western Cape Province
<i>H. bacteriophora</i>	Hb-e-nema	<i>In vitro</i> production	Germany
<i>S. yirgalemense</i>	157-C	Agricultural soil	Piketberg, Western Cape Province
<i>S. feltiae</i>	Sfel-e-nema	<i>In vitro</i> production	Germany

Wax moth larvae were cultured in the laboratory on a diet of five parts of Cerelac Nestlé regular baby cereal, bran, brown bread flour, two parts of wheat germ, yeast and one part of honey, all mixed together. Laboratory-reared FCM larvae and pupae were obtained from River Bioscience located in the Sundays River Valley, in the Eastern Cape province of South Africa.

5. Twenty-four-well bioassay protocol

A 24-well bioassay tray (Flat bottom, Nunc™, Cat. No. 144530) was used as the test arena. To obtain an even distribution in the plate, every alternate well was lined with a circular piece of filter-paper (13-mm diameter), thus using 12 wells per tray and five trays for each treatment. Each of the 12 wells was inoculated with a specific concentration of IJs in 50 µl filtered tap water. An insect was added to each of the wells, covered with a glass pane inside the lid (to prevent FCM larvae from escaping) and secured with a rubber band. The wells were closed in a plastic container lined with moistened tissue paper and placed in a growth chamber at 25 ± 2°C for 48 hours. Thereafter, mortality was determined and the infection of the insect with nematodes confirmed by dissection with the aid of a stereomicroscope.

6. Virulence assays for FCM final-instar larvae

The 24-well bioassay procedure was used to screen the six nematode species found during the survey. The isolates used include 157-C, 141-C, 106-C for *Steinernema* and 26-C, 158-C and 118-C for *Heterorhabditis* (Table 3.2.3.1). Five bioassay trays with 12 FCM last-instar larvae were used as a treatment ($n = 60$) with the corresponding control treatment ($n = 60$). For each treatment 50 IJs/50 µl filtered tap water was used and

for the control water alone. After a 48- hour exposure period to the nematodes, mortality was determined. The bioassay was repeated on a different test date with fresh nematode inoculum.

7. *Virulence assays for FCM pupae*

A pilot test with pupae showed them to be less susceptible than larvae to infection, and therefore a fourfold higher concentration of IJs was used as inoculum for pupae. The 24-well bioassay protocol was used with the same six nematode isolates as were used for the larvae. Each pupa was inoculated with 200 IJs/50 μ l of water, while the controls received water alone. Five bioassay trays with 12 FCM pupae were used as a treatment ($n = 60$) with the corresponding control treatment ($n = 60$). Death of the pupae was determined by prodding and if no movement was observed, the pupae were washed to remove surface nematodes and kept for a further two days at $25 \pm 2^\circ\text{C}$ prior to dissection in order to determine the presence of developing nematodes. The bioassay was repeated on a different test date with fresh nematode inoculum.

8. *Virulence assays for eclosing FCM adults*

Circular plastic containers (15 cm diam., height 35 cm) containing previously frozen 10 ml oven-dried river sand, plus 5 ml of sterile water, were closed and left for 24 h in a growth chamber at 25°C to allow even moisture distribution. Twenty last-instar FCM larvae were placed on top of the sand in each container. The closed containers were left for a period of seven days in a growth chamber at 25°C . This provided time for the larvae to burrow into the sand, to spin into cocoons and to turn from pre-pupae into pupae. The nematode isolates 157-C, 106-C and 118-C were added by spraying 5 ml of a concentration of 800 IJs/ml (± 22 IJs/cm²) onto the sand, using the nozzle of a spray-bottle and a 25-ml cylinder. The round containers were left open, and each was separately enclosed in a 2-litre plastic container (160 cm \times 220 cm) with a moistened paper towel in the bottom, and returned to the growth chamber for another 7 days. For each treatment, including the control, there were five replicates with 20 last-instar FCM larvae in each round container ($n = 100$). The control treatments received only water. After 14 days all live and dead moths were recorded in the outer container and the remaining pupae were retrieved by washing the sand through a sieve. In the nematode treatments, all live moths were caught and left in a petri dish with moistened filter-paper. The moths were kept till they died and were then dissected to check for infection. The procedure was repeated on a different test date and with fresh nematode inoculum.

9. *Sand bioassays with cocooned larvae*

Moist sand in plastic containers was used to determine the potential of four selected EPNs to penetrate cocooned FCM larvae. Ten last instar FCM larvae were added to 100 ml moist sand in each of five 500 ml plastic containers and closed with a lid. The containers were left at 25°C for 24 hour to give the larvae time to bury into the soil and spin into cocoons. Nematodes (200IJ/FCM larvae) were inoculated into the middle of the sand surface and after four days infection was determined by washing the cocooned larvae from the soil.

10. *Sand bioassays with pupae*

Glass petri dishes, 15 cm in diameter, were filled with 100 ml dry sterile sand, 10 ml of water added and kept at 25°C overnight for even moisture distribution. Twenty pupae were arranged on the sand in the outer circle of the petri dish and covered with 40 ml of dry sand. Nematodes were inoculated by pipetting 1 ml of IJs in the centre of the soil to give 200 IJs per pupa. For each EPN isolate, five petri dishes were used and placed in a closed plastic bag for moisture control. After a period of six days at 25°C the mortality was determined by counting the dead pupae and emerged moths. Dead pupae and moths were kept in petri dishes with moistened filter paper at 25°C for another two days and dissected to determine the presence of developing nematodes.

Moist sand in plastic containers was used to determine the potential of four selected EPNs to penetrate cocooned FCM larvae. Ten last instar FCM larvae were added to 100 ml moist sand in each of five 500 ml plastic containers and closed with a lid. The containers were left at 25°C for 24 hour to give the larvae time to burrow into the soil and spin into cocoons. Nematodes (200 IJ/FCM larvae) were entered in the middle of the sand and after four days infection was determined by washing the cocooned larvae from the soil.

11. *Soil bioassays*

Soil from three different citrus orchards in the Nelspruit area and the Eastern Cape was used to determine the infectivity of three nematode species on cocooned larvae of FCM. The containers were inoculated by spraying IJ of *H. zealandica* (SF41), *H. bacteriophora* (SF351) and *S. khoisanae* (SF 106-C) onto the surface of the soil. The nematodes were sprayed by adding 2000 IJ (58 IJ/cm²) to 5 ml of filtered tap water in a 20 ml cylinder, using the nozzle of a spray bottle to spray it evenly onto the soil surface. The containers were closed with a lid and returned to a growth chamber at 25°C . After 5 days the larvae were removed by sieving from the soil and dissected to confirm infection with nematodes.

12. *Concentration bioassay and persistence*

To determine the minimum number of IJ to apply, six concentrations (6, 12, 25, 50, 100 and 200 IJ/FCM larvae) of the selected isolate were inoculated by spraying onto unsterilized orchard soil, to which 10 FCM

larvae had been added 24 hours earlier. After 5 days at 25° C the number of infected larvae was determined by looking at the colour change to red and if unsure the larvae were dissected to confirm infection.

13. *Laboratory persistence in orchard soil*

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

14. *Study orchard used for field trials*

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

15. *Containment of insect larvae in the field trials*

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

16. *Experimental design and evaluation protocol for field trials*

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

17. *Field trial with different concentrations of H. bacteriophora (Trial 1)*

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

The cages were retrieved after 2 days in the soil and the insects removed by washing the soil from the cages through a sieve. The larvae or pupae were removed from the cocoons with the aid of a stereomicroscope and placed on moist filter paper in a 15 cm-diameter Petri dish. Dead larvae were rated as infected by their appearance. The Petri-dishes were left in a closed plastic container in a growth chamber at 25°C for a further 4 days and again evaluated for infection, confirmed by dissection.

18. *Field application of three different nematode species (Trial 2)*

Heterorhabditis bacteriophora, *H. zealandica* and *S. khoisanae* were used at 10 IJ/cm² and the fourth treatment was water only. Cages loaded with 20 FCM final instar larvae were buried 10 cm from the base of each treatment-tree just beneath the soil surface. An area of 1.5 m x 2 m around the tree was sprayed with a litre of each nematode concentration and the control received water only. The cages were left in the soil for 6 days and mortality caused by infection was determined on the same day. Cocoons were opened with the aid

of a microscope and mortality caused by infection determined by dissection or prodding for movement of pupae.

Cages loaded with unsterilized sieved orchard soil and 20 FCM larvae each were placed at the same trees 7, 14 and 21 days after the initial application of the nematodes. Mortality caused by infection was determined in an identical manner to that used for the first set of cages.

19. Field application of three different *H. zealandica* concentrations (Trial 3)

Three treatments of 5, 10 and 20 IJ/cm² and a control treatment of water only was applied by adding the appropriate number of nematodes in 1 L of water in a watering can in a 20 cm radius around the base of the tree on the same day after the cages were buried. The cages were retrieved from the soil after two days and sealed in plastic containers for another 24 hours in a growth chamber at 25 ± 2°C. Care was taken to use a different spade to dig up the cages for the control treatments. The soil was removed from the cage and then washed through a sieve to retrieve FCM larvae and cocooned insects. The cocoons were placed on a filter paper in a petri dish and opened with the aid of a stereo microscope to ensure that the larvae or pupae inside remain intact when removed. Mortality caused by infection was visually determined by the colour change in the larvae to brick red; natural death was determined by a black colouration or purification; and pupae were prodded to check for movement to confirm whether alive or not. The petri dishes were returned to the growth chamber for another 3 days and again evaluated for mortality and infection confirmed by dissection.

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

20. Horizontal and vertical movement of nematodes through soil columns

Small, 0.2 ml thin-wall PCR tubes were pierced with a hot needle to obtain 10 holes. After adding last-instar FCM larvae, the tubes were closed and placed into the tip of a 12 cm long tube, filled with sand or soil. A total of 400 IJ were pipetted on a bioassay disk, turned upside down and placed on the top of the soil column and closed with the lid to prevent the soil from drying out. Tubes were orientated horizontally and vertically and left for periods of 24, 20, 16, 12, 8 and 4 hours, starting at 24 h. All FCM larvae removed were washed and put back at 25°C for a further 4 days, after which each was dissected and the penetrated larvae counted. Tubes were placed horizontally and vertically and left for periods of 24, 20, 16, 12, 8 and 4 hours.

21. Horizontal movement of three different nematode species through three soil types

The soil columns bioassay as previously described was followed, but soil, clay and sand were used. For the purpose of the control of FCM, only horizontal movement can be regarded as of importance. This bioassay was only performed with horizontally placed columns at for 4 and 8 h.

22. Virulence of different nematode isolates at 25°C

The 24-well bioassay protocol was followed to determine the difference in infectivity of a local *H. bacteriophora* (SF351), *H. zealandica* (SF41), *Steinernema yirgalemense* (157-C) and a formulated imported *H. bacteriophora* (Hb-e-nema). Five bioassay trays with 12 FCM last-instar larvae were used as a treatment (n = 60) with the corresponding control treatment (n = 60). For each treatment, 50 IJs / 50 µl filtered tap water was used and for the control water only. After a 48-hour exposure time to the nematodes, mortality of the insect larvae was determined. Each insect was washed and put on moist filter paper for a period of two days after which each insect was dissected and the number of infected juveniles which had penetrated was determined. Each bioassay was repeated on a different test date with a fresh batch of harvested IJ.

23. Virulence of *H. bacteriophora* and *S. feltiae*

The 24-well bioassay protocol was followed to determine the virulence of local *H. bacteriophora* (SF351) and formulated imported *S. feltiae* (Sfel-e-nema) at temperatures of 25°C and 14°C. Five bioassay trays with 10 last instar FCM were used as a treatment (n = 50) with the corresponding control treatment (n = 50). For each treatment 50 IJ/50 µm filtered tap water was used and for the control water only. After 46 h at the two different temperatures, the mortality of the insect larvae was determined. Each bioassay was repeated on a different test date with a fresh batch of harvested IJ.

24. Lethal concentration of *S. feltiae* (Sfel-e-nema and Sfel) and *H. bacteriophora* (SF351) at 14°C

The 24-well bioassay protocol was followed to determine the lethal concentration of the formulated imported *S. feltiae* (Sfel-e-nema), recycled *S. feltiae* (Sfel) and local *H. bacteriophora* (SF351). Five bioassay trays with 10 last-instar FCM larvae were used as a treatment (n = 50) with the corresponding control treatment (n = 50). For each treatment, 50 IJs/50 µm filtered tap water was used, and for the control water alone. After 46 h at the two different temperatures, the mortality of the insect larvae was determined. For *H. bacteriophora*

(SF351), each larva was washed, with larvae from each treatment being placed in a petri dish with moistened filter paper and left in a growth chamber for another two days. For the first bioassay, the imported formula *S. feltiae* (Sfel-e-nema) was used, and, for the repeat on a different test date, *S. feltiae* (Sfel) recycled through a wax moth larva was used.

25. Statistical analysis

All statistical analyses were performed using Statistica 9.0 software (StatSoft Inc., 2008). 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 the lethal concentration (LC), a probit analysis (Finney, 1971) was conducted using Polo PC (LeOra Software, 1987). As almost no mortality was obtained in the controls, Abbott's formula was not used to compensate for natural death (Abbott, 1925).

Results and discussion

1. Survey

A total of 129 samples came from the Western Cape, with 20 (15.5%) samples testing positive for nematodes; 52 samples from the Eastern Cape, with eight (15.4%) testing positive; and 21 from Mpumalanga, with seven (33.3%) testing positive. Of the total of 202 samples taken, 35 (17%) of the samples tested positive for the presence of EPN (Fig. 3.2.3.1).

Identification of nematode species by means of taxonomic keys (Hominick *et al.*, 1997; Nguyen, 2007) is complicated, due to the overlap in morphological features. During the survey of citrus orchards, three species of *Steinernema* and three of *Heterorhabditis* were identified and characterised, using a combination of morphological and molecular techniques. Molecular identifications based on sequences of the ITS region were supported by morphometrics, morphological and biological observations. The steinernematids, *S. citrae*, *S. khoisanae* and *S. yirgalemense* and heterorhabditids, *H. bacteriophora*, *H. zealandica* and an undescribed *Heterorhabditis* spp. were reported.

According to Hominick (2002), during non-targeted surveys, steinernematids are generally recovered more often than are heterorhabditids. However, in the current survey, the ubiquitous *H. bacteriophora* was found to be the dominant nematode species in citrus orchards. The description of a new species for *Heterorhabditis*, *H. safricana* (Malan *et al.*, 2008) and an undescribed species reported in this survey, indicated that heterorhabditids as being more frequently recovered, although are not more diverse, than steinernematids. Other examples, from the African continent where *H. bacteriophora* was found to be the dominant species come from Kenya and Egypt (Waturu, 1998; Waturu *et al.*, 1998). In previous surveys conducted in South Africa, *H. bacteriophora* was also found to be the most common species present (Hatting *et al.*, 2009; Malan *et al.*, 2006).

Heterorhabditis zealandica has only once previously been reported in connection with natural vegetation in the Eastern Cape (Malan *et al.* 2006). In the current survey, the nematode was found in two orchards in the Western Cape and in one in Mpumalanga. However, in an extensive survey of seven geographical regions throughout South Africa that was undertaken by Hatting *et al.* (2009), no *H. zealandica* was found, indicating the species to be rare, with a limited distribution in South Africa.

Steinernema khoisanae, an endemic species for South Africa (Nguyen *et al.*, 2006), was previously recovered from grass, apple and grapevine soils in the Rawsonville, Tulbach and Villiersdorp areas in the Western Cape Province respectively (Malan *et al.*, 2006). In this study, *S. khoisanae* was identified from a citrus orchard in the Porterville area, while Hatting *et al.* (2009) reported eight isolates from the Western Cape Province.

Steinernema yirgalemense was found on the farm Perdestal in the Nelspruit area of Mpumalanga Province. This is a first report for the species in South Africa. The only other *Steinernema* species reported to be present in South Africa are *S. khoisanae* (Nguyen *et al.*, 2006) and *S. citriae* (Stokwe *et al.*, 2011). *S. yirgalemense* was described by Nguyen *et al.* (2004) from Yiglemen in Ethiopia, where it was found to be the dominant species. It was also reported from the Central Rift Valley of Kenya (Mekete *et al.*, 2005; Mwaniki *et al.*, 2008). The nematode belongs to the *bicornutum*-group of six described species, of which the exsheathed IJ has two hornlike structures in the cephalic region (Nguyen *et al.*, 2007). Currently, the nematode has not been reported outside Africa.

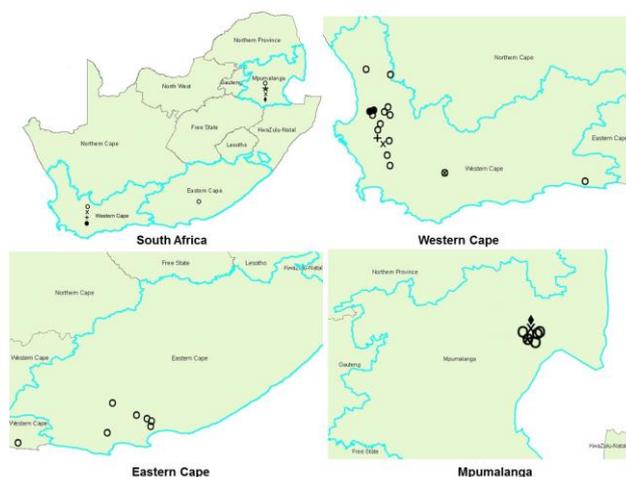


Fig. 3.2.3.1. Occurrence and distribution of entomopathogenic nematodes in citrus orchards of the Western Cape, Eastern Cape and Mpumalanga provinces, of South Africa. Key: o = *H. bacteriophora*; x = *H. zealandica*; ♦ = *Heterorhabditis* sp.; + = *S. khoisanae*; * = *S. yirgalemense*; ● = *Steinernema citrae*.

2. Molecular characterisation of nematodes found during the survey

Sequences for all nematode isolates were deposited in GenBank. The accession numbers are indicated in Table 3.2.3.2. Thirty-one (89%) of the isolates were heterorhabditids. The species identified using molecular techniques were *H. bacteriophora* (27), *H. zealandica* (3) and an unknown species of *Heterorhabditis* (1). *Heterorhabditis bacteriophora* occurred in all three provinces. As can be seen in Table 3.2.3.2, *H. zealandica* (118-C, 130-C, 160-C) has been found only in the Western Cape and in Mpumalanga, and the unknown species of *Heterorhabditis* (158-C) has only been found in Mpumalanga. Of the isolates, four (11.4%) were steinernematids, representing *S. khoisanae* (106-C), *S. yirgalemense* (157-C) and two isolates of *S. citrae* (SF141-C, SF143-C).

Table 3.2.3.2. Species and isolates of entomopathogenic nematodes from a survey of citrus orchards in areas from three provinces of South Africa, with GenBank accession numbers.

Province	Area	Species	Isolate	Genbank accession number
Western Cape:	Citrusdal	<i>H. bacteriophora</i>	42-C	EU796073
	Citrusdal	<i>H. bacteriophora</i>	111-C	FJ217350
	Citrusdal	<i>H. bacteriophora</i>	113-C	EU049290
	Citrusdal	<i>H. bacteriophora</i>	153-C	EU699435
	Citrusdal	<i>H. bacteriophora</i>	154-C	EU860187
	Citrusdal	<i>H. bacteriophora</i>	201-C	FJ360728
	Citrusdal	<i>H. bacteriophora</i>	175-C	FJ360727
	Montagu	<i>H. bacteriophora</i>	117-C	EU860183
	Montagu	<i>H. zealandica</i>	118-C	EU860184
	Morreesburg	<i>H. zealandica</i>	130-C	EU031650
	Piketberg	<i>H. bacteriophora</i>	149-C	FJ235076
	Piketberg	<i>S. citrae</i>	143-C	FJ235074
	Porterville	<i>H. bacteriophora</i>	104-C	EU850800
	Porterville	<i>H. bacteriophora</i>	142-C	EU860186
	Porterville	<i>H. bacteriophora</i>	147-C	EU715292
	Porterville	<i>S. khoisanae</i>	106-C	EU683802
	Porterville	<i>S. citrae</i>	141-C	EU740970
Riviersonderend	<i>H. bacteriophora</i>	190-C	FJ217349	
Trawal	<i>H. bacteriophora</i>	150-C	FJ360729	
Wellington	<i>H. bacteriophora</i>	26-C	EU740971	
Mpumalanga:	Nelspruit	<i>H. bacteriophora</i>	63-C	EU848594
	Nelspruit	<i>H. bacteriophora</i>	65-C	EU848595
	Nelspruit	<i>H. bacteriophora</i>	66-C	EU848596
	Nelspruit	<i>H. bacteriophora</i>	67-C	EU850799
	Nelspruit	<i>H. bacteriophora</i>	159-C	EU18483
	Nelspruit	<i>Heterorhabditis</i> sp.	158-C	FJ235075
	Nelspruit	<i>H. zealandica</i>	160-C	EU718483
Nelspruit	<i>S. yirgalemense</i>	157-C	EU625295	
Eastern Cape:	Addo	<i>H. bacteriophora</i>	29-C	EU740972
	Kirkwood	<i>H. bacteriophora</i>	51-C	EU796074
	Kirkwood	<i>H. bacteriophora</i>	20-C	EU074157
	Knysna	<i>H. bacteriophora</i>	56-C	FJ217351
	Patensie	<i>H. bacteriophora</i>	89-C	EU715293
	Patensie	<i>H. bacteriophora</i>	136-C	EU860185
	Sundays River Valley	<i>H. bacteriophora</i>	17-C	FJ217352

3. Molecular characterisation of nematodes

Analysis of the ITS rDNA region, based on maximum parsimony, placed the isolate 157-C in the *bicornotum*-group with *S. yirgalemense*, the isolate 141-C falls within the *feltiae*-group along with *S. citrae* 143-C while the isolate 106-C clustered with *S. khoisanae* in the *glaseri*-group (Fig. 3.2.3.2).

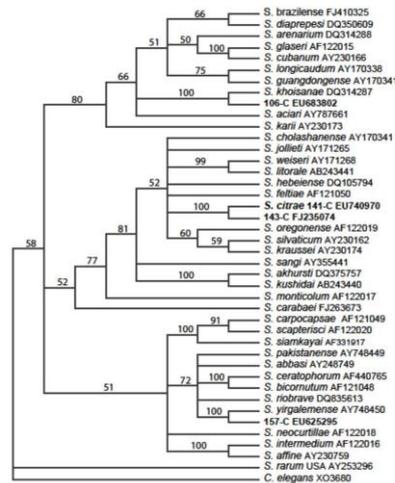


Fig. 3.2.3.2. Phylogenetic relationship of the three *Steinerhema* isolates found during the survey based on analysis of the ITS rDNA regions. Numbers indicated at the nodes represent bootstrap proportion values (50% or more, 1000 replicates). Numbers after each species and isolate indicate the GenBank Accession numbers.

The analysis of all available sequences of known *Heterorhabditis* species from Genbank with those generated during the survey, based on MP, are indicated in Fig. 3.2.3.3. The unknown isolate 158-C of *Heterorhabditis* clustered in the *indica*-group close to *H. indica* and *H. gerrardi*. Pairwise distance comparison showed 158-C to differ from *H. indica* by 14 base pairs and from *H. gerrardi* with 11 base pairs. Isolates 118-C, 130-C and 160-C clustered with *H. zealandica*. The rest of the *Heterorhabditis* isolates (27) found during the survey group with *H. bacteriophora* in the *megidis*-group.

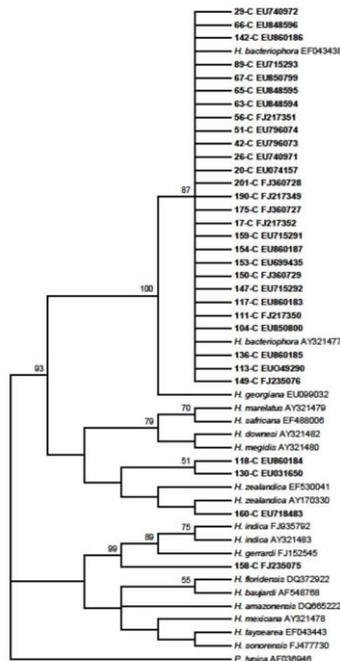


Fig. 3.2.3.3. Phylogenetic relationships of *Heterorhabditis* strains found during the survey based on analysis of the ITS rDNA regions. Numbers at the nodes represent bootstrap proportions (50% or more, 1000 replicates). Numbers after each species and isolate indicate the GenBank Accession numbers.

4. Morphometric and morphological identification

The morphometric and morphological characters of the IJs and males of isolate 26-C used in bioassays resemble the original description of *H. bacteriophora* by Poinar (1976). The IJ of the original description of *H. bacteriophora* is characterised by the body length and E% (Table 3.2.3.3).

The body length of 588 μm (512-671) of the original description of *H. bacteriophora* is longer than for isolate 26-C, with a body length of 546 μm (474-625). However, the E% (112) of *H. bacteriophora* and that of 26-C are (113) very similar. The pouch housing the bacteria can also be clearly observed in the IJ of all isolates. The spicule and gubernaculum length of the male also falls within the range of the original description, as well as the D% (Table 3.2.3.3). Bacteria of this isolate turned freshly killed wax moth larvae a reddish colour.

The characteristics of the IJ of isolate 118-C were compared with the original description of *H. zealandica* by Poinar, 1990. The body length of 625 μm was, however, found to be shorter than the original description of 685 μm (570-740) (Table 3.2.3.3). The shortened body length caused the range to shift and such characteristics as the EP and tail length with sheath to be smaller than the original description (Table 3.2.3.3). However, the E% was found to fall within the range for the species. For the first-generation male of 118-C the lengths of the spicule and gubernaculum were found to fall well within the range of the original description (Table 3.2.3.3). The spicule of the male of isolate 118-C has a rostrum that is not visible for the isolate 26-C. The anterior lumen of the intestine of isolate 118-C, which is wide, contains bacteria, in comparison with isolate 26-C, where the bacterial chamber was found to be clearly visible. Bacteria of this isolate turned freshly killed wax moth larvae a steel-grey colour.

Table 3.2.3.3. Comparative morphometrics of the infective juveniles of *Heterorhabditis* and *Steinernema* found during the survey. Measurements are in μm , except the indexes, in form: mean \pm SD (range).

Species/Isolate	<i>Heterorhabditis</i> sp. (26-C)	<i>H. bacteriophora</i> ¹	<i>Heterorhabditis</i> sp. (118-C)	<i>H. zealandica</i> ²	<i>Heterorhabditis</i> sp. (158-C)	<i>H. indica</i> ³	<i>Steinernema</i> sp. (106-C)	<i>S. khoisanae</i> ⁴	<i>Steinernema</i> sp. (157-C)	<i>S. yirgalemense</i> ⁵	<i>S. citrae</i> ⁶
N	25	15	25	25	25	25	25	25	25	25	25
L	546 (474-625)	588 (512-671)	625 (449-686)	685 (570-740)	528 (484-563)	528 (479-)	1055 (911-1129)	1062 (904-1159)	584 (523-650)	635 (548-693)	754 (623-849)
A	23 (21-25)	25 (17-30)	25 (24-26)	25 (24-26)	25 (24-28)	26 (25-)	31 (27-38)	35 (32-37)	22 (20-24)	21 (20-25)	30 (25-34)
B	4.9 (4.6-5.5)	4.5 (4.0-5.1)	5.2 (4.8-6.2)	4.9 (4.2-5.0)	5.3 (4.9-8.1)	4.5 (4.3-)	7.8 (6.8-8.5)	7.7 (6.6-8.4)	6.0 (5.5-6.7)	5.2 (4.8-5.9)	6 (5.1-7.1)
c	6.3 (5.9-6.8)	6.2 (5.5-7.0)	6.6 (6.1-7.1)	6.7 (6.2-6.7)	5.9 (5.0-8.1)	5.3 (4.5-)	12.2 (11.0-13.4)	12.5 (10.6-13.8)	9.75 (8.97-)	10.3 (9.2-11.2)	15 (13-17)
c ¹	5.9 (5.3-6.6)	-	5.9 (5.5-6.2)	-	6.6 (4.9-7.1)	-	4.1 (2.8-4.7)	3.9 (3.3-4.7)	3.3 (2.9-3.3)	3.3 (2.9-3.3)	3.3 (2.9-3.3)
Greatest body diam.	24 (22-27)	23 (18-31)	25 (23-29)	27 (22-30)	21 (19-23)	20 (19-22)	29 (30-36)	31 (27-34)	27 (24-30)	29 (24-44)	26 (13-28)
EP	99 (92-106)	103 (87-110)	103 (90-112)	112 (94-123)	91 (79-113)	98 (88-)	95 (85-104)	96 (87-101)	46 (44-50)	51 (45-59)	56 (49-64)
NR	78 (68-88)	85 (72-93)	85 (75-94)	100 (90-107)	74 (70-87)	82 (72-85)	106 (100-113)	109 (101-118)	78 (71-96)	88 (82-93)	98 (88-110)
ES	111 (101-119)	125 (100-139)	120 (110-129)	140 (135-147)	103 (99-108)	117 (109-)	136 (127-150)	140 (130-155)	97 (91-103)	121 (115-128)	125 (118-137)
Hemizonion	91 (83-96)	-	97 (88-105)	-	83 (76-93)	-	131 (118-142)	-	60 (55-67)	-	-
Tail length with	87 (79-94)	98 (83-112)	94 (84-104)	102 (87-119)	86 (80-92)	101 (93-)	87 (77-93)	85 (76-97)	60 (55-67)	62 (57-67)	71 (63-81)
Tail without	53 (46-57)	-	67 (56-92)	-	66 (56-76)	-	-	-	35 (31-42)	-	-
Hyaline portion	-	-	-	-	-	-	49 (39-56)	-	15 (13-18)	-	30 (25-34)
Anal body	14 (13-16)	-	16 (14-18)	41	13 (12-13)	-	21 (18-29)	20 (15-25)	15 (13-18)	19 (17-21)	14 (13-17)
D% = EP/ES x	88 (81-94)	84 (76-92)	70 (62-75)	80 (70-84)	92 (83-111)	84 (79-)	70 (62-75)	68 (60-73)	48 (44-52)	42 ^a (38-48)	44 (39-58)
E% = EP/T x	113 (105-122)	112 (103-130)	110 (103-120)	109 (103-109) ^a	107 (85-124)	94 (83-)	110 (103-120)	112 (95-128)	78 (67-87)	83 (67-98)	110 (85-132)
H% = H/T x	-	-	-	-	-	-	57 (49-58)	-	58 (53-65)	-	43 (37-50)
Mucron	A	A	A	A	A	A	A	A	A	A	P

References: *Isolates recovered during this survey; ¹after Poinar, 1976; ²after Poinar, 1990; ³after Poinar, *et al.*, 1992; ⁴after Nguyen *et al.*, 2006; ⁵after Nguyen, *et al.*, 2005; ⁶after - data not available in original description

EP = distance from anterior end to excretory pore, NR = distance from anterior end to nerve ring; ES = distance from anterior end to end of

^aAdded after the Nguyen, 2007

Table 3.2.3.4. Comparative morphometrics of the male of *Heterorhabditis* and *Steinernema* found during the survey. Measurements are in μm , except the indexes, in form: mean \pm SD (range).

Species/Isolate	<i>Heterorhabditis</i> sp. (26-C)	<i>H. bacteriophora</i> ¹	<i>Heterorhabditis</i> sp. (118-C)	<i>H. zealandica</i> ²	<i>Heterorhabditis</i> sp. (158-C)	<i>H. indica</i> ³	<i>Steinernema</i> sp. (106-C)	<i>S. khoisanae</i> ⁴	<i>Steinernema</i> sp. (157-C)	<i>S. yirgalemense</i> ⁵	<i>S. citrae</i> ⁶
n	20	15	20	15	20	15	20	20	20	25	20
L	696	820	888	914	653	721	1841	1809	1560	1566	1154
	(566-830)	(780-960)	(792-998)	(848-1044)	(542-758)	(537-788)	(1496-2031)	(1428-2248)	(1254-1908)	(1331-1777)	(1028-1402)
Greatest body diam.	42	43	47	41	38	42	128	108	135	112	103
	(34-48)	(38-46)	(42-54)	(36-45)	(33-56)	(36-45)	(87-145)	(90-132)	(111-138)	(97-138)	(87-113)
EP	111	121	128	139	101	123	163	146	95	86	81
	(98-128)	(114-130)	(113-146)	(130-150)	(84-114)	(109-138)	(137-180)	(122-168)	(76-113)	(74-107)	(64-92)
NR	68	72	77	-	71	75	106	98	106	108	106
	(53-93)	(65-81)	(68-87)	-	(63-78)	(72-85)	(96-119)	(90-110)	(87-115)	(98-136)	(92-119)
ES	103	103	105	118	92	101	175	180	138	148	139
	(90-105)	(99-105)	(97-114)	(110-128)	(86-98)	(93-109)	(155-186)	(163-183)	(132-165)	(132-165)	(123-155)
TR	65	79	90	-	71	91	-	520	-	278	-
	(48-87)	(59-87)	(64-115)	-	(72-85)	(35-144)	-	(310-586)	-	(205-331)	-
Tail length	23	28	31	35	26	28	46	36	29	20	25
	(19-30)	-	(22-35)	(30-41)	(23-30)	(24-32)	(41-55)	(30-40)	(21-37)	(17-27)	(17-31)
Anal body diam.	20	23	23	20	19	23	50	40	39	38	25
	(17-24)	(22-25)	(21-30)	(18-24)	(17-21)	(19-24)	(48-52)	(33-45)	(31-37)	(32-45)	(17-31)
Spicule length (SP)	42	40	51	51	42	43	77	85	69	64	65
	(36-49)	(36-44)	(43-56)	(48-55)	(35-38)	(34-48)	(70-82)	(70-88)	(62-72)	(51-77)	(57-80)
Gubernaculum length	21	20	20	22	19	21	43	43	39	48	44
(GL)	(17-23)	(18-25)	(15-25)	(19-25)	(15-24)	(18-23)	(34-49)	(34-48)	(31-47)	(42-54)	(32-59)
D% = EP/ES x 100	119	117	124	118	110	121	94	85	69	58	58
	(108-143)	-	(109-148)	-	(88-120)	-	(80-109)	(71-99)	(54-82)	(50-66)	(47-67)
E% = EP/T x 100	448	-	416	-	391	-	-	-	334	-	-
	(334-611)	-	(151-580)	-	(303-464)	-	-	-	(239-509)	-	-
SW% = SP/ABD x 100	207	174	214	181	221	187	153	203	134	171	198
	(173-261)	-	(163-269)	(160-209)	(185-271)	-	(137-167)	(167-227)	(110-212)	(121-213)	(156-233)
GS% = GL/SP x 100	49	50	39	44	47	49	52	70	57	74	68
	(43-56)	-	(33-50)	-	(38-54)	-	(44-62)	(60-80)	(44-72)	(65-85)	(48-89)

References: *Isolates recovered during this survey; ¹after Poinar, 1976; ²after Poinar, 1990; ³after Poinar, *et al.*, 1992; ⁴after Nguyen *et al.*, 2006; ⁵after Nguyen, *et al.*, 2005; ⁶after Stockwe *et al.*, 2010

- data not available in original description

EP = distance from anterior end to excretory porte, NR = distance from anterior end to nerve ring; ES = distance from anterior end to end of esophagus;

^aAdded after the Nguyen, 2007

Isolate 158-C was compared with the original description of *H. indica* (Poinar *et al.*, 1992). The body length of the IJ of 158-C was found to be the exact length (528 μm) of the original description of *H. indica* (Poinar *et al.*, 1992). Apart from *H. taysaerae*, this is one of the smallest heterorhabditids. The EP of isolate 158-C was found to be shorter (94 μm) in comparison with *H. indica* (107 μm), while the tail (86 μm) did not fall within the range indicated in the original description, causing the D% and E% to differ (Table 3.2.3.3). The D% and E% separate this species from the other two heterorhabditids found during the survey. The intestinal pouch filled with symbiotic bacteria was clearly visible. However, in the male, the length of the spicula was exactly the same (43 μm), while the length of the gubernaculum was similar, falling within the range originally described for *H. indica*. The gubernaculum of isolate 158-C is also approximately 50% of the length of the spicula, as found in the original description. This nematode isolate turned freshly killed wax moth larvae a chocolate-brown colour.

The steinernematid isolate, 106-C, was compared with the original description of *S. khoisanae* (Nguyen *et al.*, 2006). In comparing the IJ, it was found that the body length, the EP, the length of the tail, the D% and E% fell in the range of the original description for *S. khoisanae* (Table 3.2.3.4). For the first-generation male, the lengths of the spicule and the gubernaculum were found to overlap (Table 3.2.3.3). The bacteria of this isolate were found to turn the wax moth larvae a tan colour with black spots.

The revised description of *S. yirgalemense* (Nguyen *et al.*, 2007) was used to compare the morphological characteristics of the steinernematid isolate 157-C. As the length of the IJ was generally shorter, most of the other features were shorter than in the original description (Table 3.2.3.3), although most fell in the range of the original description. In the first-generation male, the length of the spicula compared well, although the gubernaculums were shorter. Horn-like structures on the lip region were observed, using a research microscope at 100 x oil magnification. This isolate turned freshly killed wax moth larvae a light yellow colour.

The two Isolates 141-C and 143-C were described in full as a new steinernematid species for South Africa (Stokwe *et al.*, 2011). The morphometric characteristics of the IJ and the first-generation male are indicated in Tables 3.2.3.2 and 3.2.3.3.

5. Virulence assays for FCM final-instar larvae

Last-instar FCM larvae were found to be highly susceptible to the six isolates of the nematode species used, with mortality ranging between 77% and 100% (Fig. 3.2.3.4). As there were no significant interactions between the test dates and the treatments, data from the two test dates were pooled and analysed, using a one-way ANOVA that showed a significant effect ($F_{(5, 54)} = 7.848$; $P < 0.001$) of the treatment on percentage larval mortality. A bootstrap multiple comparison showed that *S. yirgalemense* (157-C) gave greater control of last-instar FCM larvae on both test dates (100%), than did *S. citrae* (141-C), *H. bacteriophora* (26-C), *S. khoisanae* (106-C) and *Heterorhabditis* sp. (158-C) ($P < 0.001$), while the latter species did not differ significantly from each other. Efficacy of *S. yirgalemense*, did not differ from that of *H. zealandica* (118-C) ($P = 0.398$).

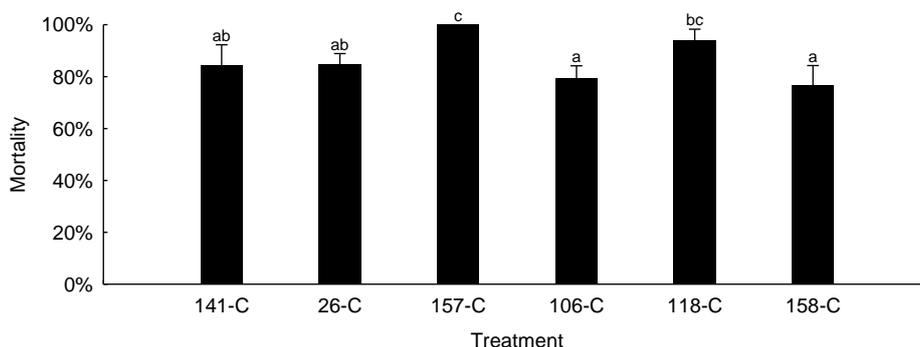


Fig. 3.2.3.4. Mean percentage (Abbott's corrected) mortality (95% confidence intervals) of FCM last instar larvae inoculated with 50 IJ/insect of *Steinernema* sp. (141-C), *Heterorhabditis bacteriophora* (26-C), *S. yirgalemense* (157-C), *S. khoisanae* (106-C), *H. zealandica* (118-C) and *Heterorhabditis* sp. (158-C) after 48 h at 25°C. Different letters above the vertical bars indicate significant differences (one-way ANOVA ($F_{(5, 54)} = 7.848$; $P < 0.001$)).

6. Virulence assays for FCM pupae

Pupae of FCM were found to be susceptible to all isolates of the six nematode species, with mean percentage mortalities ranging between 20% and 74% (Fig. 3.2.3.5). As there were no significant interactions between the test dates and the treatments, data from the two test dates were pooled and a one-way ANOVA

showed significant differences between the treatments ($F_{(5, 54)} = 7.783$; $P < 0.001$). The *S. yirgalemense* (157-C) mean mortality of 74% did not differ ($P = 0.0525$) from that of *S. citrae* (141-C) with mortality of 42%, *H. bacteriophora* (26-C) ($P = 0.128$) with mortality of 49%, and *H. zealandica* (118-C) ($P = 0.128$) with mortality of 52%. *Steinernema yirgalemense* (157-C), however, was found to differ significantly ($P > 0.001$) from *S. khoisanae* (106-C), with mortality of 20%, and the *Heterorhabditis* sp. (158-C), with mortality of 27%.

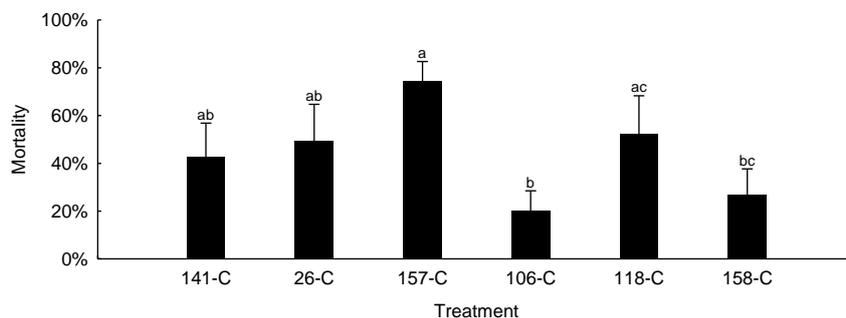


Fig. 3.2.3.5. Mean percentage (Abbott's corrected) mortality (95% confidence intervals) of FCM pupae, 48 h after being inoculated with 50 IJ/insect of *Steinernema* sp. (141-C), *Heterorhabditis bacteriophora* (26-C), *S. yirgalemense* (157-C), *S. khoisanae*, *H. zealandica* (118-C) and *Heterorhabditis* sp.(158-C) at 25 °C. Different letters above the vertical bars indicate significant differences (one-way ANOVA: ($F_{(5, 54)} = 7.783$; $P < 0.001$).

7. Virulence assays against emerging moths in sand

The percentage mortality of both cocooned pupae and emerging moths was high for all three nematode species tested (Fig. 3.2.3.6). As there were no significant interactions between the test dates and the treatments, data from the two test dates were pooled and a one-way ANOVA showed significant differences between the treatments used ($F_{(2, 27)} = 15.209$; $P < 0.001$). *S. yirgalemense* (157-C) was found to be the most effective nematode (93.5%), and differed significantly ($P = 0.017$) from *H. zealandica* (82.7%), which, again, differed significantly ($P = 0.011$) from *S. khoisanae* (65.4%), which was found to be the least effective of the three species.

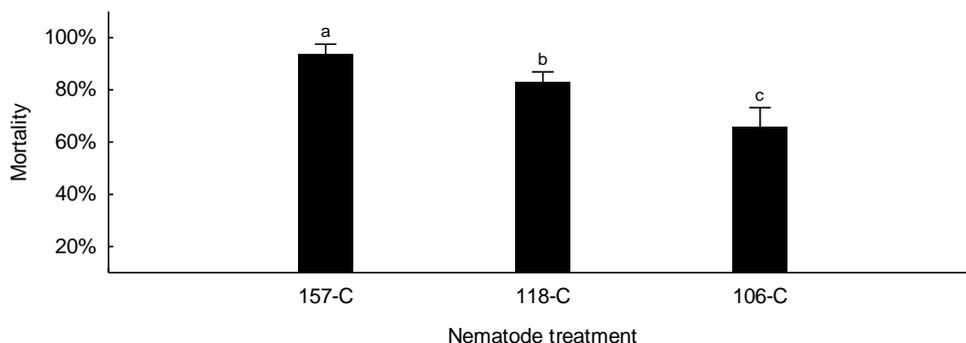


Fig. 3.2.3.6. Mean percentage (Abbott's corrected) mortality (95% confidence intervals) of cocooned FCM pupae and emerging moths in sand, seven days after being inoculated with *Steinernema yirgalemense* (157-C), *Heterorhabditis bacteriophora* (118-C) and *Steinernema khoisanae* (106-C) at a concentration of 20 IJ/cm². Different letters above the vertical bars indicate significant differences (one-way ANOVA: ($F_{(2, 27)} = 15.209$, $P < 0.001$).

The percentage mortality of the pupae in the sand was analysed separately (Fig. 3.2.3.7). As there were no significant interactions between the test dates and the treatments, data from were from the two dates were pooled and show significant differences ($F_{(2, 27)} = 5.115$; $P = 0.013$) between the treatments. The pupal mortality in the sand for *S. yirgalemense* (42.4%) and *H. zealandica* (36.1%), did not differ ($P = 0.78$). Both species, however, were found to differ significantly from *S. khoisanae* (16.9%) (Fig. 3.2.3.7). The percentage of fully emerged moths that were infected with nematodes was 51% for *S. yirgalemense*, 47% for *H. zealandica* and 49% for *S. khoisanae*.

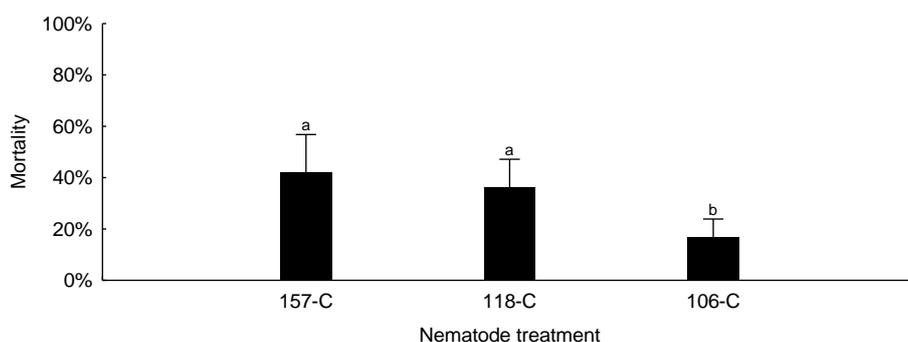


Fig. 3.2.3.7. Mean percentage (Abbot's corrected) mortality (95% confidence intervals) of cocooned FCM pupae in sand inoculated with *Steinernema yirgalemense* (157-C), *Heterorhabditis bacteriophora* (118-C) and *Steinernema khoisanense* (106-C). Different letters above the vertical bars indicate significant differences (one-way ANOVA: ($F_{(2, 27)} = 5.115$, $P = 0.013$).

8. Sand bioassays with cocooned larvae

The FCM larvae in sand were found to be in tightly woven cocoons with sand particles clinging to the outside. The highest percentage infection was obtained for *H. zealandica* and *H. bacteriophora* (Fig. 3.2.3.8.). The other two isolates were discarded for future testing against FCM. These results also indicate that the cocoons were no barrier for nematode infection.

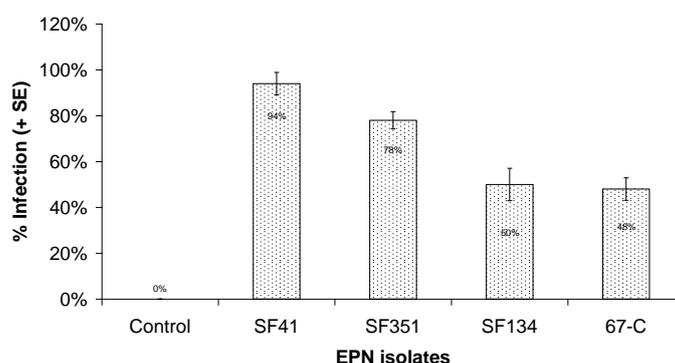


Fig. 3.2.3.8. Infectivity of four EPN isolates to cocooned FCM larvae in sterilized sand.

9. Sand bioassays with pupae

Species and isolates which caused 100% mortality of larvae and above 40% mortality of pupae were selected from the fast screening bioassays. These isolates were tested in the sand bioassays (Table 3.2.3.4). It was found that pupae, as well as moths that emerged from the sand during the test period, were infected with nematodes. Some nematode isolates gave a combined percentage mortality for pupae and moths of up to 96%. The overall mortality was used to select five isolates to be used against FCM in further studies.

Nematodes performed exceptionally well in the sand bioassay, because of the long exposure period and the inability of emerged moths to escape contact with the nematodes.

Table 3.2.3.4. Combined percentage mortality of pupae and emerged moths, and pupae and moths alone, for selected EPN isolates in a sand laboratory bioassay.

Species	EPN Isolate	Combined % mortality for pupae and emerged	% Mortality of pupae	% Mortality of emerged moths
<i>Heterorhabditis</i>	SF351*	93	13	82
<i>H. bacteriophora</i>	SF10	70	23	47
<i>H. bacteriophora</i>	SF134	62	41	20
<i>H. bacteriophora</i>	SF1	58	19	39
<i>H. bacteriophora</i>	67-C*	87	38	52
<i>H. bacteriophora</i>	17-C	73	16	60

<i>H. zealandica</i>	SF41*	78	40	37
<i>H. zealandica</i>	SF379*	89	20	74
<i>H. zealandica</i>	SF593*	96	33	66
<i>Steinernema khoisanae</i>	SF87	57	34	23

*Final selected EPN isolates for FCM control

10. Soil bioassays

All three of the selected EPN species performed well in all three soil types and infectivity was high (Fig. 3.2.3.9). *Heterorhabditis bacteriophora* (SF351) gave 100% control in all three soil types followed by *H. zealandica*. *Steinernema khoisanae* was included in the study to give an indication of persistence. The nematode did not perform well in soil type 1, but in the other soil types infectivity was comparable in the other two isolates. The nematodes performed even better in unsterilized soil than in the sand. After a period of 100 days some infection was still obtained in the soil for *S. khoisanae* and *H. bacteriophora*. Heterorhabditids are not known to persist for long periods in the soil in comparison with steinernematids.

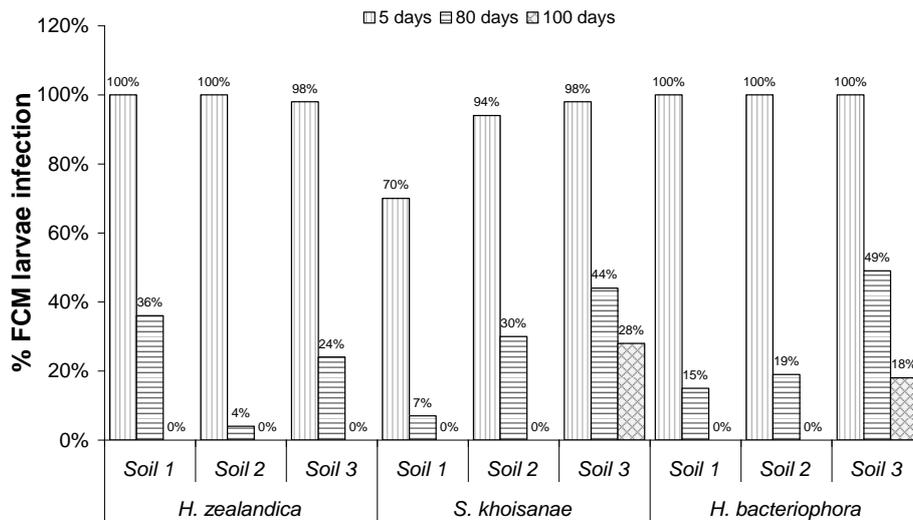


Fig.3.2.3.9. Infection of FCM larvae by three EPN species after five, 80 and 100 days in three types of unsterilized citrus orchard soil.

11. Concentration bioassay

Concentrations of 25-200 IJ/FCM gave no statistical significant differences between the mean percentage infectivity. Between 6 and 16 IJ/FCM larva, there were also no statistical differences, but it differed statistically from the four higher concentrations (Fig. 3.2.3.10.)

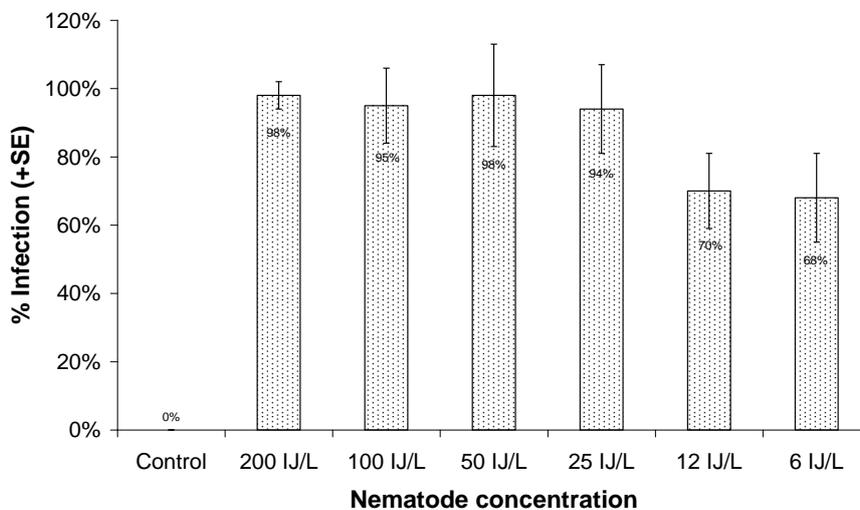


Fig.3.2.3.10. Six different concentrations of the selected EPN isolate (SF351) were used to test for infectivity of cocooned FCM in unsterilized soil.

12. Laboratory persistence

For *H. bacteriophora* (SF351) a 100% infection was found up to day 25 with a concentration of 50 and 200 IJ/FCM larva. With half the concentration the infection was still 100% up to 15 days after which it started to decline. With a concentration of 25 IJ/FCM larvae, infection stayed the same up to 35 days (Fig. 3.2.3.11).

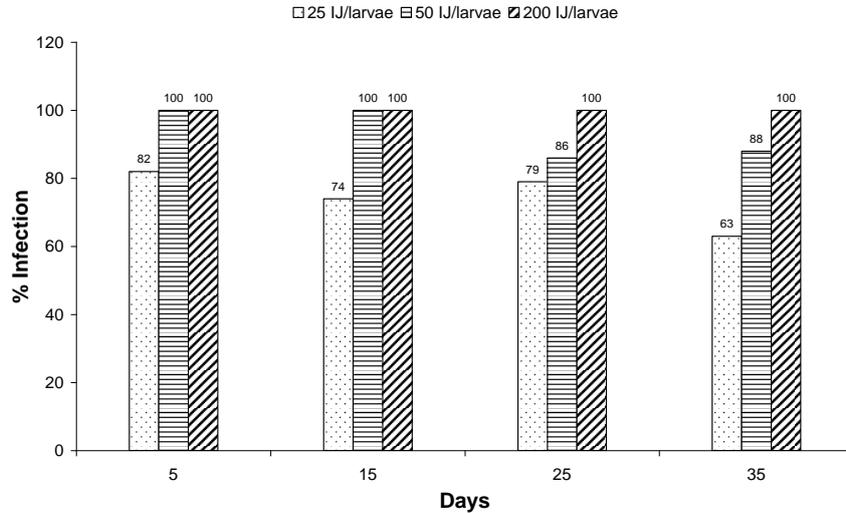


Fig. 3.2.3.11. Three concentrations (25, 50 and 200 IJ/FCM larvae) of SF351 were inoculated onto sterilized citrus orchard soil to determine the infectivity after a period of 5, 15, 25 and 35 days.

13. Field trial with different *H. bacteriophora* concentrations (Trial 1)

The three nematode concentrations of 20, 40 and 60 IJ/cm² caused a high level of mortality of FCM larvae, ranging from 90-99% (Fig. 3.2.3.12). No significant differences were found between the three *H. bacteriophora* concentrations applied. There were also no significant differences in mortality after 2, 3 and 6 days in the soil. An endemic nematode population was detected at three trees in trial 1 of the controls (4%). This endemic nematode was identified as *H. bacteriophora*.

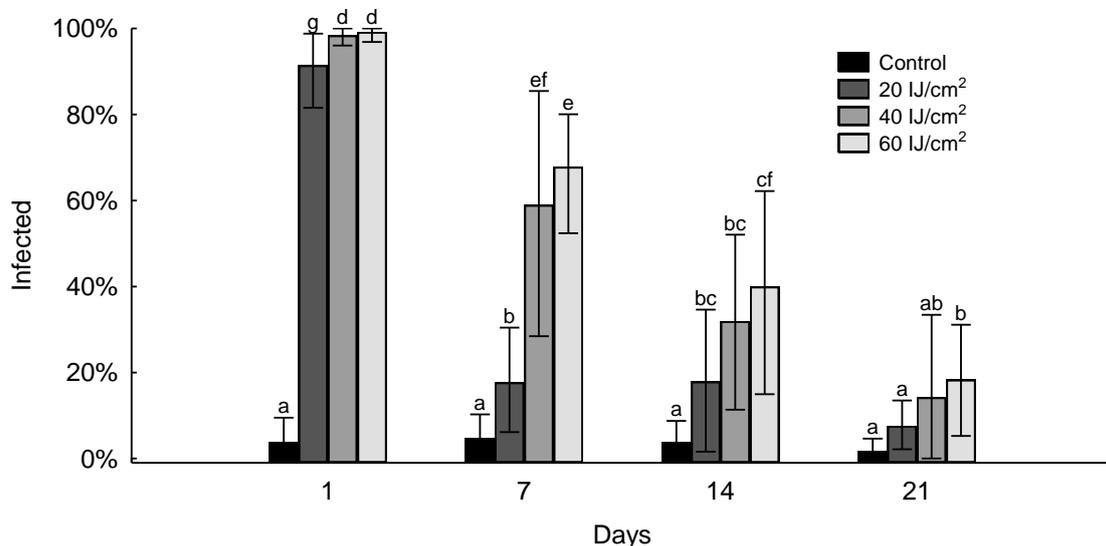


Fig. 3.2.3.12. Mean percentage mortality (95% confidence intervals) of FCM larvae after field application of 0, 20, 40 and 60 *H. bacteriophora* IJ/cm² evaluated on day 1-21. Different lettering above vertical bars indicates significant differences (Repeated Measures ANOVA; $F_{(9, 79)} = 15.87$; $P < 0.001$).

Usually results in field trail are somewhat disappointing but, in this trial better that expected results were obtained. Concentrations should be lowered to get an optimum field concentration.

The results for persistence of the three nematode species was analysed using Repeated Measures ANOVA of the percentage mortality for treatments over days, using the compound symmetry assumption of correlations over days. There was a significant interaction between days and treatment ($F_{(9, 84)} = 5.14$; $p < 0.001$) which were interpreted with Bonferroni's multiple comparison (Fig. 3.2.3.13).

On day 6, 35 and day 49 there were no significant differences between the control and the *S. khoisanae*-treatment. There were, however, a significant differences ($p = 0.028$) between the percentage nematode infection for day 6 (21%) and day 21 (53.8%) and for day 21 (53.8%) and 49 (6.25%) with $p < 0.001$. There was no significant difference between the *S. khoisanae*-treatment for day 21 and day 35 ($p = 1$). For the *H. bacteriophora*-treatment there was a significant difference between the controls for each evaluation day except for day 49 (Fig. 3.2.3.13). There was a significant difference ($p < 0.0001$) between day 6 (80.62%) and day 21 (62.48%), but no significant difference between any of the other days. For the *H. zealandica*-treatment there were significant differences between the controls and each evaluation day up to day 49 (Fig. 3.2.3.13). There were significant differences ($p < 0.001$) between day 6 (62.65%) and day 21 (82.0%) and day 35 (68.91%) and day 49 (37.86%).

After 21 days the mortality of *S. khoisanae* increased to 45%, but dropped to as low as 6% after 49 days. Mortality for *H. zealandica* increased to 72% on day 35, but dropped to 27% on day 49. Mortality for *H. bacteriophora* increased for day 21 to 84% and dropped to 72% for day 25 to 28% for day 49. On day 49 all species dropped significantly to levels under 30% (Fig. 3.2.3.13). During the period of 49 days that the cages were in the soil, minimum temperature recorded was 7°C, maximum temperature was 32°C and mean temperature of 17°C (Table 3.2.3.5).

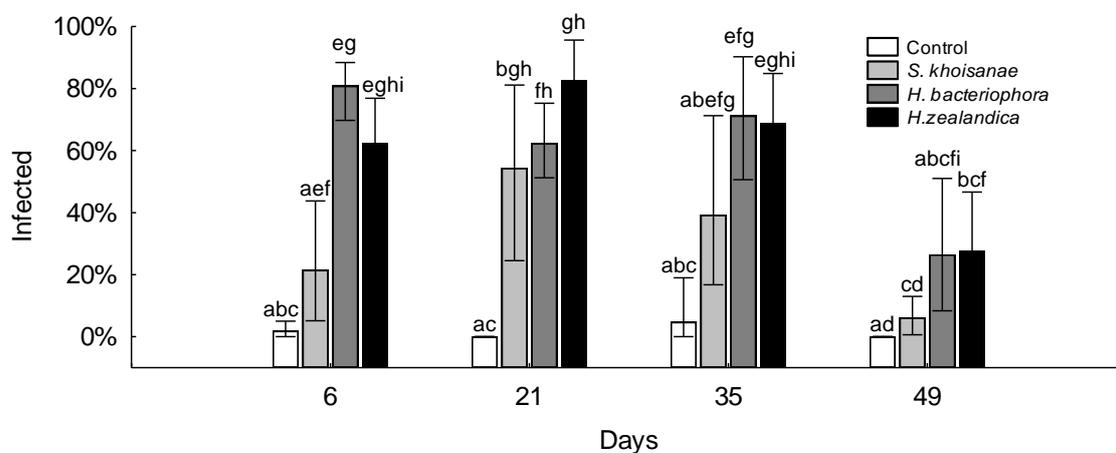


Fig.

3.2.3.13. Percentage mortality (95% confidence level) for FCM larvae, 6, 21, 35 and 49 days after initial inoculation with *Steinernema khoisanae*, *Heterorhabditis zealandica* and *H. bacteriophora* at a concentration of 10 IJ/cm². Larvae were evaluated after 6 days in cages in the soil and on the same day of removal (Trial 2). Repeated measures ANOVA of variance, $F(9, 84) = 5.15$; $p < 0.001$). Different letter above vertical bars indicated statistical significance.

After a period of three months when the cages were left in the soil a mean infection rate for all treatments of 44% was obtained (Fig. 3.2.3.14). The results were analysed using a One-way ANOVA with no significant differences (One-ANOVA; $F_{(3, 27)} = 0.12$; $p = 0.95$) between the nematode and the control treatments. The can be ascribed to the patchy distribution of the nematodes.

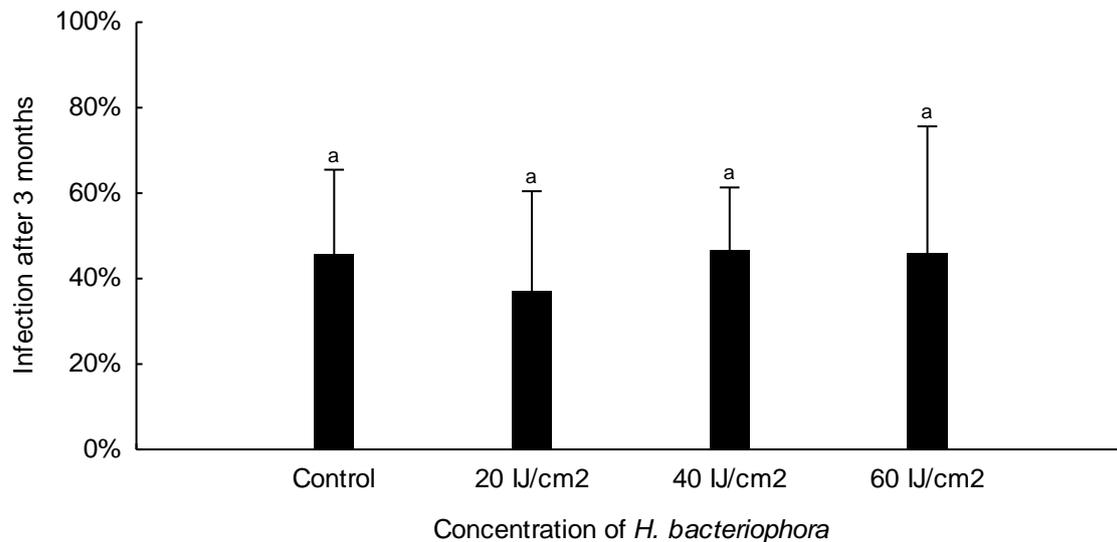


Fig. 3.2.3.14. Mean percentage mortality of FCM larvae (95% confidence level) in cages, three months after inoculation with four concentrations of *H. bacteriophora*, 0, 20, 40 and 50 IJ/cm² three months earlier. Different lettering above vertical bars indicates significant differences (One-way ANOVA; $F_{(3, 27)} = 0.12$; $p = 0.95$).

14. Field application of three different species (Trial 2)

Evaluation of three nematode species: On day 6, 35 and 49 there were no significant differences between the control-treatment and the *S. khoisanae*-treatment (Fig. 3.2.3.15). There were no significant differences between the *S. khoisanae*-treatment for day 21 and day 35. For the *H. bacteriophora*-treatment there was a significant difference between the controls for each evaluation day except for day 49. For the *H. zealandica*-treatment there was a significant difference between the controls and each evaluation day up to day 49. After 21 days the mortality of *S. khoisanae* increased to 54% but dropped to as low as 6% after 49 days. Mortality for *H. zealandica* increased to 72% on day 35, but dropped to 27% on day 49. Mortality for *H. bacteriophora* increased at day 21 to 84% and dropped to 72% on day 35 and to 28% on day 49. On day 49 mortality for all species dropped significantly to levels under 30%. Ambient temperature for day 49 of the period the cages were in the soil was a minimum of 7°C, a maximum of 32°C and a mean temperature of 17°C (Table 3.2.3.6).

When the percentage mortality for day 2 and day 6 were evaluated for the controls and *S. khoisanae* treatments, there were no significant differences (Fig. 3.2.3.14). However, for *H. bacteriophora* and *H. zealandica* treatments, there was a significant difference between the two evaluation times. For *H. bacteriophora* the percentage infection increased from 59.49% to 80.62% and for *H. zealandica* from 48.18% to 62.65%.

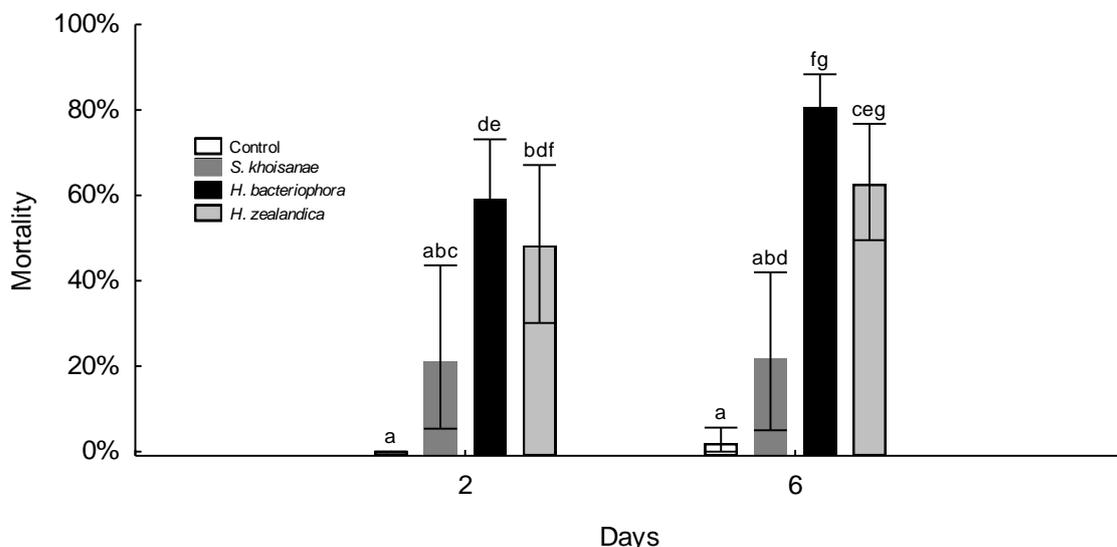


Fig. 3.2.3.15. Mean mortality (95% confidence intervals) after application of *S. khoisanae*, *H. zealandica* and *H. bacteriophora* at a concentration of 10 IJ/cm² and for controls. Cages were left in the soil, insects removed

and evaluated for infection after 2 days in the soil and another 4 days in the laboratory. Different letters above vertical bars indicate significant differences (Repeated Measures ANOVA of variance, $F_{(3,28)} = 13.26$; $p = 0.0001$).

After 35 days the percentage mortality of all three species of FCM declined to a significantly lower level by day 49 (Fig. 3.2.3.16).

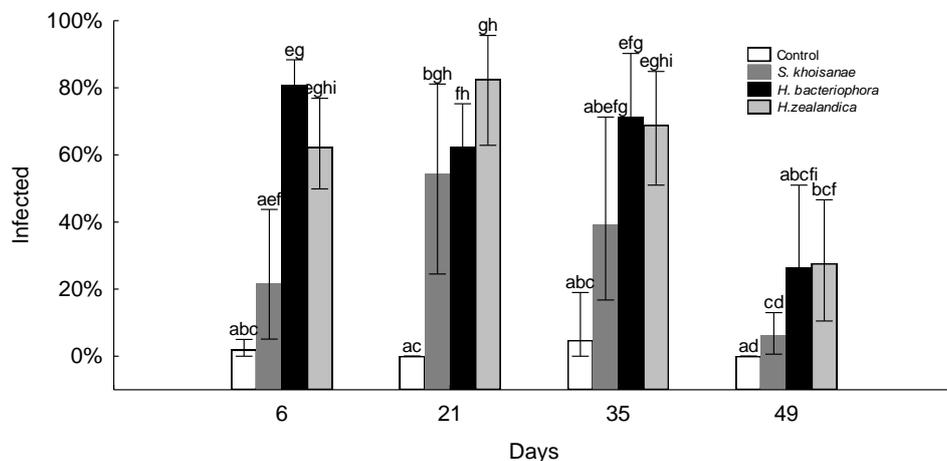


Fig. 3.2.3.16. Percentage mortality (95% confidence level) for FCM larvae, 6, 21, 35 and 49 days after initial inoculation of the species, *S. khoisanae*, *H. zealandica* and *H. bacteriophora* at concentrations of 10 IJ/cm². Repeated measures ANOVA of variance, $F_{(9, 84)} = 5.15$; $p < 0.001$). Different letters above vertical bars indicate statistical significance.

Table 3.2.3.6. Minimum, maximum and mean temperatures for the period cages were left in the soil.

Periods (days) during which cages were buried	Minimum °C	Maximum °C	Mean °C
1-6	11.28	36.90	23.20
15-21	11.38	30.31	19.10
29-35	11.38	38.32	22.42
43-49	7.03	31.93	17.01

Percentage infectivity of the different treatments after 12 months showed that the treatments did not differ significantly from one another (Fig. 3.2.3.17). Percentage mortality for FCM larvae was generally very low and variable. Only *H. zealandica* showed a significant difference from the control with a mean infection of 40%.

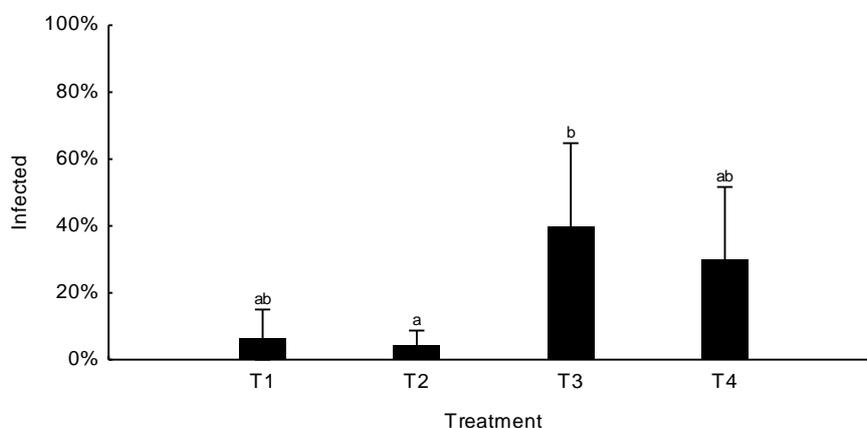


Fig. 3.2.3.17. Mean mortality (confidence limits 95%) of four treatments (water only, *H. khoisanae*, *H. bacteriophora* and *H. zealandica*) 12 months after application of 10 IJ/cm². Different letters above vertical bars indicate significant difference (One-way ANOVA, $F_{(3, 28)} = 4.23$, $p = 0.014$).

15. Field application of three different *H. zealandica* concentrations (Trail 3)

A Repeated Measures ANOVA of variance on the percentage larvae infected was done for no treatment on day 1, and for the four treatments of 0, 5, 10 and 20 IJ/cm² over days 7, 21 and 35. There was a significant interaction between days and treatments ($F_{(9, 63)} = 4.72$; $P < 0.001$). Since the residuals are not normally distributed a bootstrap multiple comparison was done (Fig. 3.2.3.18).

Baseline sampling before any nematodes were applied showed mortality caused by endemic nematodes which ranged from 2% to 13% with no significant difference between the different trees used for treatments. On day 7 after application there were no significant differences in the percentage mortality between the three concentrations of *H. zealandica* applied, which were 85% for 5 IJ/cm², 80% for 10 IJ/cm² and 87% for 20 IJ/cm². There were, however, significant differences between the control and the nematode treatments at $p < 0.001$ (Fig. 3.2.3.18). For the concentration 10 IJ/cm² there were, however, no significant differences between any of the treatments and the control. The percentage mortality for baseline sampling, day 21 and day 35 showed great variation but no significant differences between each other.

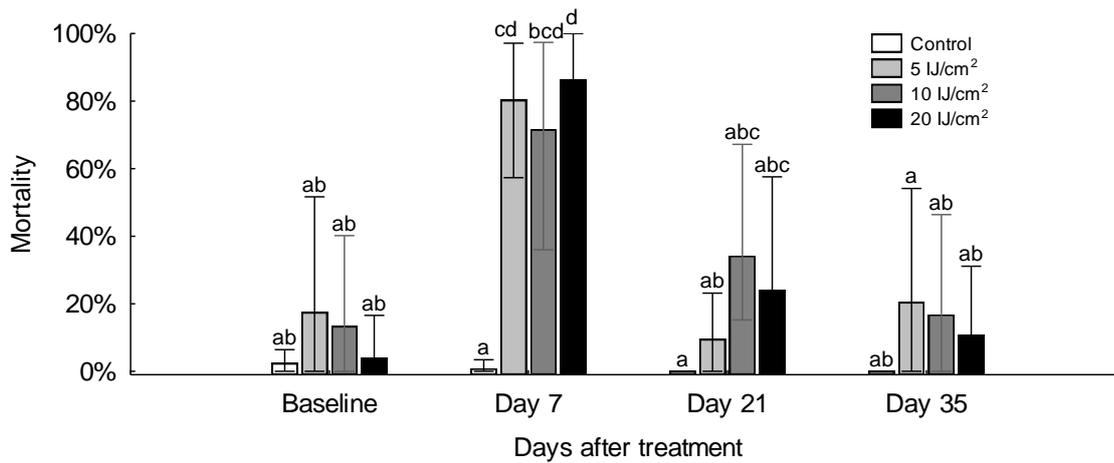


Fig. 3.2.3.18. Percentage mortality (95% confidence level) for FCM larvae, 7, 21 and 35 days after the initial inoculation of *H. zealandica* at concentrations of 0, 5, 10 and 20 IJ/cm² and evaluated before application of nematodes then after 7, 21 and 35 days in cages in the soil and evaluated on the same day of removal (Trial 3). Repeated measures ANOVA of variance, $F_{(9, 84)} = 5.15$; $p < 0.001$). Different letter above vertical bars indicated statistical significance.

When the percentage mortality for day 2 and day 6 were evaluated for the controls and *S. khoisanus* treatments, there were no significant differences (Fig. 3.2.3.19). However, for *H. bacteriophora* and *H. zealandica* treatments, there was a significant difference between the two evaluation times. For *H. bacteriophora* the percentage infection increased from 59.49% to 80.62% and for *H. zealandica* from 48.18% to 62.65%.

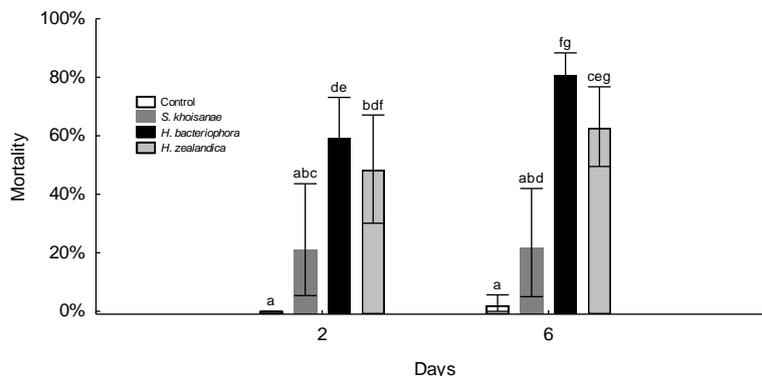


Fig. 3.2.3.19. Mean mortality (95% confidence intervals) of controls and after application of *S. khoisanus*, *H. zealandica* and *H. bacteriophora* at a concentration of 10 IJ/cm². Cages were left in the soil, insects removed and evaluated for infection after 2 days in the soil and another 4 days in the laboratory. Different letters above vertical bars indicate significant differences (Repeated Measures ANOVA of variance, $F_{(3,28)} = 13.26$; $p = 0.0001$).

16. Horizontal and vertical movement of nematodes through soil columns

Movement of nematodes through a 15 cm soil column showed a 100% control shortening the time from 24 to 8 hours. Some infection was obtained after only 4 h (Fig. 3.2.3.20).

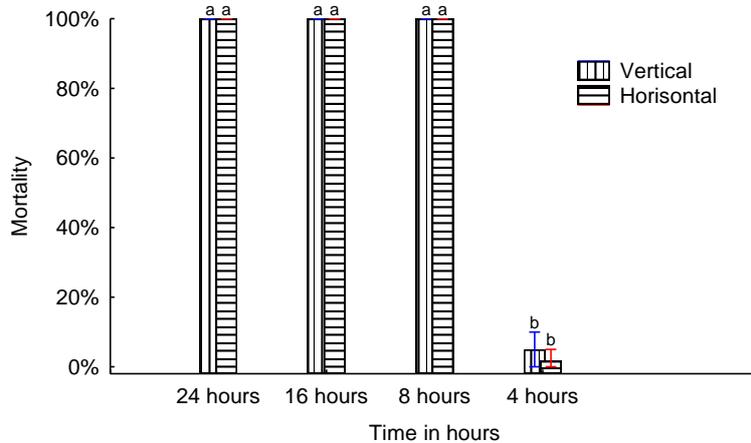


Fig. 3.2.3.20. Mean percentage infection (95% confidence limit) of FCM last instar larvae in vertical and horizontally placed 15 cm sand columns after inoculation with 400 IJ of *H. bacteriophora* (Factorial ANOVA, $F_{(3,88)} = 1.1579$, $p = 0.33048$). Different lettering above bars indicates significant differences.

No significant difference in penetration was found between vertical and horizontal movement of nematodes (Fig. 3.2.3.21) in 15 cm sand columns. Nematodes were able to move over a distance of 15 cm within 4 h to infect FCM larvae.

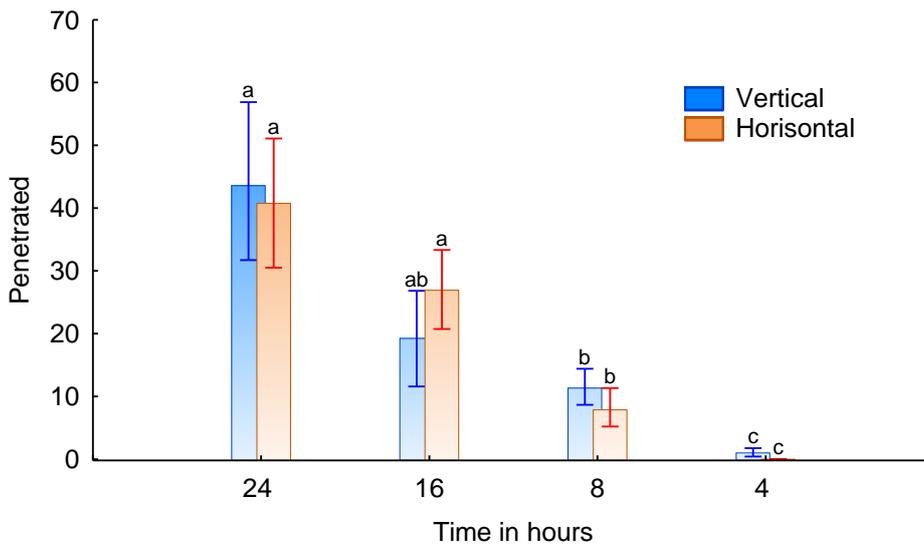


Fig. 3.2.3.21. Mean percentage penetration (95% confidence limit) of FCM last instar larvae in vertically and horizontally placed 15 cm sand columns after inoculation with 400 IJ of *H. bacteriophora* (Factorial ANOVA, $F_{(3,88)} = 0.09926$, $p = 0.4002$). Different lettering above bars indicates significant differences.

17. Horizontal movement of three different EPN species through three soil types

Steinernema yirgalemense (157-C) and *S. feltiae* (recycled) caused 100% mortality of FCM larvae after 8 h in sand, loam and clay soil. *Heterorhabditis bacteriophora* (SF 351) caused 77% mortality in loam and 58% mortality in clay soil, while *S. yirgalemense* and *S. feltiae* gave 40% and 70% respectively. *Steinernema feltiae* caused 70% mortality in loam and 100% mortality in clay soil (Fig. 3.2.3.22).

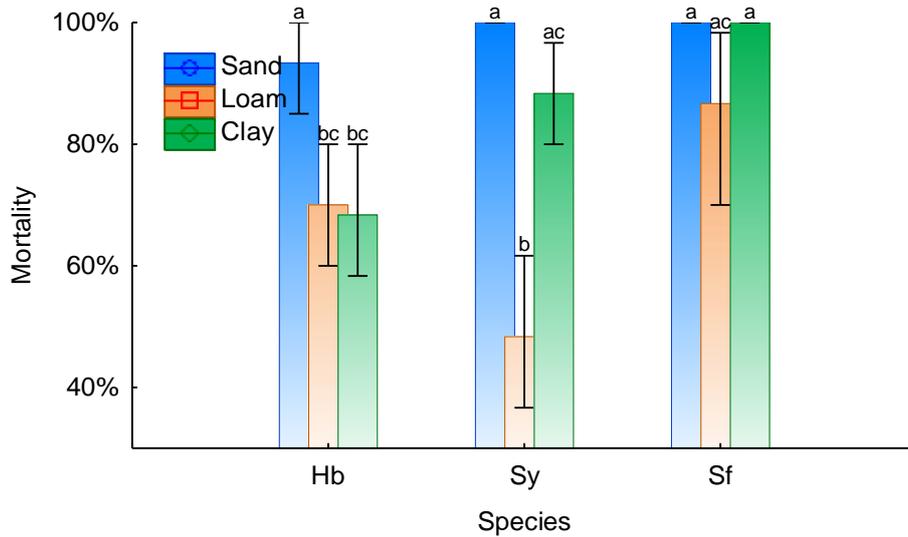


Fig. 3.2.3.22. Mean percentage mortality (95% confidence interval) 8 h after inoculation with 400 IJ of *H. bacteriophora* (Hb), *S. yirgalemense* and *S. feltiae* (Sf) moving through a 15 cm column of sand, loam and clay soil (Factorial ANOVA, $F_{(4,99)} = 7.825$, $p = 0.0002$). Different lettering above bars indicates significant differences.

Good penetration was obtained by *S. feltiae* in all soil types with the highest penetration rate in sand, clay and loam (Fig. 3.2.3.23). A factorial ANOVA showed significant differences in penetration rate for *S. feltiae* in comparison to *H. bacteriophora* and *S. yirgalemense*.

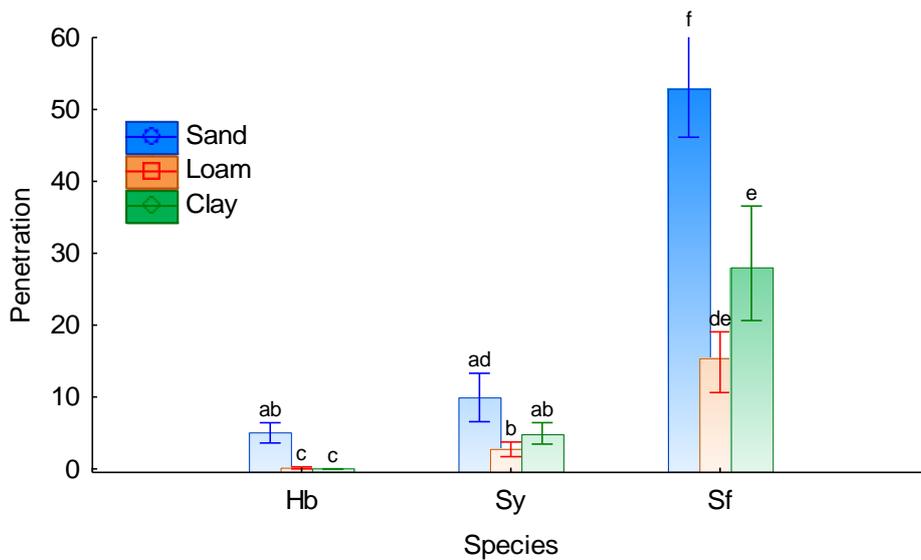


Fig. 3.2.3.23. Mean penetration of FCM larvae (95% confidence interval) with *H. bacteriophora* (Hb), *S. yirgalemense* and *S. feltiae* (Sf) after inoculation with 400 IJ of soil in a 15 cm column of sand, loam and clay soil and movement of IJs through the soil after 8 h (Factorial ANOVA, $F_{(4,99)} = 17.678$, $p < 0.005$). Different lettering above bars indicates significant differences.

After 4h *S. feltiae* gave significant differences in mortality in comparison with the other two species in all three soil types (Fig. 3.2.3.24).

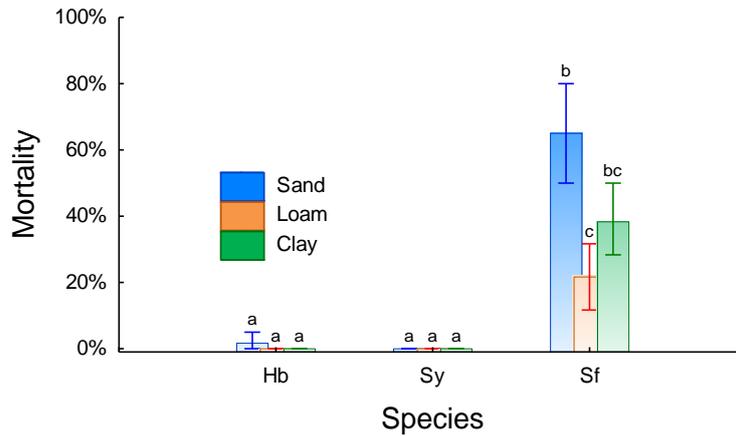


Fig. 3.2.3.24. Mean percentage mortality (95% confidence interval) 4 h after inoculation with 400 IJ of *H. bacteriophora* (Hb), *S. yirgalemense* and *S. feltiae* (Sf) after moving through a 15 cm column of sand, loam and clay soil after 4 h (Factorial ANOVA, $F_{(4,99)} = 11.089$, $p = 0.0002$). Different lettering above bars indicates significant differences.

Only low numbers of nematodes had penetrated larvae after 4h in horizontal columns for all three nematode species. In sand, *S. feltiae* showed significantly better penetration in comparison with the other soil types and the other two nematode species (Fig. 3.2.3.25).

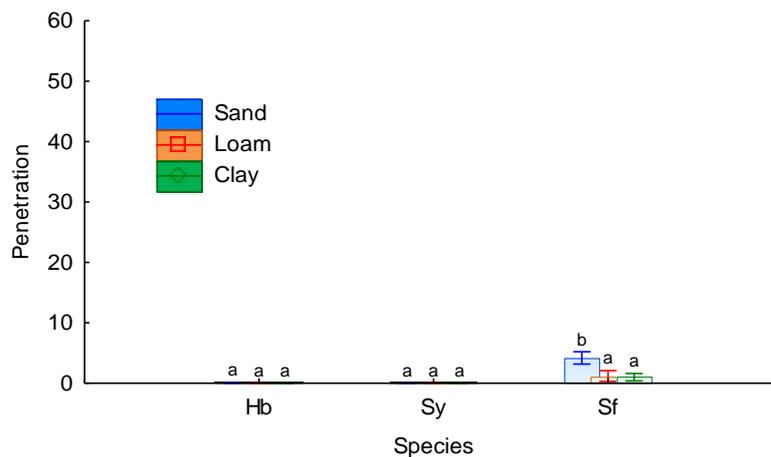


Fig. 3.2.3.25. Mean penetration of FCM larvae (95% confidence interval), 4 h after inoculation of soil with 400 IJ of *H. bacteriophora* (Hb), *S. yirgalemense* and *S. feltiae* (Sf) after moving through 15 cm columns of sand, loam and clay soil after 4h (Factorial ANOVA, $F_{(4,99)} = 7.825$, $p < 0.0005$). Different lettering above bars indicates significant differences.

18. Virulence of different nematode isolates at 25°C

Last-instar FCM larvae were found to be 100% controlled with *S. yirgalemense* (157-C), which differed significantly from the other three isolates tested. The endemic *H. bacteriophora* (SF351), with a percentage mortality of 87%, did not differ significantly from the formulated imported *H. bacteriophora*, with a mortality of 76%. However, the formulated *H. bacteriophora* differed significantly from both *H. zealandica* and *S. yirgalemense*, which differed significantly from each other (Fig. 3.2.3.26).

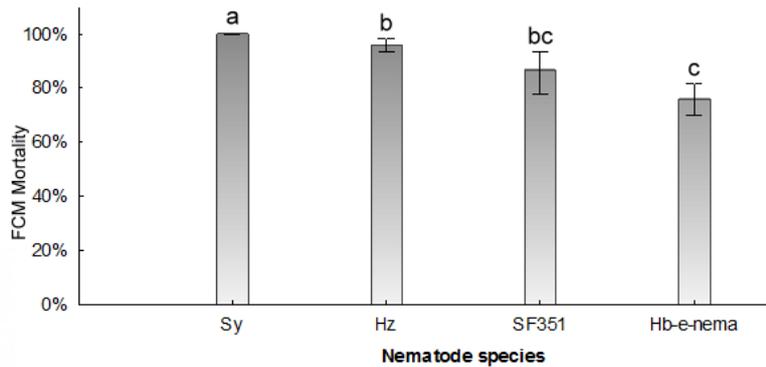


Fig. 3.2.3.26. Mean percentage mortality (95% confidence interval) of FCM last-instar larvae inoculated with 50 IJs/insect of *S. yirgalemense* (157-C), *H. zealandica* (SF41), *H. bacteriophora* (SF351) and formulated *H. bacteriophora* (Hb-e-nema) after a period of 48 h at 25°C. Different letters above vertical bars indicate significant differences.

The efficacy of the nematodes, measured by mortality of FCM, is reflected in the number of nematodes which penetrated. For *S. yirgalemense* and *H. zealandica*, there was no difference in the number of nematodes which penetrated, and they were found to differ significantly from the local *H. bacteriophora*. The lowest number of nematodes penetrated with the formulated *H. bacteriophora*, differing significantly from the local isolate, which, in turn, differed significantly from *S. yirgalemense* and *H. zealandica* (Fig. 3.2.3.27).

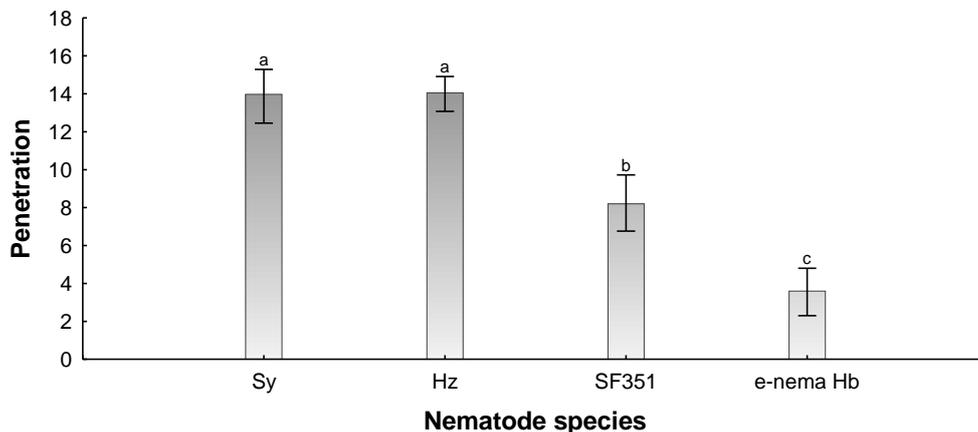


Fig. 3.2.3.27. Mean penetration (95% confidence interval) of IJs into FCM last-instar larvae, inoculated with 50 IJs/insect of *S. yirgalemense* (157-C), *H. zealandica* (SF41), *H. bacteriophora* (SF351) and formulated *H. bacteriophora* (Hb-e-nema) after a period of 48 h at 25°C. Different letters above vertical bars indicate significant differences.

In a previous study conducted by Malan *et al.* (2011), it was shown that *S. yirgalemense* and *H. zealandica* performed significantly better than did local *H. bacteriophora* against last-instar FCM larvae. The current study showed comparable results regarding the potency of local *H. bacteriophora*. However, the formulated imported *H. bacteriophora* was the least effective of the species tested, with a mortality of 76%, as could be expected from an *in vitro* cultured formulated product. However, the formulated product did not differ significantly in mortality from the locally produced *H. bacteriophora*, although a significantly lower number of nematodes managed to penetrate the FCM larvae.

19. Virulence of Hb and Sf

At a temperature of 25°C, 100% control of last-instar FCM larvae was found with *S. feltiae* and 94% control with *H. bacteriophora* with no significant difference ($P = 0.21$) between the two. In comparison, very low virulence of both the formulated *S. feltiae* and local *H. bacteriophora* was found at 14°C (Fig. 3.2.3.28).

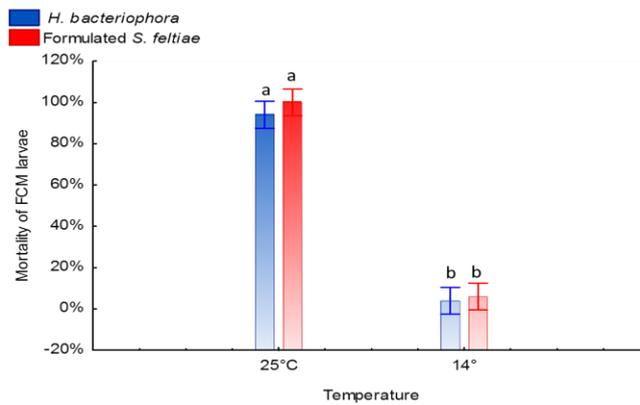


Fig. 3.2.3.28. Mean mortality (95% confidence interval) of FCM last-instar larvae, inoculated with 50 IJs/insect of *H. bacteriophora* (SF351) and formulated *S. feltiae* after a period of 48 h at 25°C. Different letters above vertical bars indicate significant differences.

20. Lethal concentration

Analysis of the two trials showed significant interaction between the main effects of formulated and recycled *S. feltiae* ($F_{(4, 39)} = 18.165$; $P = 0.005$). In the first trial, formulated *S. feltiae*, and in the second trial, *S. feltiae*, recycled though wax moth larvae were used. With the imported formulated *S. feltiae*, significant differences between the two trials were obtained at concentrations of 50 and 100 IJs. However, at higher concentrations of 25, 200 and 400 IJs, no difference in mortality was found (Fig. 3.2.3.29). The difference observed can be ascribed to the poor condition of the imported nematodes (with the ice packs having melted during transportation, resulting in the consignment having not been at a low temperature when received).

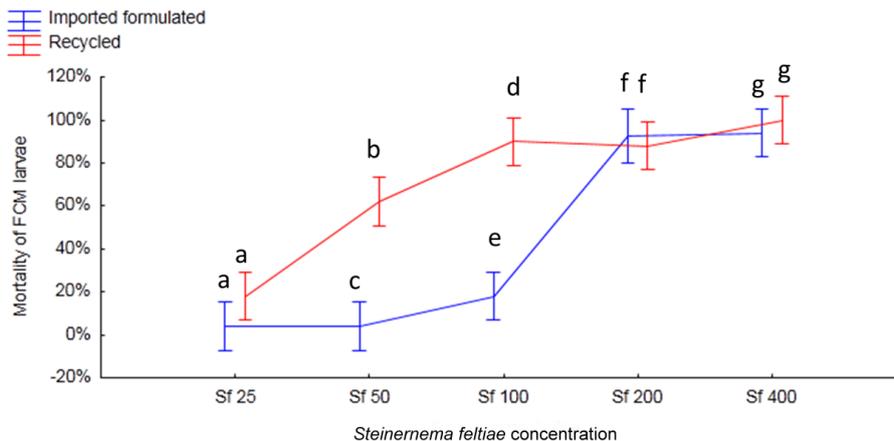


Fig. 3.2.3.29. Percentage mortality (95% confidence interval) recorded for FCM larvae after exposure to different concentrations of *S. feltiae* as an imported formulation and as a recycled product at 14°C for a period of 24 h. Different letters above vertical bars indicate significant differences.

The results show that *H. bacteriophora* (SF351) caused no mortality after 48 h at any of the concentrations used (Fig. 3.2.3.30). For *S. feltiae* (combined Sfel-e-nema and Sfel), the results illustrated a positive relationship between insecticidal activity and nematode concentration, with an accumulative increase in mortality as nematode concentrations increased. At a concentration of 25 IJs/insect 11% mortality was obtained, which was significantly higher than that which was achieved for the control ($P < 0.001$), with no mortality. Between concentrations of 25, 50 and 100 IJs/insect no significant difference was detected, but a significant increase in mortality was recorded from 100 to 200 IJs/insect with mortality of 89% and 97%, respectively (Fig. 3.2.3.30).

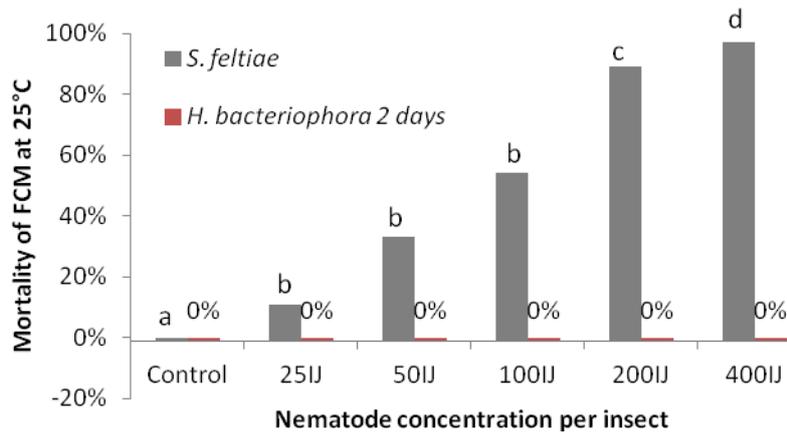


Fig. 3.2.3.30. Percentage mortality recorded for FCM larvae after exposure to different concentrations of *H. bacteriophora* (SF351) and *S. feltiae* at 14°C for a period of 24 h. Different letters above vertical bars indicate significant differences.

A Probit analysis showed that for *S. feltiae* the data fitted the model well ($\chi^2 = 3.396$; d.f. = 2; $P = 0.38$), indicating a positive relationship between nematode concentration and insecticidal activity at 14°C. The LC_{50} , LC_{90} and LC_{99} values were 78, 242 and 333 IJ/insect respectively (Fig. 3.2.3.31).

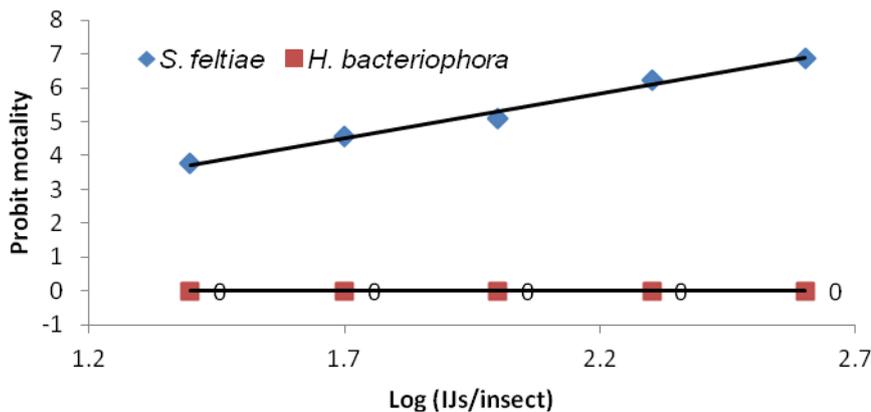


Fig. 3.2.3.31. Probit mortality of FCM last-instar larvae after exposure to different concentrations of *H. bacteriophora* (SF351) and *S. feltiae* at 14°C for a period of 24 h.

As no mortality was found for FCM at 14°C, the live larvae were washed (to make sure all surface nematodes were removed) and kept for another two days at 25°C to determine whether they were, indeed, infested with nematodes. Figure 3.3.3.32 shows that, even though the larvae were not killed by *H. bacteriophora*, they were infested with them, with the concentration of 25 IJs/FCM showing 57% infection resulting in increasing mortality with higher concentrations of nematodes, up to 97% at a concentration of 400 IJ.

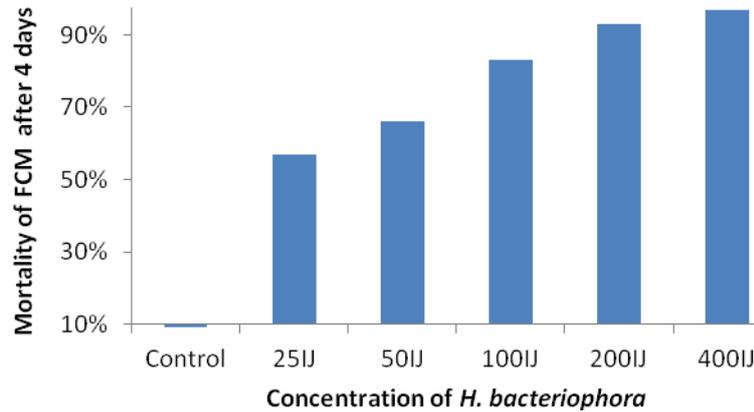


Fig. 3.2.3.32. Percentage mortality of *H. bacteriophora* after two days exposure to different concentrations of IJs, followed by being washed and left on moistened filter paper for another two days at 25°C.

The results from the bioassay clearly indicated that *S. feltiae* showed insecticidal activity at a temperature as low as 14°C within a period of 48 h, in comparison to a local strain of *H. bacteriophora* which showed no insecticidal activity at that temperature. A previous study with codling moth (*Cydia pomonella*) showed that low temperatures (17°C for 10 h and 12°C for 14 h) negatively affected larvicidal activity ($\leq 3\%$) of six endemic isolates tested (De Waal *et al.*, 2011). As virulence is regarded as the foremost prerequisite for a nematode isolate to be considered as having biocontrol potential against an insect pest, *S. feltiae* would be regarded as very quick and effective at 14°C using higher concentrations.

Discussion

Nematodes have not previously been used for the biological control of FCM, and this is the first study on their possible use as a biological control agent against a key pest of citrus in South Africa. Both *S. yirgalemense* and *H. zealandica* showed promise as biological control agents in laboratory bioassays against FCM larvae, pupae and emerging moths. In addition, *H. zealandica* has also been found to be highly virulent against codling moth (*Cydia pomonella*, L.) diapausing larvae residing beneath the bark of apple trees as an aerial application (De Waal *et al.*, 2010, 2011). The biology of FCM offers a window of opportunity for application of EPN to the soil. The last-instar larvae, the cocooned pupae and the emerging moth are in the soil for a total period of approximately two weeks or longer, depending on the temperature, during which they can be targeted using EPN (Daiber, 1979a, 1979b, 1980, 1989).

The larvae of FCM were found to be highly susceptible to low concentrations of all isolates of the six species of EPN found during the survey. Of all species, *S. yirgalemense* performed the best with the highest mortality, followed by *H. zealandica*, which was the same South African isolate as that which was used against codling moth (De Waal *et al.*, 2010, 2011). In this study pupae of FCM were found to not be as susceptible as larvae and needed fourfold the concentration (200 IJ/pupa) to give mortality of between 20-75% in comparison with larvae where a concentration of 50IJ/larvae gave mortality of 77-100%. The same pattern of efficacy against pupae as for the larvae were found between the isolates of the six species, with *S. yirgalemense* and *H. zealandica* performing the best and *S. khoisanae* the worst. In contrast, Anbesse *et al.* (2008) tested *S. yirgalemense* for the control of grubs of the barley chafer, *Coptognathus curtippennis*, in Ethiopia, and reported an overall mortality of only 16%, using double the nematode concentration (100 IJ/insect).

In the sand bioassays the length of *S. khoisanae* (IJ > 1 000 μm), was considered to perhaps provide an advantage in mobility. However, in overall performance, *S. yirgalemense* killed the highest percentage of pupae and moths in the sand bioassay, followed by *H. zealandica* and *S. khoisanae*. In comparison with 75% infection of pupae in the 24-well bioassays, *S. yirgalemense* was able to infect just 40% of the pupae in sand before they eclosed as moths. *Heterorhabditis zealandica* performed similarly against pupae in bioassays. The percentage infected fully eclosed moths was the almost the same for all three species. However, for *S. khoisanae* infection for moths was relatively high in comparison with the number of infected pupae.

It is noteworthy that moths infected with nematodes in the sand bioassay were able to emerge normally and to fly away from the sand surface, before being trapped in the surrounding bigger container. In natural

conditions, the moths would be able to fly away for at least 24 h before they would be killed by septicaemia. Such a finding means that EPN are able to distribute to other localities via aerial transport of infected emerging moths. If they fall onto the soil in a moist environment, the life cycle of the nematode can be completed. This same type of phoresy was observed by Kaya *et al.*, (1982) for adults of the beet armyworm (*Spodoptera exigua*) infected with *S. carpocapsae* when they emerged from soil. They concluded that the infection of adults during eclosion may aid in dispersal and the subsequent mortality could increase the effectiveness of the nematode.

The results of the current study showed the six nematode species found during the survey to be effective against FCM soil stages in laboratory bioassays, with clear differences between the species. The same pattern for mortality with the different species was reflected in all the host life stages tested, with *S. yirgalemense* and *H. zealandica* causing the highest level of mortality and *S. khoisanae* the lowest. Nematode species were found to be highly virulent against last-instar FCM larvae and emerging moths, while pupae were not as susceptible to nematode infection. Since FCM is a key pest of citrus in South Africa, it would be advisable to test the nematodes in field trials in an integrated pest management (IPM) system for the control of FCM. The pest species concerned is also a multivoltine species, with up to six generations per year (Newton, 1998), offering year-round availability of a host for EPN, providing the possibility of persistence in citrus orchards.

Conclusion

The limited research conducted to present indicates that nematodes may well be exceptionally good biological control agents for use against FCM. However there is still much research that needs to be done to ensure the effective application of these nematodes in the field.

Future research

Research on EPNs and FCM should be on going, especially with regard to the application technology especially in situations with no sprinkler irrigation. The dynamics, timing of applications and the amount of water is also an important factor that needs further research. The persistence of nematodes is also an important topic for future research as this information will indicate the frequency with which application would be needed.

Technology transfer

Scientific publications

1. Malan, A.P., Nguyen, K.B. De Waal, J. & Tiedt, L., 2008. *Heterorhabditis safricana* n. sp. (Rhabditidae: Heterorhabditidae), a new entomopathogenic nematodes from South Africa. *Nematology* 10 (3): 281-396.
2. Stokwe, N.F., Malan, A.P., Nguyen, B.K., Knoetze, R. & Tiedt, L. 2011. *Steinernema citrae* n. sp. (Rhabditida: Steinernematidae), an new entomopathogenic nematode from South Africa. *Nematology* 13 (5): 567-587.
3. Malan, A.P., 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.
4. Malan, A.P., Knoetze, R., Tiedt, L. 2013. *Heterorhabditis noenieputensis* n. sp. (Rhabditida: Heterorhabditidae), a new entomopathogenic nematode from South Africa. *Journal of Helminthology* (accepted).

Popular publications

Malan, A.P. & Moore, S.D., 2012. Entomopathogenic nematodes show excellent potential to control soil stages of false codling moth and can use the adult moth for aerial transport over long distances. *SA Fruit Journal* 10 (6): 64-67.

International oral presentations

Malan, A. P. & Moore, S.D., 2010. Field trials with entomopathogenic nematodes for the control of false codling moth (*Thaumatotibia leucotreta*). Proceedings of the Society of Invertebrate Pathology's Annual Meeting, Trabzon, Turkey, 11-15 July (Presentation).

National oral presentations

1. Malan, A.P. & Moore, S.D., (2007). Potential of entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae for the control of false codling moth. 16th Symposium of the Nematological Society of Southern Africa.

2. Malan, A. P. & Moore, S.D., 2008. Entomopathogenic nematodes for the control of false codling moth. IPM meeting (oral presentation)
3. Malan, A.P. & Moore, S.D., (2008). Potential of entomopathogenic nematodes for the control of false codling moth, *Thaumatotibia leucotreta*, (Lepidoptera: Tortricidae) in laboratory bioassays. CRI Symposium
4. Malan, A.P. & Moore, S.D., (2009). Control of false codling moth with entomopathogenic nematodes in field trials. 19th Symposium of the Nematological Society of Southern Africa, 17-20 May.
5. Malan, A.P. & Moore, S.D., (2009). Field trials with entomopathogenic nematodes for the control of false codling moth. 16th Entomological congress of the Entomological Society of Southern Africa, 5-7 July, 2009.
6. Malan, A.P. & Moore, S.D., (2009). Laboratory bioassays and field application of entomopathogenic nematodes for the control of false codling moth (*Thaumatotibia leucotreta*). South African Journal of Plant and Soil (3) 27: 278. (19th Symposium of the Nematological Society of Southern Africa, Hazyview, 17-20 May).
7. Malan, A. P. & Moore, S.D., (2010). Field trials with entomopathogenic nematodes for the control of false codling moth (*Thaumatotibia leucotreta*). The 6th Citrus Research Symposium held at the Champagne Sports Resort in the Drakensberg 15-18 August 2010.
8. Malan, A.P. & Moore, S.D. 2012. Movement of entomopathogenic nematodes in different soil types. Citrus Symposium. 7th Citrus Research Symposium at the Champagne Sports Resort, Drakensberg, 19-22 Augustus 2012.

National poster

De Waal J Y, Malan A P & Ferreira T (2007). Influence of temperature on the infectivity of entomopathogenic nematodes (*Steinernema* and *Heterorhabditis*) to false codling moth, *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae). *South African Journal of Plant and Soil* 24 (4), 257.

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

Project 955 (April 2009 – July 2013) by Sean Moore, Wayne Kirkman (CRI), Tanya Fullard and Martin Hill (RU)

Summary

Laboratory trials were conducted to compare the efficacy of different pheromone isomer blends for disrupting the ability of adult male FCM to locate virgin female FCM and thus mate. Unfortunately after numerous attempts, an appropriate technique and protocol for conducting these trials could not be developed and the study was thus aborted. A field trial was conducted to compare various mating disruption and attract and kill products – both those commercially available and experimental products. Mating disruption “overkill”, a combination of Isomate and Checkmate (the latter applied fortnightly), was the most effective treatment in reducing both moth catches and fruit infestation. Evaluation of this trial is not yet complete.

Opsomming

Laboratoriumproewe is uitgevoer om verskillende feromoon isomeer mengsels te vergelyk in hulle effektiwiteit om 'n volwasse mannetjie VKM se vermoë om 'n ongepaarde wyfie VKM op te spoor te verstuur en dus om te paar. Na verskeie pogings, was dit ongelukkig nie moontlik om 'n geskikte tegniek en protokol te ontwikkel om hierdie proewe uit te voer nie en dus is dié studie gestaak. 'n Veldproef is uitgevoer om

verskeie paringsontwrigting en lok-en-vrek produkte te vergelyk – albei dié wat kommersieel beskikbaar is en eksperimentele produkte. Paringsontwrigting “oordosis”, ‘n kombinasie van Isomate en Checkmate (ten laaste elke tweede week toegedien), was die mees doeltreffende behandeling, albei wat afname in mot vangstes en vrugbesmetting betref. Evaluasie van hierdie proef is nog nie voltooi nie.

3.2.5 FINAL REPORT: Amelioration of the post-harvest cold treatment regime for FCM with the use of carbon dioxide

Project 965 (April 2009 – March 2012) by J. Terblanche, L. Boardman (SU), T.G. Grout, V. Hattingh, P.R. Stephen and K.C. Stoltz (CRI)

Summary

Stellenbosch University

Understanding the low temperature biology of false codling moth (FCM) *Thaumatotiba leucotreta*, is of critical importance to post-harvest control. This research has examined the low temperature tolerance and cold hardiness of 4-5th instar larvae of FCM using a range of approaches (molecular, whole-animal). Apart from determining important baseline parameters related to temperature and gas stress, all of which are useful to pest management and post-harvest control, this work addressed the key issue of how modified atmospheres, especially elevated CO₂ typically in use, may best be used to enhance post-harvest mortality rates. Specifically, we tested the potential mode of action of low temperature sterilization, and how additional combinations of stressors might alter biochemical responses and, ultimately, survival. In general, the work showed that low temperatures elicit changes in body water content, cell membrane phospholipid composition and heat shock protein 70. By contrast, the addition of elevated carbon dioxide or low oxygen resulted in a decrease in body water content, and in the case of carbon dioxide a decrease in glucose and no change in membrane fluidity (indicated by fatty acid chain length). Overall, these and additional experiments suggest low oxygen should be avoided in post-harvest treatments as it may result in cross tolerance to low temperature exposure. High carbon dioxide, on the other hand, may be a more viable method for reducing post-harvest cold sterilization time and obtaining the same mortality levels as in longer treatments, possibly by limiting the changes in membrane composition that are needed to survive long term, low temperature exposures. Additionally, a high temperature pre-treatment may be considered prior to cold sterilization, as there does not appear to be an overlap in mechanisms of high and low temperature tolerance in FCM. Further work on infested fruit would naturally be required to confirm this suggestion.

CRI

Research on the effect of CO₂ on false codling moth (FCM) larvae in fruit 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 100% mortality in different trials whereas 30% CO₂ in air for 12 h at 25°C caused 8% or less mortality and 60% CO₂ in air for 12 h at 25°C caused 36% or less mortality. Mortality in the latter treatment did not increase when fumigated for 24 h. Combinations of 12 h fumigation with 30% CO₂ 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% CO₂ 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. It is therefore clear that a 24 h break after the CO₂ stress improved survival in the following cold treatment. Treating exposed FCM eggs with 60% CO₂ in air for 24 h at 25°C reduced egg hatch by 87%. Although this project has now terminated, further research will be conducted using 60% CO₂ for 24 h and varying the interval between fumigation and cold treatment in project 913 on fumigation.

Opsomming

Stellenbosch Universiteit

Vir die suksesvolle na-oes beheer van die valskodlingmot (VKM), *Thaumatotiba leucotreta*, is ‘n goeie begrip van VKM se lae temperatuur biologie noodsaaklik. Hierdie navorsing is die lae temperatuur toleransie en koudhardheid strategieë van die 4-5de instar larwes van VKM ondersoek, deur gebruik te maak van verskeie benaderings (molekulêre, hele dier). Afsien van die bepaling van fundamentele parameters met betrekking tot temperatuur en gas stres, belangrik vir plaag- en na-oes beheer, spreek hierdie werk nog ‘n kern kwessie aan, naamlik die gebruik van verhoogde koolstofdioksied (CO₂) algemeen toegepas in die praktyk, in ‘n gemodifiseerde atmosfeer vir die verbetering van na-oes mortaliteit. Ons het spesifiek die potensiële modus van aksie van lae temperatuur sterilisasie ondersoek, sowel as hoe addisionele kombinasies van stressors dalk biochemiese reaksies beïnvloed en uiteindelik dus ook oorlewing. Grotendeels het die werk getoon dat lae temperatuur veranderinge in liggaamswater-inhoud, selmembraan fosfolipied samestelling en hitte-skok

proteïen 70 veroorsaak. In teenstelling hiermee, het die toevoeging van verhoogde CO₂ of verlaagde suurstof gelei tot 'n afname in liggaamswater-inhoud. In die geval van koolstofdiksied was daar 'n afname in glukose en geen verandering in membraanvloeibaarheid nie (aangedui deur vetsuurketting lengte). Omvatlik stel hierdie, asook die addisionele eksperimente voor, dat lae suurstof vermy moet word in na-oes behandeling omdat dit moontlike kruis-toleransie tot gevolg kan hê by blootstelling aan lae temperature. Hoë koolstofdiksied is dalk 'n meer haalbare manier om tegelykertyd 'n verkorting in die tyd van na-oes koue sterilisasie te hê asook dieselfde aantal sterftes as in langer behandelings. Dit is moontlik as gevolg van beperkte veranderinge in die samestelling van die membraan nodig vir die oorlewing van lae temperature oor die langtermyn. Verder, kan 'n hoë temperatuur behandeling voor koue sterilisasie dalk oorweeg word omdat dit voorkom asof daar nie oorvleueling is tussen hoë en lae temperatuur toleransie in VKM nie. Verdere werk op besmette vrugte word natuurlik vereis om hierdie voorstel te bevestig.

CRI

Navorsing oor die effek van CO₂ op valskoddingmot (VKM) larwes in vrugte het getoon dat Valencias net so effektief soos nawel-lemoene kunsmatig besmet kan word deur die plasing van stukkie eierblaaie op die vrugte. Kort 5-dag koue behandelings by -0.5°C het van 40 tot 100% mortaliteit in verskillende proewe veroorsaak, waar 30% CO₂ in die lug vir 12 h by 25°C, 8% minder mortaliteit veroorsaak het, en 60% CO₂ in die lug vir 12h teen 25°C, 36% of minder mortaliteit veroorsaak het. Mortaliteit in laasgenoemde behandeling het nie verhoog nie wanneer daar vir 24 h berook is. Kombinasies van 12 h beroking met 30% CO₂ gevolg deur onmiddellike waksing en 'n 5-dag koue behandeling, het ongeveer 60% mortaliteit tot gevolg gehad, maar as die koue behandeling met 24 h ná waks verminder is, het die mortaliteit met omtrent 35% verminder. Die gevolglike vlak van mortaliteit was laer as met die koue behandeling alleen. Kombinasies van 12 h beroking met 60% CO₂ gevolg deur 'n onmiddellike waks en 'n 5-dag koue behandeling het ongeveer 90% mortaliteit tot gevolg gehad, maar as die koue behandeling met 24h ná waks vertraag is, het die mortaliteit met omtrent 25% verminder. In hierdie geval was die gevolglike mortaliteit met die koue behandeling ná die interval steeds hoër as die koue behandeling alleen. Dit is dus duidelik dat 'n 24 h pouse ná die CO₂ stres, die oorlewing in die koue behandeling wat gevolg het, verbeter het. Behandeling van blootgestelde VKM-eiers met 60% CO₂ in lug vir 24 h by 25°C het die uitbroei van eiers met 87% verminder. Alhoewel hierdie projek nou gestaak is, sal verdere navorsing gedoen word deur 60% CO₂ vir 24 h te gebruik, en die interval tussen beroking en koue behandeling sal afgewissel word in projek 913 oor beroking.

Introduction

The only practical post-harvest disinfestation treatment currently available for FCM in citrus, is a 22 d cold treatment at a temperature of -0.6°C. This treatment has detrimental effects on fruit quality, resulting in colour loss in Clementines and unacceptably high levels of chilling injury in lemons and grapefruit. Within the international phytosanitary trade regulatory environment, there is an increasing sensitivity to FCM. Consequently most new markets that are opened require FCM disinfestation procedures and there is a risk that some existing markets may in future also insist on the inclusion of such measures. There is therefore an urgent need to develop alternative post-harvest disinfestation treatments for FCM in citrus that are both less detrimental to fruit quality and are of shorter duration to make them compatible with export to markets where the shipping time is less than 22 d.

Whereas exposing fruit to high levels of CO₂ for long periods does have insecticidal effects, such protracted exposure also has detrimental effects on fruit quality. However, it has previously been established that exposure of fruit to elevated CO₂ levels (10%) for shorter periods (approximately 3 days) does have a pre-conditioning effect, making the fruit less susceptible to subsequent chilling injury (K Lesar and P Cronjè – pers comm.). Spanish researchers (Alonso et al. 2005) reported promising preliminary results with the combination of a 20 h 90% CO₂ shock treatment, followed by reduced intensity cold treatment, as a potential disinfestation treatment for Medfly larvae in citrus fruit.

Whereas a major shortcoming of most potential disinfestation treatments tested is that insecticidal efficacy and fruit damage are in most cases closely associated, this combination treatment may actually combine increased insecticidal efficacy with a reduction in the sensitivity of fruit to cold damage.

If such a pre-cold, CO₂ shock treatment, were to have a potentiating effect on the efficacy of subsequent cold treatment, it may be possible to reduce the severity of the cold treatment component (reduced duration or increased temperature) and still maintain the requisite Probit 9 level of efficacy.

Objectives

1. To better understand the physiology behind FCM's susceptibility to cold and shock treatments in order to design more effective cold treatments.
2. To determine whether CO₂ shock treatments can increase cold-susceptibility of FCM larvae and allow for shorter cold treatments than the 22 or 24 d currently required.

Materials and methods

Physiological assays 2009-2012 (SU)

Firstly, the low temperature tolerance of FCM was investigated using standard thermal physiology assays. The critical thermal minimum (CT_{min}, lowest temperature at which larvae are active), supercooling point (SCP, the temperature at which the larva freezes at) and the lower lethal temperature were determined. In addition, plasticity of the SCP was investigated using different ramp rates and inoculation with water and orange juice.

Secondly, using a recently developed real-time technique, thermolimit respirometry (Lighton and Turner 2004), we simultaneously assessed metabolic rate, activity, critical thermal minima (CT_{min}) and survival under cooling conditions in normal atmospheric gases and different O₂ concentrations in FCM larvae (following e.g. Klok et al. 2004). This served to better comprehend the conditions which elicit FCM spiracle opening while maintaining metabolic rate and allowing activity, and is critical to determining the mode of action of gas treatments as a form of post-harvest control.

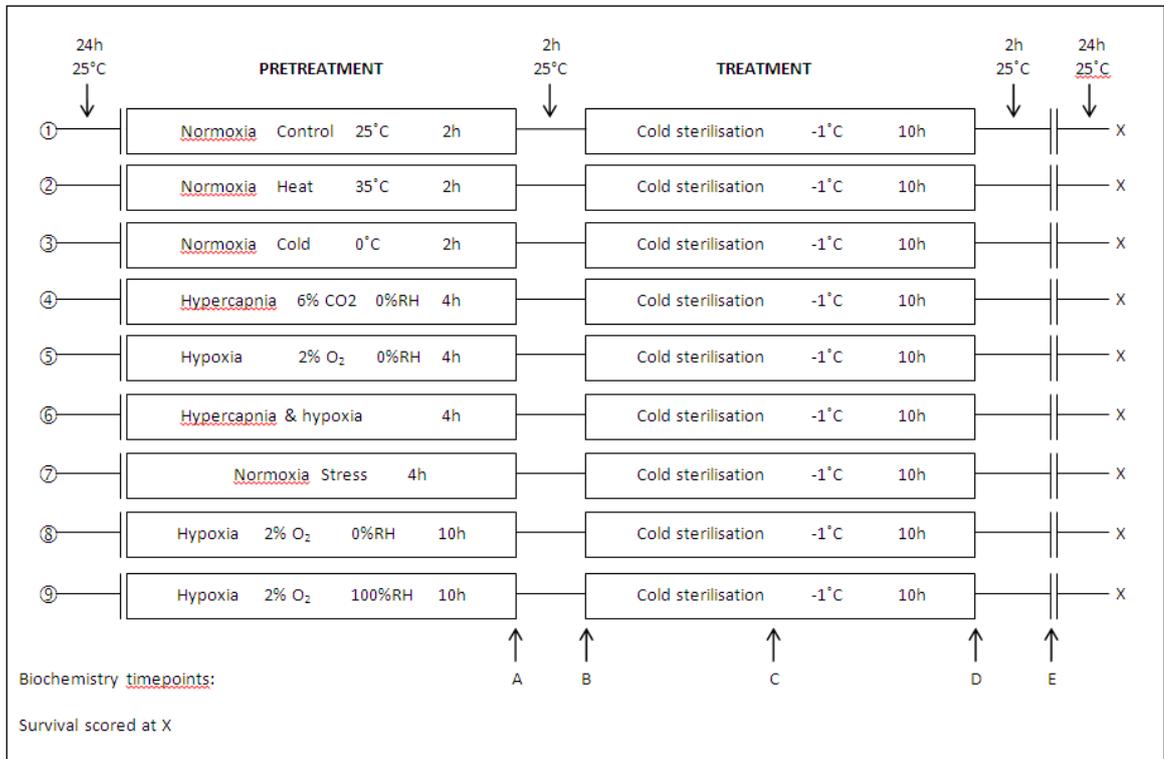
Thirdly, given that the water balance physiology of the FCM larvae appears to be an integral part of understanding the underlying physiology, we ran a set of baseline experiments that investigated water loss rates, time to death under either starvation or desiccation, and plasticity of thermal tolerance in order to better characterise the study organisms' thermal biology in relation to fluctuating stress.

Lastly, 18 experimental regimes consisting of controlled atmospheres (O₂ and or CO₂) and temperature treatments prior to a low temperature exposure at -1°C were conducted (Fig. 3.2.5.1, referred to as: original 9 experiments, additional CO₂ and cold combination experiments, and additional longer-term experiments). The original 9 experiments consisted of a treatment (including a handling control, a stress control (not thermal nor gas stress), temperature or gas exposures), all followed by 2 hours of recovery at 25°C, a 10h cold exposure at -1°C, 2 hours of recovery at 25°C and 24 hours of recovery at 25°C. The original 9 treatments were: 1) Control: 2h at 25°C, 2) Stress: Shaken for 4h at 25°C, 3) Cold: 2h at 0°C, 4) Heat: 2h at 35°C, 5) Hypercapnia: 6% CO₂ for 4h at 25°C, 6) Hypoxia: 2% O₂ for 4h at 25°C, 7) Hypoxia and Hypercapnia: 2% O₂ and 6% CO₂ for 4h at 25°C, 8) Hypoxia dry: 2% O₂ for 10h at 25°C with silica gel (<10% RH), 9) Hypoxia wet: 2% O₂ for 10h at 25°C with dH₂O soaked cotton wool (>80% RH). After these experiments were completed, the ordering/combinations of the CO₂ and cold pre-treatments was investigated, as well as six additional treatments that were identified as high priorities for future work: 1) 24 hours of 6% CO₂ at 4°C, followed by 25°C at normal atmospheres for 24 h, and then cold sterilisation of -1°C for 5 days; 2) 24 h of 6% CO₂ at 4°C, followed by -1°C for 5 days; 3) 24 h of 6% CO₂ 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; 4) 24 h of 6% CO₂ at 4°C, followed by 25°C for 3 days, followed by cold sterilisation of -1°C for 5 days; 5) 24 h of 6% CO₂ at 4°C combined with shaking or vibration stress, followed by 25°C for 24 h, followed by cold sterilisation of -1°C for 5 days; 6) 35°C for 4 h followed by 25°C for 1 day, followed by cold sterilisation of -1°C for 5 days.

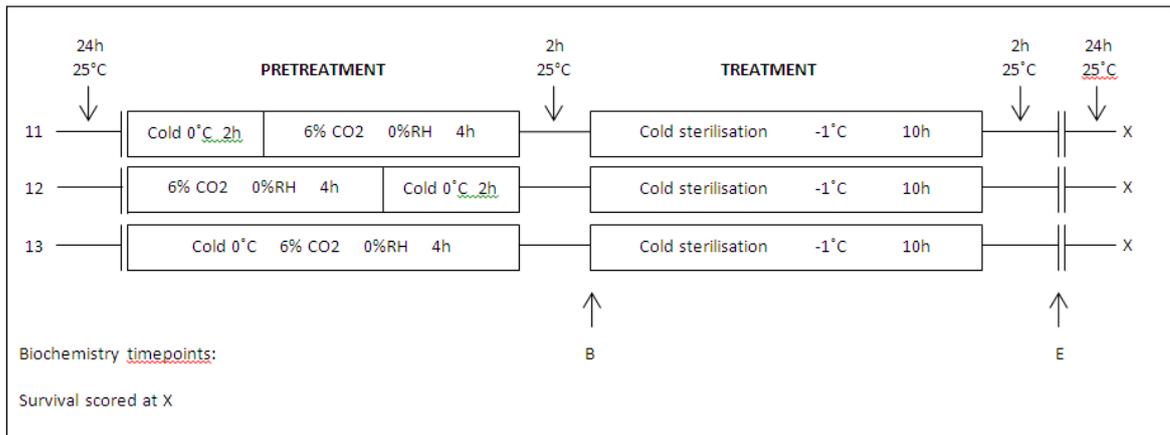
The whole animal responses and biochemistry of individual larvae was assessed at several time points. Measured variables included:

- Larval survival, pupation and emergence rates
- Gravimetric determination of body water and body lipid content
- Haemocyte, fat tissue and silk gland tissue viability determined using fluorescent microscopy and LIVE/DEAD sperm viability kit, Invitrogen, Molecular Probes Inc. (Yi and Lee 2003) to determine compromised cell function following pre-treatments
- Total protein concentration and heat shock protein 70 (HSP70) was determined using BCA assay, Western blotting and ELISA (e.g. Karl et al., 2009) to determine if common stress pathways are responsible for FCM mortality responses to typical phytosanitary treatments
- Cryoprotective sugars and polyols were measured using gas chromatograph mass spectrometer (GC-MS) to identify the cryoprotective polyols used by FCM, and quantify the changes in cryoprotectant concentration and membrane phospholipid fatty acid composition in response to the various stressors
- Membrane fatty acid composition was determined using GC-MS as an indicator for membrane fluidity

Original 9 experiments



Additional 3 treatments:
CO₂ and cold combinations



Additional 6 treatments:
Longer-term

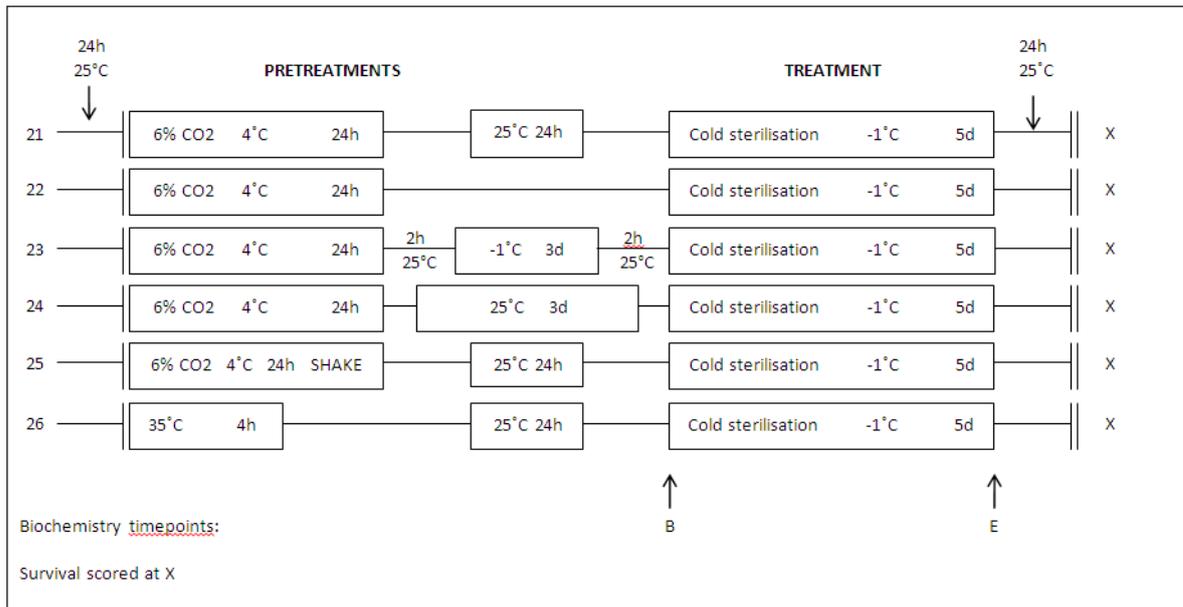


Figure 3.2.5.1. Details of the 18 experimental regimes that have been completed.

Infested fruit research 2009-10 (CRI)

In order to work in parallel with the physiological assays, fruit infested with late (4th or 5th) instar larvae were exposed at 25°C to 6% CO₂ for 4 h, 2% O₂ for 4 h or a mixture of the two gases for 4 h. Fumigation took place in custom-made gas-proof boxes that allowed for approximately 10 navel oranges each time. Fruit was infested artificially by placing pieces of wax paper with 50 eggs on each fruit, then allowing the larvae to penetrate the fruit and develop to the correct stage before treatment started. With this number of eggs we were hoping for around 10 larvae per fruit. However, the low oxygen or no oxygen treatments resulted in the larvae leaving the fruit and these mixtures would be impractical or expensive to implement on a large scale. Further research was delayed as no navel oranges (thought to be preferred by FCM) were available.

Further infested fruit research 2010-13 (CRI)

After a meeting with Paul Cronjè to discuss possible uses of CO₂ to reduce chilling injury we decided to work with increased concentrations of CO₂ in air using larger containers (280 L) than previously. This required the use of a gas analyser to measure concentrations of 10 to 60% in a chamber as could be implemented in a degreening room at a packhouse. Almost an entire season of research was lost while trying to acquire a reliable analyser capable of measuring such high CO₂ levels. Once the apparatus was available the approach to the research was to try progressively longer fumigation periods (6 h, 12 h and 24 h) with increasing concentrations of CO₂ (10, 30 and 60%). The temperature during fumigation was kept close to ambient (22-25°C). Fruit were infested by placing a square of an egg-sheet with 40 to 50 eggs on each fruit in a ventilated container and allowing the FCM larvae to hatch and penetrate the fruit naturally. Even though fruit were washed with post-harvest fungicides, many fruit were lost to fungal infections so higher numbers of fruit needed to be infested in order to have sufficient fruit to fumigate when larvae had at least developed to the third instar. After fumigation, fruit were sometimes dipped in a fruit wax suspension after being left to stand for up to an hour. Some fruit were then moved to a cold room at -0.5°C for 5 days. Infested, non-fumigated fruit were used for a control, half of these were cold treated and half were held at ambient for 24 h after the other fruit went into the cold room, before cutting and evaluating mortality. After the cold treatment, the fruit were removed to ambient temperature for 24 h before being cut and larval mortality determined.

In Experiment 1, egg sheet squares were placed on 20 navel oranges per treatment on 22 July 2011 and held at 25°C until 4 August when they were fumigated in the larger fumigation chambers at 25°C with either 10% or 30% CO₂ for 6 h. This was followed immediately by a cold treatment at -0.5°C for 5 days without waxing. Control fruit that were not cold treated were cut on 5 August and larval mortality determined. The other fruit that received a cold treatment was moved to 25°C for a day after the cold treatment was complete so that the larvae could return to 25°C before evaluation of mortality on 10 August 2011.

In Experiment 2 the same method was used as for Experiment 1. Egg sheet squares were placed on navel oranges on 12 August 2011, fruit were fumigated with either 30 or 60% CO₂ for 12 h at 25°C on 25 August. After fumigation, fruit were dipped in a suspension of fruit wax within 20 min of being removed from the gas. The fruit were then moved directly to the cold room. Fruit that did not have a cold treatment were dissected on 26 August and cold-treated fruit were dissected on 31 August after being warmed up to room temperature.

By this time of the year there were no fresh navel oranges available and there was a high degree of waste when using old navel oranges that had been in cold storage, so a small experiment (3) was conducted to see whether Valencia oranges would also be suitable for FCM. Squares from FCM egg sheets were placed on 10 navel oranges and 10 Valencias on 16 September 2011 and the fruit stored at 25°C to compare larval development. On 27 September the fruit were dissected and the larvae counted and measured.

After the results of Experiment 3 showed that Valencia oranges were also suitable for this research a further experiment (4) was conducted along the same lines as Experiments 1 and 2. Fruit were infested on 13 October 2011, stored at 25°C then fumigated on 28 October with either 30% CO₂ or 60% CO₂ for 12 h. After fumigation, all fruit were waxed by dipping, then some were held for 24 h at 25°C before going to the cold treatment at -0.5°C for 5 d while other fruit went immediately from waxing to the cold treatment. The controls were evaluated on 30 October and the treatments without a gap and with a gap between fumigation and cold treatment were evaluated on 4 and 5 November, respectively. This was the last experiment that could be conducted in 2011.

In 2012 *ad hoc* cold treatment research on fruit flies had to be conducted for Japan and this reduced the amount of labour available for this research. Only three trials were conducted with CO₂ and FCM in 2012 and these focused more on determining what the maximum mortality was that could be expected with 60% CO₂ alone after 24 h fumigation, before using this combination with short cold treatments.

Experiment 5 was conducted with FCM in Shamouti oranges. Fruit were infested on 13 July 2012 and incubated at 25°C for 10 days before being fumigated on 23 July. Fumigation was at 60% CO₂ for 24 h at 25°C with some fruit then being cold treated at -0.5°C for 5 d. The fruit were not waxed before cold treatment. The fruit that were not cold treated were evaluated on 25 July and those that were cold treated were evaluated on 30 July.

Experiments 6 and 7 were conducted on FCM eggs to determine what percentage of them would hatch if exposed to 60% CO₂ for 24 h. Fruit were not used in these experiments but squares of egg sheet (10 mm x 10 mm) were placed in the lid from a small petri dish (38 mm diameter) which was placed on a coating of polybutene in the base of a larger petri dish (90 mm diameter). Ten dishes were used per treatment. The dishes were left open for fumigation on 25 July and 29 August and after 24 h fumigation at 25°C they were held for a further 7 d at 25°C to allow all the eggs to hatch. The numbers of larvae hatched and caught in the polybutene were then determined.

Results and discussion

Low temperature tolerance

The low temperature tolerance of *T. leucotreta* larvae has been published as “False codling moth *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera, Tortricidae) larvae are chill susceptible” in *Insect Science* (Boardman et al., 2012). The published abstract can be found below, with two key figures from the paper (Fig. 3.2.5.2 and 3.2.5.3).

This study reports on the low temperature tolerance and cold hardiness of larvae of false codling moth, *Thaumatotibia leucotreta*. We found that larvae have mean critical thermal minima (lower limits of activity) of 6.7°C which was influenced by feeding status. The effects of low temperature exposure and duration of exposure on larval survival were assessed and showed that the temperature at which 50% of the population survives is $-11.5 \pm 0.3^\circ\text{C}$ after 2 h exposure. The supercooling point (SCP, i.e., freezing temperature) was investigated using a range of cooling rates and under different conditions (feeding and hydration status) and using inoculative freezing treatments (in contact with water or orange juice). The SCP decreased significantly from -15.6°C to -17.4°C after larvae were fasted for 24 h. Twenty-four hour treatments at either high or low relative humidity (95.9% or 2.4%) also significantly decreased SCP to -17.2°C and -18.2°C respectively. Inoculative freezing (by water contact) raised SCP from -15.6°C to -6.8°C which could have important implications for post-harvest sterilization. Cooling rates did not affect SCP which suggests that there is limited phenotypic plasticity of SCP during the larval life-stage, at least over the short time-scales investigated here. In conclusion, larvae of *T. leucotreta* are chill-susceptible and die upon freezing. These results are important in understanding this pest's response to temperature variation, understanding pest risk status and improving post-harvest sterilization efficacy.

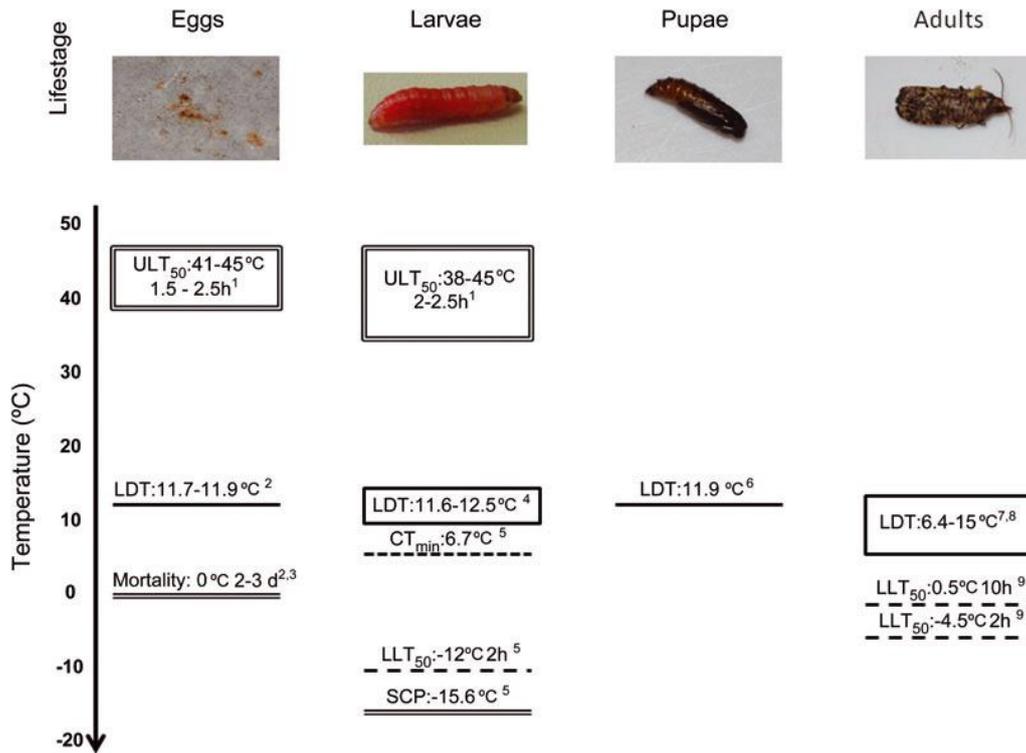


Figure 3.2.5.2. Temperature tolerance of *T. leucotreta* for each of the life-stages taken from published studies. Gaps in information occur where the figure is left blank. Sources are: ¹Johnson and Neven, 2010; ²Daiber, 1979a; ³Blomefield, 1978; ⁴Daiber, 1979b; ⁵This study; ⁶Daiber, 1979c; ⁷Daiber, 1975; ⁸Daiber, 1980; ⁹Stotter and Terblanche, 2009. ULT₅₀, upper lethal temperature (that results in 50% mortality); LLT₅₀, lower lethal temperature (that results in 50% mortality); LDT, lower developmental threshold; CT_{min}, critical thermal minima; SCP, supercooling point.

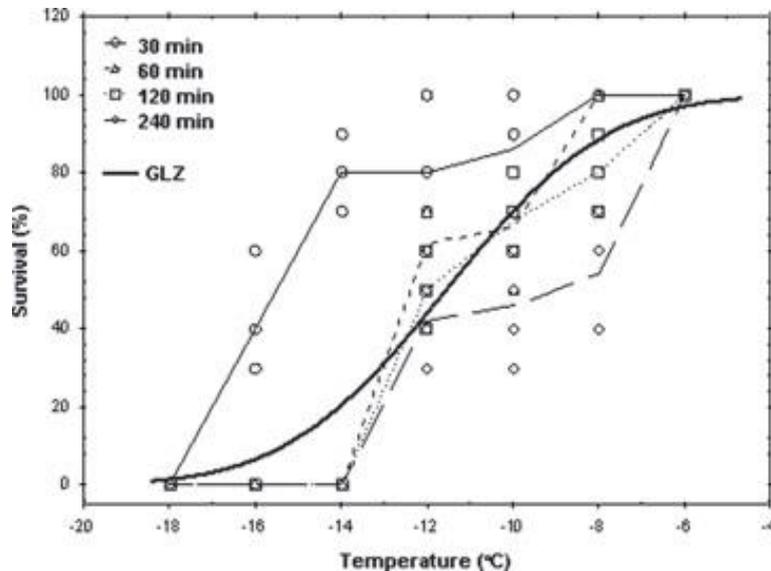


Figure 3.2.5.3. Survival (%) of *T. leucotreta* under different low temperatures during different durations of exposure. Each data point represents a single replicate ($n = 10$), while lines indicate mean survival for each duration. GLZ = generalized linear model fit.

Chill coma recovery time (CCRT) after 2 h at 0°C was 618 ± 63 s. In addition, we found that CT_{min} and CCRT are affected by developmental acclimations ($H_{2,80} = 56,9411$; $P < 0.001$; $H_{2,86} = 8,0384$; $P = 0.018$). Therefore, CT_{min} and CCRT are plastic responses. No rapid cold hardening response has been detected, although this result needs additional verification.

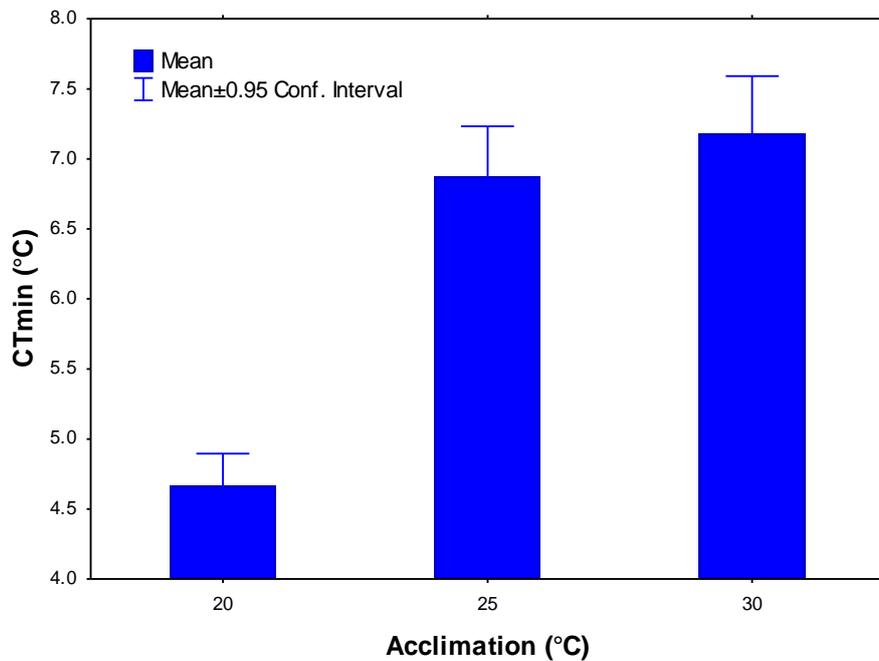


Figure 3.2.5.2. Mean CTmin (\pm 95% CI) after developmental acclimation at one of three temperatures. Larvae acclimated at 20°C have a significantly lower CTmin ($P < 0.0001$).

Thermolimit respirometry

We have completed thermolimit respirometry (TLR) under 4 different gas conditions (2.5%, 10%, 21%, 40% O₂) with a minimum of 10 successful TLR runs per gas. Data extraction and analysis is ongoing, but preliminary results suggest that there is no difference between mean, maximum or minimum VCO₂ (i.e. metabolic rate) under the varying gas conditions ($P > 0.05$). These initial results indicate that the change in O₂ does not result in significant changes in metabolism, nor a detectable switch to anaerobic metabolism at low O₂. 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.

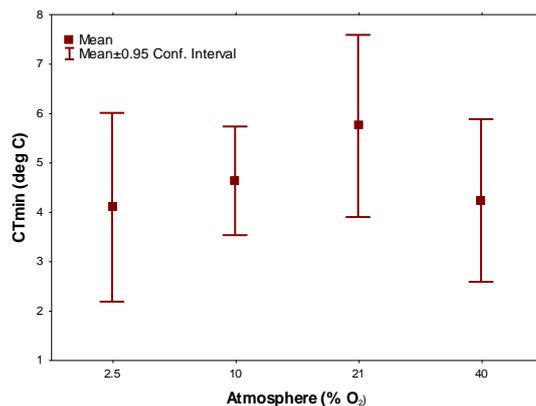


Figure 3.2.5.3. Mean CTmin measured using thermolimit respirometry (TLR) was not affected by altered atmosphere ($P > 0.38$).

Water loss rates and time to death experiments

Time to death experiments under either starvation or starvation with desiccation showed that at low relative humidity, larvae survive at least 10 days without access to food. Larvae survive longer without added desiccation ($Z = 2.535$, $P < 0.05$) – median of 12.5 days ($n = 16$; Min: 9, Max: 25) vs. with desiccation: Median = 10 days ($n = 14$; Min: 5, Max: 18)). At death, larvae have lost approx. 50% of their initial body mass.

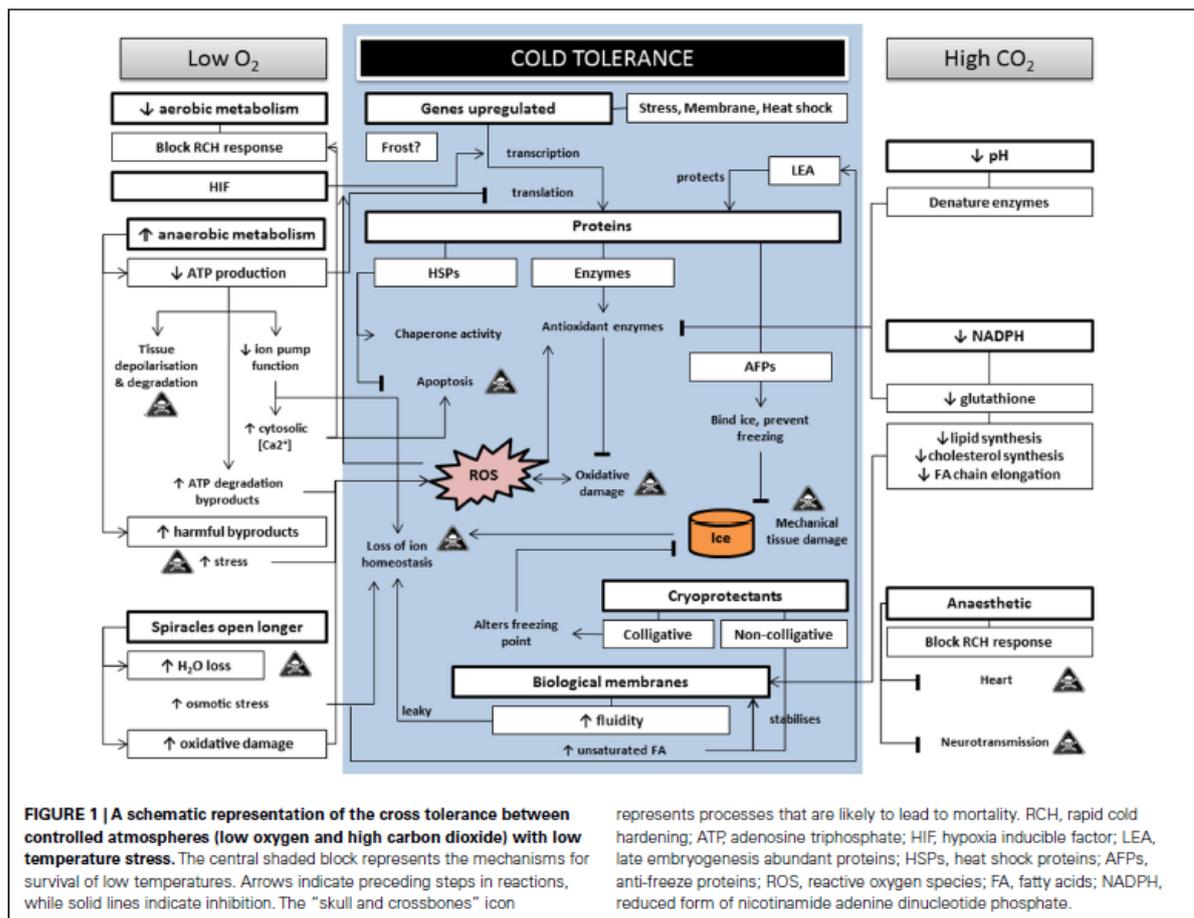
Cuticular and respiratory water losses (CWL & RWL) were calculated from carbon dioxide and evaporative water loss data obtained from larvae in a gas analyser at 15°C and 25°C. At 15°C, mean V_{H_2O} = 0.77 mg/h (CWL = 0.57 mg/h; RWL = 0.20 mg/h) while at 25°C, mean V_{H_2O} = 0.84 mg/h (CWL = 0.66 mg/h; RWL = 0.18 mg/h). Therefore, unless larvae were actively regulating water loss, larvae would lose 18-20 mg H_2O per day and would die within 3 days at these temperatures.

These data were used in Boardman et al., under revision, *Journal of Insect Physiology*.

Controlled atmospheres

A literature review of the theoretical overlap between controlled atmospheres and low temperatures was conducted and published in *Frontiers in Physiology* as “Interactions between controlled atmospheres and low temperature tolerance: A review of biochemical mechanisms” (Boardman et al., 2011). The published abstract can be found below with the summary figure from the paper.

Controlled atmosphere treatments using carbon dioxide, oxygen and/or nitrogen, together with controlled temperature and humidity, form an important method for postharvest sterilization against insect-infested fruit. However, in insects, the cross tolerance and biochemical interactions between the various stresses of modified gas conditions and low temperature may either elicit or block standard stress responses which can potentiate (or limit) lethal low temperature exposure. Thus, the success of such treatments is sometimes erratic and does not always result in the desired pest mortality. This review focuses on the biochemical modes of action whereby controlled atmospheres affect insects’ low temperature tolerance, making them more (or occasionally, less) susceptible to cold sterilization. Insights into the integrated biochemical modes of action may be used together with the pest’s low temperature tolerance physiology to determine which treatments may be of value in postharvest sterilization.



We have completed all proposed 18 experimental regimes (see Figure 3.2.5.1, referred to as: original 9 experiments, additional CO_2 and cold combination experiments, and additional longer-term experiments). All of the assays have been completed and the data extraction and analysis are in various stages of completion.

Original 9 experiments

The results of the initial 9 experiments have been analysed and are currently being written up for submission. For the results presented, the pretreatments are abbreviated as: C – handling control, SC – stress control, LT – low temperature, HT – high temperature, HC – hypercapnia, HO – hypoxia, HCHO – hypercapnia and hypoxia, HOL – hypoxia (low relative humidity), HOH – hypoxia (high relative humidity).

Table 3.2.5.1. Summary of results of generalized linear models, for the effects of experiment and timepoint on all the measured variables. Analyses were run independently. Significant effects are highlighted in bold font.

Variable	Effect	DF	Wald χ^2	P-value
<i>Survival</i>				
	Experiment	9	2734.69	< 0.0001
<i>Pupation rate</i>				
	Experiment	9	118.77	< 0.0001
<i>Emergence rate</i>				
	Experiment	9	308.22	< 0.0001
<i>Haemolymph viability</i>				
	Experiment	8	51.32	< 0.0001
	Timepoint	1	0.03	0.86
	Experiment*Timepoint	8	33.41	< 0.0001
<i>Fat tissue viability †</i>				
	Experiment	9	172.64	< 0.0001
	Timepoint	1	4.27	0.04
	Experiment*Timepoint	8	10.44	0.24
<i>Silk gland tissue viability †</i>				
	Experiment	9	110.74	< 0.0001
	Timepoint	1	2.04	0.15
	Experiment*Timepoint	8	13.66	0.09
<i>Body water content (mg, as percentage of start mass)</i>				
	Experiment	8	808.16	< 0.0001
	Timepoint	5	11.78	0.04
	Experiment*Timepoint	40	50.47	0.12
<i>Body lipid content (mg, as percentage of start mass)</i>				
	Experiment	8	278.23	< 0.0001
	Timepoint	5	20.87	< 0.001
	Experiment*Timepoint	40	101.94	< 0.001
<i>Membrane phospholipid composition (UFA:SFA)</i>				
	Experiment	8	13.84	0.09
	Timepoint	2	126.89	< 0.0001
	Experiment*Timepoint	16	27.30	0.04
<i>Average fatty acid chain length</i>				
	Experiment	8	5.19	0.74
	Timepoint	2	6.47	0.04
	Experiment*Timepoint	16	22.08	0.14
<i>Total protein concentration</i>				
	Experiment	8	91.67	< 0.001

	Timepoint		5	38.50	< 0.001	
	Experiment*Timepoint		40	54.10	0.07	
<i>HSP70 expression (relative absorbance)</i>						
	Experiment		8	182.87	< 0.001	
	Timepoint		5	10.84	0.05	
	Experiment*Timepoint		40	30.92	0.85	
<i>Fructose</i>						
	Experiment		8	2.54	0.96	
	Timepoint		2	0.13	0.94	
	Experiment*Timepoint		16	9.69	0.88	
<i>Glucose</i>						
	Experiment		8	5.30	0.73	
	Timepoint		2	13.68	0.001	
	Experiment*Timepoint		16	13.99	0.60	
<i>Maltose</i>						
	Experiment		8	13.83	0.09	
	Timepoint		2	41.09	< 0.0001	
	Experiment*Timepoint		16	7.74	0.96	
<i>Sorbitol</i>						
	Experiment		8	8.80	0.36	
	Timepoint		2	4.46	0.11	
	Experiment*Timepoint		16	12.70	0.69	
<i>Trehalose</i>						
	Experiment		8	2.33	0.97	
	Timepoint		2	7.74	0.02	
	Experiment*Timepoint		16	14.23	0.58	

† Chi-squared values presented

Table 3.2.5.2. A summary of significant differences found relative to the handling control. Arrows indicate the direction of a significant effect. Where no arrow is given, results were statistically indistinguishable from the control.

Timepoint	SC	LT	HT	HC	HO	HCHO	HOL	HOH
Survival								
tX		↑	↓	↑	↑	↑		↑
Haemocyte viability								
tB							↓	
tX								↓
Body water content								
tA		↑	↓	↓	↓	↓	↓	
tB		↑	↓	↓	↓	↓	↓	↓
tC		↑	↓	↓	↓	↓	↓	↓
tD		↑	↓	↓	↓	↓	↓	↓
tE	↓	↑	↓	↓	↓	↓	↓	↓
tX	↓	↑	↓	↓	↓	↓	↓	↓
Body lipid content								
tA		↓		↑	↑	↑		
tB					↑	↑		
tC		↓						
tD		↓					↑	
tE	↑		↑		↑	↑	↑	↑
tX		↓	↑	↑	↑	↑	↑	↑
UFA:SFA								
tB		↑					↑	
tE								
tX								
Fatty acid chain length								
tB		↓						
tE								
tX	↑	↑	↑		↑	↑	↑	↑
Total protein content								
tA	↓				↓	↓		↓
tB	↓			↓				↓
tC					↓			
tD	↓			↓	↓	↓	↓	↓
tE	↓				↓			
tX					↓	↓	↓	↓
HSP70								
tA	↓			↓	↓	↓		↓
tB	↓	↑		↓		↓		
tC	↓				↓	↓		
tD					↓	↓		
tE	↓	↑			↓	↓	↓	
tX		↑	↓		↓	↓		
Glucose								
tB				↓				
tE								
tX								
Maltose								
tB								
tE								
tX				↓				
Sorbitol								
tB	↓	↓	↓	↓	↓			↓
tE								
tX								

C – handling control, SC – stress control, LT – low temperature, HT – high temperature, HC – hypercapnia, HO – hypoxia, HCHO – hypercapnia and hypoxia, HOL – hypoxia (low relative humidity), HOH – hypoxia (high relative humidity).

NS differences for pupation rates, emergence rates, silk gland viability, tissue viability, fructose or trehalose

Table 3.2.5.3. Survival, pupation and emergence rates after 9 treatments. Sample sizes (n) are given in brackets. Survival, pupation and emergence rates were all significantly affected by treatment (GLZ: $\chi^2 = 2734.69$, $\chi^2 = 118.77$ and $\chi^2 = 308.22$ respectively, $df = 9$, $P < 0.0001$ in all cases).

Treatment	Survival % (n)	Pupation % (n)	Emergence % (n)
C	97.1 (280)	74.3 (74)	90.9 (55)
SC	97.9 (190) *	66.7 (84)	91.1 (56)
LT	98.9 (182) *	82.1 (78)	89.1 (64)
HT	95.7 (187) *†	79.2 (72)	80.7 (57)
HC	99.5 (200) *	67.5 (83)	92.9 (56)
HO	99.5 (196) *	78.6 (84)	84.8 (66)
HCHO	99.0 (203) *	68.2 (85)	82.8 (58)
HOL	98.0 (201) ‡	63.2 (95) ‡	90.0 (60)
HOH	99.5 (196) *	72.9 (85)	96.8 (62) ‡

C – handling control, SC – stress control, LT – low temperature, HT – high temperature, HC – hypercapnia, HO – hypoxia, HCHO – hypercapnia and hypoxia, HOL – hypoxia (low relative humidity), HOH – hypoxia (high relative humidity). * denotes groups that were significantly different from C. † denotes where HT differed from LT; ‡ denotes where controlled atmosphere treatments differed from HO.

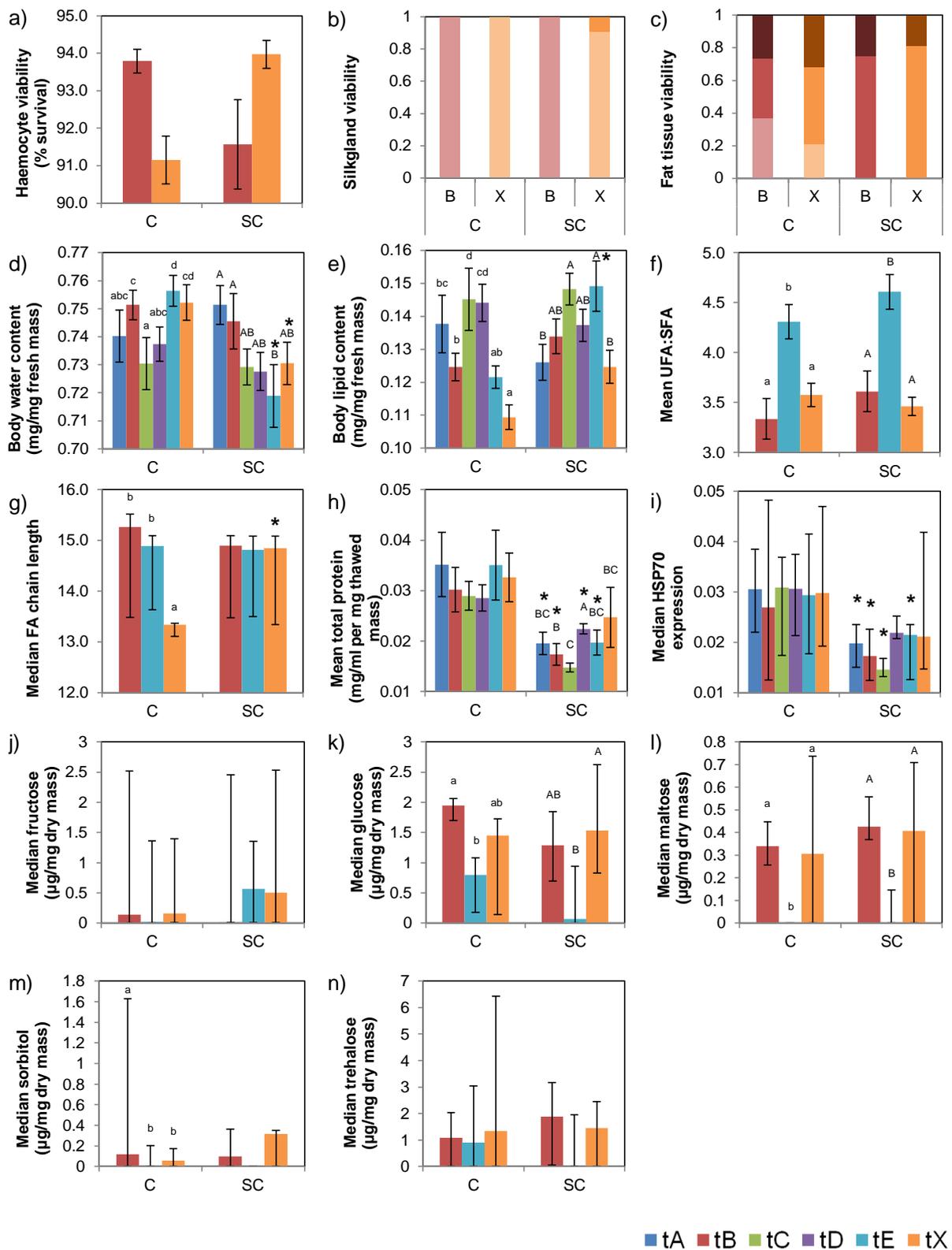


Figure 3.2.5.4. Handling control (C) and stress control (SC) results from a) body water content, b) body lipid content, c) total protein concentration, d) HSP70 expression, e) UFA:SFA, f) fatty acid chain length, g) haemocyte viability, h) silk gland viability, i) fat tissue viability, j-k) cryoprotectant concentrations. * denotes a timepoint that was significantly different from the same timepoint in experiment C. Significant differences between timepoints within each experiment are indicated by different letters (small letters, C and capital letters, SC).

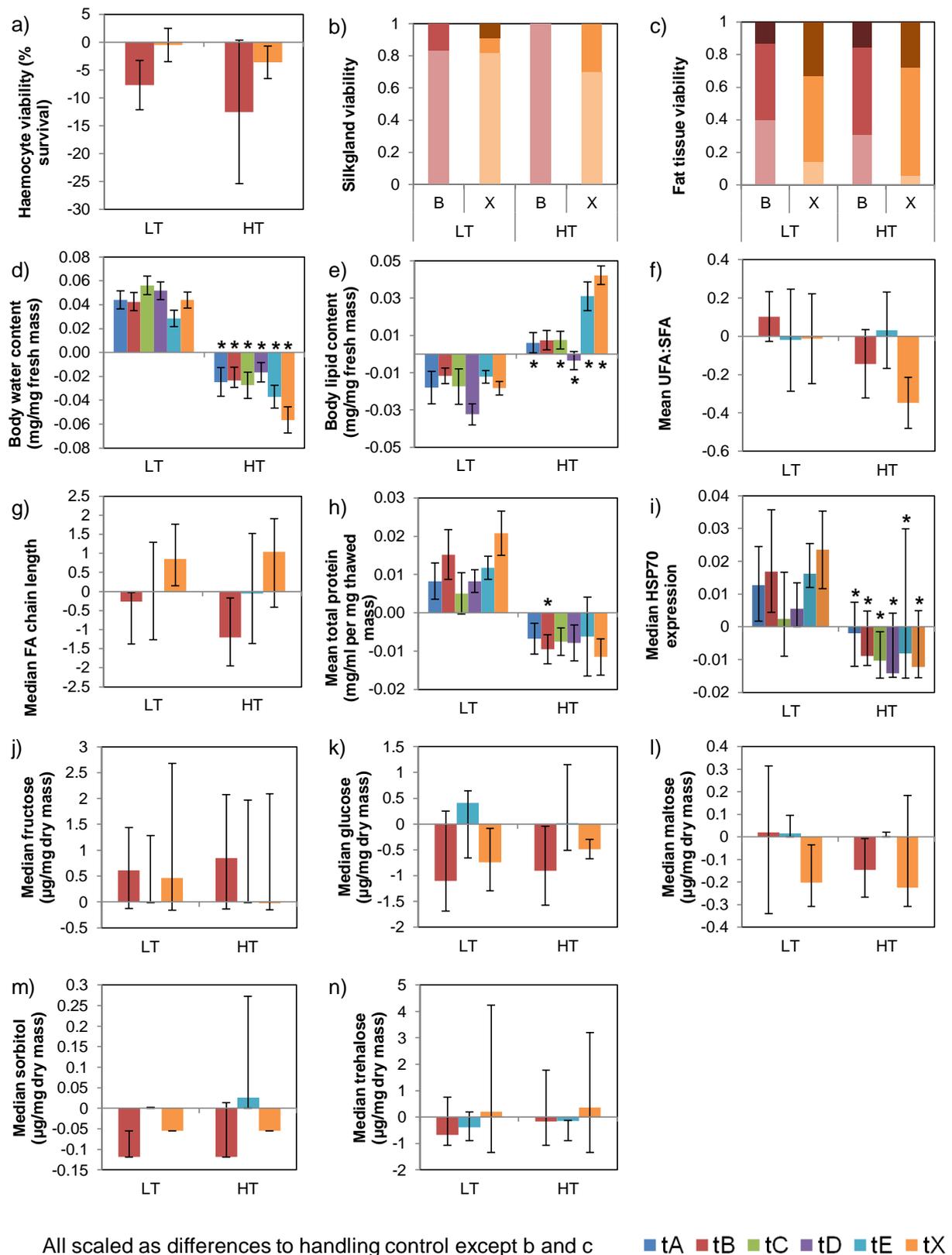
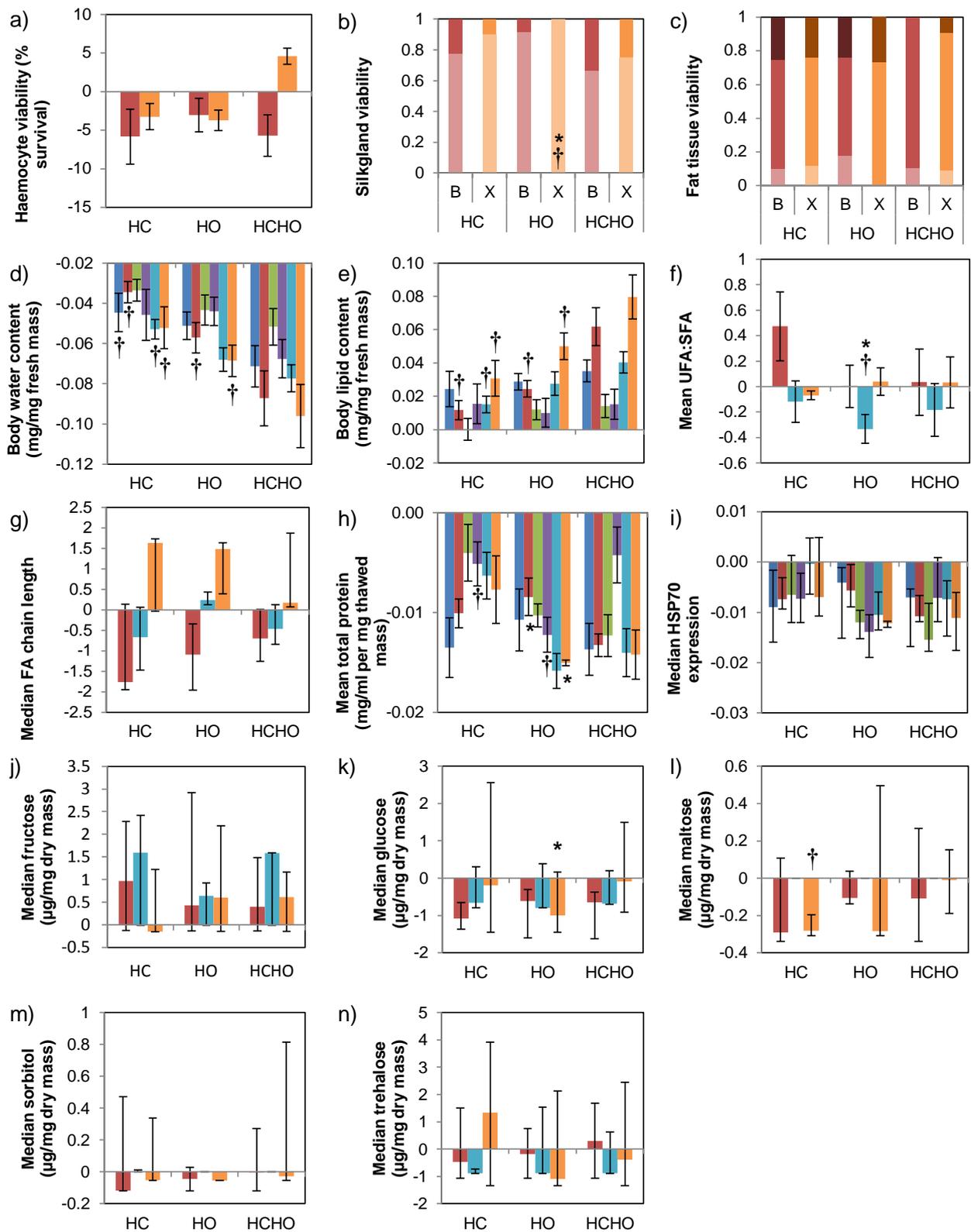


Figure 3.2.5.5. Low temperature (LT) and high temperature (HT) results scaled as relative differences to the handling control for a) body water content, b) body lipid content, c) total protein concentration, d) HSP70 expression, e) UFA:SFA, f) fatty acid chain length, g) haemocyte viability, h) silk gland viability, i) fat tissue viability, j-k) cryoprotectant concentrations.* denotes a timepoint in HT that was significantly different from same timepoint in LT.



All scaled as differences to handling control except b and c

■ tA ■ tB ■ tC ■ tD ■ tE ■ tF

Figure 3.2.5.6. Controlled atmosphere results after hypercapnia (HC), hypoxia (HO) or both hypercapnia and hypoxia (HCHO) scaled as relative differences to the handling control for a) body water content, b) body lipid content, c) total protein concentration, d) HSP70 expression, e) UFA:SFA, f) fatty acid chain length, g) haemocyte viability, h) silk gland viability, i) fat tissue viability, j-k) cryoprotectant concentrations. * denotes a timepoint in HO that was significantly different from same timepoint in HC. † denotes a timepoint in HC or HO that was significantly different from same timepoint in HCHO.

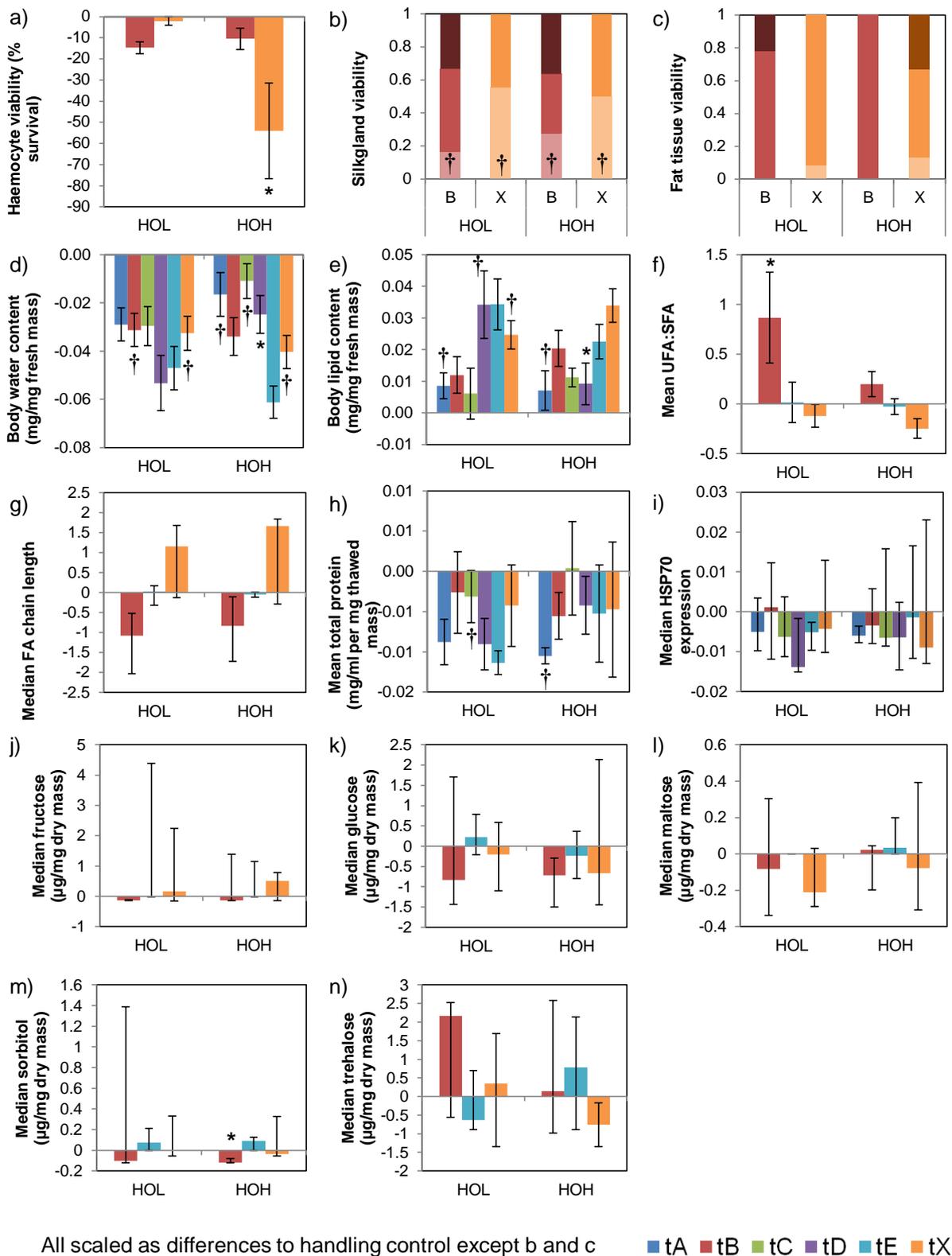


Figure 3.2.5.7. Results from longer term hypoxia treatments at low (HOL) or high (HOH) relative humidities scaled as relative differences to the handling control for a) body water content, b) body lipid content, c) total protein concentration, d) HSP70 expression, e) UFA:SFA, f) fatty acid chain length, g) haemocyte viability, h) silk gland viability, i) fat tissue viability, j-k) cryoprotectant concentrations. * denotes a timepoint in HOH that was significantly different from same timepoint in HOL. † denotes a timepoint in HOL or HOH that was significantly different from same timepoint in HO.

Additional CO₂ and cold combination

The results for these experiments are in the process of being analysed.

Table 3.2.5.4. Survival, pupation and emergence rates after additional CO₂ and cold combination pre-treatments. Sample sizes (n) are given in brackets.

Experiment	Treatment	Survival % (n)	Pupation % (n)	Emergence % (n)
11	Cold then CO ₂	100 (93)	60.9 (64)	87.2 (39)
12	CO ₂ then cold	99.2 (121) *	75.7 (111)	91.7 (84)
13	Combination	100 (119)	77.8 (90)	87.1 (70)

* denotes groups that were significantly different from control (2 h at 25°C)

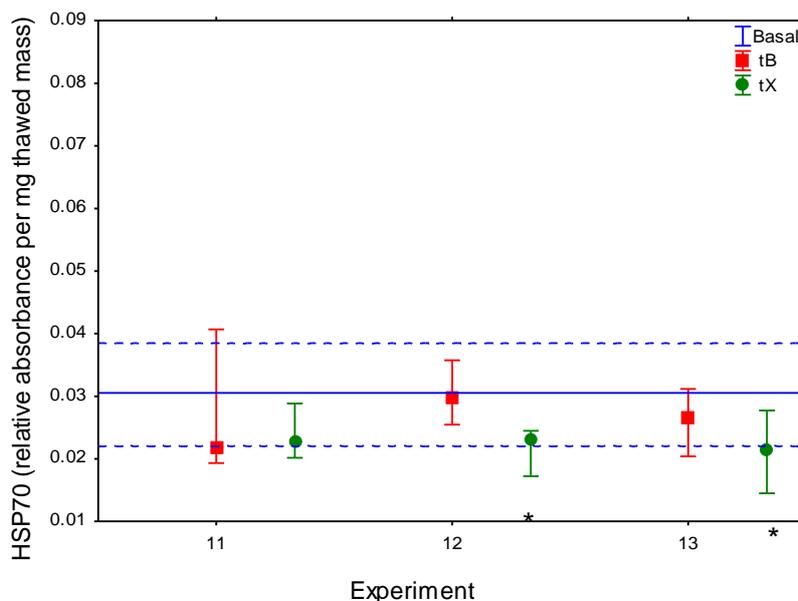


Figure 3.2.5.8. Median HSP70 (with non-outlier ranges) at tB and tX after CO₂ and cold combination pre-treatments. Blue lines indicate the basal (i.e. control) HSP70 amount. * indicate where results differed from basal amount

Preliminary results indicate that the order of these treatments did not matter - survival, pupation and emergence rates were not significantly different from one another, and all resulted in high larval survival. HSP70 was significantly lower than basal amounts at tX after CO₂ then cold, and the combination.

Additional longer term experiments

The results for these experiments are in the process of being analysed.

Table 3.2.5.5. Survival, pupation and emergence rates after the additional longer term experiments. Sample sizes (n) are given in brackets.

Treatment	Survival % (n)	Pupation % (n)	Emergence % (n)
1	35.2 (88)	42.1 (19)	25.0 (8)
2	56.3 (87)	25.0 (36)	44.4 (9)
3	0 (82)		
4	78.8 (85)	46.4 (56)	30.8 (26)
5	77.7 (94)	38.3 (60)	65.2 (23)
6	80.9 (94)	42.2 (64)	77.8 (27)

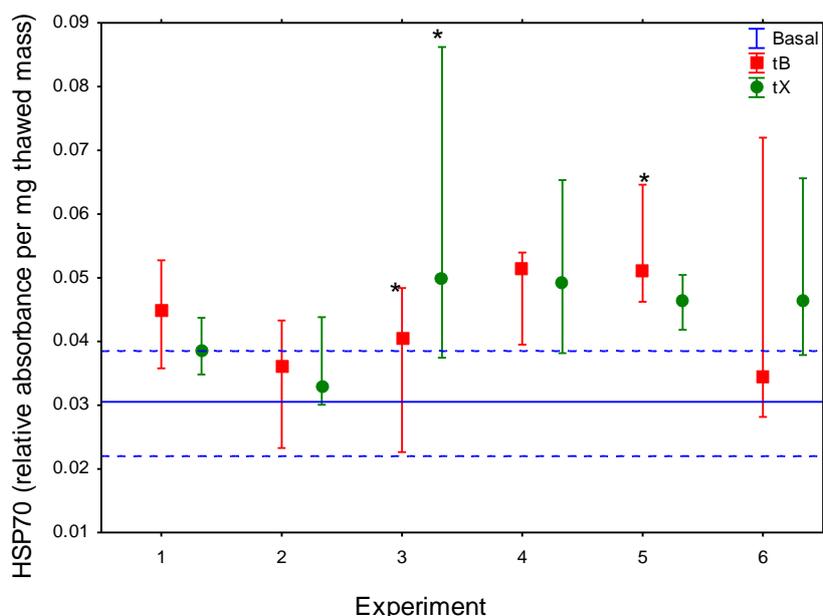


Figure 3.2.5.9. Median HSP70 (with non-outlier ranges) at tB and tX after the additional longer term experiments. Blue lines indicate the basal (i.e. control) HSP70 amount. * indicate where results differed from basal amount. tB and tX of experiment 23 are also significantly different from one another.

All the longer term experiments reduced survival, pupation and emergence rates. Additional treatment 3 resulted in 100% mortality, although the mechanism is currently unclear. The increased HSP70 at tX could be detrimental to the insect, and may result in upregulation of apoptotic pathways resulting in programmed cell death.

Infested fruit research (CRI)

When investigating the combination of CO₂ fumigation and cold treatments at -0.5°C for 5 days the effect of the cold alone on FCM larvae inside oranges varied tremendously from 40% mortality (Table 3.2.5.9) to 100% mortality (Table 3.2.5.10). Further experiments will have to be done with a less severe cold treatment in order to detect changes in mortality due to fumigation. CO₂ at 30% for 12 h had a negligible effect on FCM larval mortality (Tables 3.2.5.7 and 3.2.5.9) but when this amount of CO₂ for 6 h was immediately followed by a 5 day cold treatment the mortality of FCM larvae was 26.7% compared with 61.4% for the cold only (Table 3.2.5.6), which was significantly different (P<0.05). A similar, but not so dramatic, result was obtained when 30% CO₂ for 12 h was immediately followed by cold treatment with the resultant mortality being 57.3% compared with 70.8% for cold alone (Table 3.2.5.7). However, when the same treatment was repeated a similar mortality of 62.1% was obtained with the CO₂ followed by cold, but this time the cold alone had a lower mortality of 40.4% (Table 3.2.5.9). It therefore seems that 30% CO₂ for only 6 h can make the larvae less susceptible to the following cold treatment, but the 12 h treatment with this dose of CO₂ is more detrimental to the larvae and does not give them much assistance in surviving the cold treatment. When a 1-day gap was introduced between the fumigation with 30% CO₂ for 12 h and the cold treatment, the mortality dropped to 25.3% which was less than the effect of cold only (Table 3.2.5.9) and similar to the effect of a 6 h treatment of the same dosage followed by cold. It therefore appears that the 1 day interval allows the larvae to recover and prepare for the cold after the 12 h treatment to the same extent as the 6 h treatment with no interval between fumigation and cold. This led to the evaluation of 60% CO₂ in further experiments.

The comparison between navel oranges and Valencias as substrates for FCM infestations in the laboratory showed that Valencias could be used in these experiments (Table 3.2.5.8) so this allowed research to be conducted later in the year beyond the navel orange season.

The use of 60% CO₂ for 12 h caused significantly more mortality than 30% CO₂ for 12 h and when the former treatment was followed by a cold treatment it also caused significantly more mortality than the latter treatment followed by cold (Table 3.2.5.9). Having a 1-day interval between the 60% CO₂ treatment and the cold treatment caused a significant drop in mortality to the level obtained with 30% CO₂ followed immediately by cold, but this still caused significantly more mortality than cold alone (Table 3.2.5.9). Fumigating FCM larvae with 60% CO₂ for 24 h did not appear to cause any more mortality than for 12 h (Table 3.2.5.10) but these two treatments need to be repeated in the same trial. It is also possible that fumigation for 24 h may have adverse effects on fruit quality so this must be investigated.

When FCM eggs were fumigated with CO₂ at 60% for 24 h there was an 88% reduction in hatch (Table 3.2.5.11) based on the numbers in the control in Experiment 6 and a similar 87% reduction in hatch in Experiment 7 (Table 3.2.5.12), so this would be an extra benefit of fumigation if there was a delay in the fruit entering the cold chain.

Table 3.2.5.6. Gas treatment of FCM-infested navel oranges 04/08/2011

Treatments	Dead larvae	Total larvae	Mortality (%)	Corrected mortality
Control (no treatment)	8	197	4.1 c	
Cold ONLY at -0.5°C for 5 d	143	227	63.0 a	61.4
10% CO ₂ (6h) + cold (-0.5) for 5 d	99	161	61.5 a	59.9
30% CO ₂ (6h) + cold (-0.5) for 5 d	56	189	29.6 b	26.7

Means followed by the same letter were not significantly different ($\alpha = 0.05$) (Tukey's HSD)

Table 3.2.5.7. Gas treatment of FCM-infested navel oranges 25/08/2011

Treatments	Dead larvae	Total larvae	Mortality (%)	Corrected mortality
Control (no treatment)	9	199	4.5 d	
Cold ONLY at -0.5°C for 5 d	155	215	72.1 b	70.8
30% CO ₂ (12h) only	21	173	12.1 d	8.0
30% CO ₂ (12h) + cold (-0.5) 5 d	125	211	59.2 b	57.3
60% CO ₂ (12h) only	69	179	38.5 c	35.6
60% CO ₂ (12h) + cold (-0.5) 5d	259	276	93.8 a	93.5

Fruit were waxed by dipping in 20 min after gassing before going into cold, i.e. no gap.

Table 3.2.5.8. Results of dissecting 10 Navels and 10 Valencias to compare FCM larval development.

Fruit type	Total larvae	Size range	Mean length
Navels	88	3-13 mm	7.8 mm
Valencias	152	3-9 mm	6.4 mm

Table 3.2.5.9. Treatment of FCM-infested Valencias 28/10/2011

Treatments	Dead larvae	Total larvae	Mortality (%)	Corrected mortality
Control (no treatment)	7	189	3.7 e	
Cold ONLY at -0.5°C for 5 d	78	183	42.6 c	40.4
30% CO ₂ (12h no gap) + Cold (-0.5)	120	189	63.5 b	62.1
30% CO ₂ (12h 1-day gap) + Cold	46	164	28.0 d	25.3
30% CO ₂ only 12h	9	200	4.5 e	0.8
60% CO ₂ (12h no gap) + Cold (-0.5)	168	195	86.2 a	85.6
60% CO ₂ (12h 1-day gap) + Cold	108	164	65.9 b	64.5
60% CO ₂ 12h only	37	180	20.6 d	17.5

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

Fruit were waxed by dipping after gas treatment.

Table 3.2.5.10. Susceptibility of FCM larvae in Shamouti oranges fumigated with CO₂ on 23/7/2012

Treatments	Dead larvae	Total larvae	Mortality (%)
Untreated control at 25°C	0	115	0.0 c
Cold only at -0.5°C for 5 d	148	148	100.0 a
60% CO ₂ 24 h at 25°C	41	115	35.7 b
60% CO ₂ 24 h at -0.5°C for 5 d	127	134	94.8 a

Means followed by the same letter were not significantly different ($\alpha = 0.05$) (Tukey's HSD)
Fruit were not waxed after fumigation and before cold treatment.

Table 3.2.5.11. Susceptibility of FCM eggs to CO₂ 25/7/2012

Treatments	Total no. hatched eggs	Percentage hatch relative to control
Untreated control	1112	-
60% CO ₂ 24 h only	134	12.1

$t = 8.564$; $df = 18$; $P < 0.0001$

Table 3.2.5.12. Susceptibility of FCM eggs to CO₂ 30/8/2012

Treatments	Total no. hatched eggs	Percentage hatch relative to control
Untreated control	1 828	-
60% CO ₂ 24 h only	244	13.3

$t = 21.947$; $df = 18$; $P < 0.0001$

Conclusions

Cold tolerance

- Larval activity ceases from 6.7 to 3.1°C but is influenced by a range of factors (e.g. feeding status and experimental methodology)
- The effects of low temperature exposure and duration of exposure on larval survival were assessed and showed that the temperature at which 50% of the population survives is $-11.5 \pm 0.3^\circ\text{C}$ after 2 h exposure
- Combined mortality-freezing temperature assays clearly showed that larvae which freeze die readily while those which do not freeze survive
- Larvae typically freeze between -15.6°C and -18.2°C
- Inoculation by water raised SCP from -15.6°C to -6.8°C which could have important implications for post-harvest sterilization
- Cooling rates did not affect SCP which suggests that there is limited plasticity of SCP during the larval life-stage
- CT_{min} and chill coma recovery time were found to be plastic responses, while no rapid cold hardening response was detected

Thermolimit respirometry

- Neither CT_{min} nor SCP was significantly affected by the altered atmosphere which indicates that the mode of action of gas treatments is likely unrelated to a change in cold tolerance
- A change in O₂ does not result in significant changes in metabolism

Water balance & time to death

- At low relative humidity, larvae survived at least 10 days without access to food, and when they died, larvae had lost approx. 50% of their initial body mass

Controlled atmospheres

- FCM larvae have HSP70, though it is only upregulated after low temperature pre-treatments
- Mannose, trehalose, glucose, sorbitol and maltose were identified as the cryoprotective sugars used by FCM to supercool
- The additional 6 longer-term treatments resulted in a significant increase in mortality relative to the initial 9 treatments
- 24 h of 6% CO₂ 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

- Where high survival was observed after controlled atmosphere pre-treatments, larvae generally completed the transition into adults
- High temperature, low O₂ and high CO₂ decrease BWC, even under high relative humidities in the case of low O₂
- Low temperature pre-treatment increases BWC, ratio of unsaturated to saturated fatty acids and HSP70 – all of which likely allow greater survival of subsequent low temperature exposure
- Sorbitol is probably used to buffer thermal and osmotic stress in FCM
- Death after high temperature is likely because of a lack of cross tolerance between high and low temperature tolerance in FCM
- Total protein decreased after exposure to low O₂, possibly indicating a lack of ATP available for gene translation
- 10h Low O₂ (dry conditions) treatments resulted in significantly more haemocyte death, compared to the control
- Fat tissue is the most susceptible to damage, but damage to fat tissue is not necessarily lethal in FCM
- The ordering of CO₂ and cold has little effect on survival, but may affect HSP70
- Prolonged upregulation of HSP70 may be detrimental in FCM (as per additional 6 longer-term treatment 3)
- In general multiple non-lethal stress (e.g. pretreatments with low temperatures and low O₂) makes FCM less susceptible to low temperatures through cross tolerance and overlaps between molecular mechanisms
- High CO₂ over longer timescales may prevent FCM from increasing their membrane fluidity (e.g. decreasing fatty acid chain lengths) which is required to withstand low temperature stress
- Shorter exposure to CO₂ is likely buffered by glucose and maltose

Infested fruit research

- Exposure of FCM larvae in oranges to 30% or less CO₂ for 12 h did not increase mortality.
- Exposing FCM larvae in oranges to 30% CO₂ for 12 h followed immediately by a 5 day cold treatment sometimes resulted in more mortality than with the cold treatment alone, but if the cold treatment was delayed for 24 h the resultant mortality was lower than with the cold treatment alone.
- Exposing FCM larvae in oranges to 60% CO₂ for 12 h followed 24 h later by a 5 day cold treatment caused less mortality than the same CO₂ treatment without the 24 h gap, but not less mortality than cold alone.
- Further research should be conducted with 60% CO₂ to avoid reducing the efficacy of the cold treatment.

Future research

Although this project has now ended, research on the use of CO₂ as a shock treatment before a shortened cold treatment will continue under project 913 which covers fumigants for various pests. Careful evaluation of these combined treatments on rind condition, shelf life and taste will be required.

Technology transfer

Publications

Interactions between controlled atmospheres and low temperature tolerance: A review of biochemical mechanisms. Boardman, L., Sørensen, J.G., Johnson, S.A., Terblanche, J.S. 2011. *Frontiers in Physiology*, 2(92). doi: 10.3389/fphys.2011.00092

False codling moth *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera, Tortricidae) larvae are chill-susceptible. Boardman, L., Grout, T.G., Terblanche, J.S. 2012. *Insect Science*, 19: 315-328.

Physiological responses to fluctuating thermal and hydration regimes in the chill susceptible insect, *Thaumatotibia leucotreta*. Boardman, L., Sørensen, J.G., Terblanche, J.S. Under revision. *Journal of Insect Physiology*.

Biochemical responses to temperature and controlled atmospheres. In preparation. Boardman, L., Sørensen, J.G., Grout, T.G., Terblanche, J.S.

Presentations

Heat shock protein 70 response to low temperature and elevated carbon dioxide in the false codling moth, *Thaumatotibia leucotreta*. Boardman, L., Sørensen, J.G., Grout, T.G., Terblanche, J.S. Society of Experimental Biologists Annual Meeting, Salzburg, Austria, 2012. Oral presentation.

Biochemical responses of false codling moth, *Thaumatotibia leucotreta*, to low temperature and controlled atmospheres. Boardman, L., Sørensen, J.G., Grout, T.G., Terblanche, J.S. 7th Citrus Research Symposium, Champagne Sports Resort, South Africa, 2012. Oral presentation.

Cross tolerance between modified atmospheres and low temperature in insects. Boardman, L., Sørensen, J.G., Grout, T.G., Terblanche, J.S. Society for Integrative and Comparative Biology Annual Meeting, San Francisco, USA, 2013. Oral presentation - part of Symposium entitled: "Physiological Responses to Simultaneous Shifts in Multiple Environmental Stressors: Relevance in a Changing World".

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Literature applicable to physiology

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

Project 976 (April 2010 – March 2015) by Wayne Kirkman and Sean Moore (CRI)

Summary

The objective of this study is to investigate post-harvest techniques to detect cryptic pests in citrus fruit. The micro focus X-ray unit at NECSA was prepared and calibrated to deliver the highest possible quality images. Tungsten was the most effective target, and aluminium the best filter. The optimum energy level was determined to be 70 KV and 40 W. With micro focus computed tomography (μ CT), images were captured at 500 projections per scan, with an exposure time of 4 seconds. FCM infestation could not be detected two days after infestation of Delta Valencias using X-ray radiography, as many of the larvae were still in the albedo. Microfocus radiography detected 60% of infestation after 10 days, 77% after 7 days, and 40% 4 days after infestation. Micro focus tomography detected 100% of infestation. Oranges were X-rayed just prior to infestation, and then again at 1, 2 and three days after infestation, positioned in exactly the same way as

before. This was done in an attempt to detect differences in Gray values between the healthy and infested fruit, using the healthy fruit as a base/template. Results were skewed due to fruit not being placed in identical positions before and after infestation. Imaging algorithms are being developed in collaboration with NECSA, Mafroda (USA) and the University of Valencia, to automatically classify μ 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 continued with Tastetech (NZ) to investigate detection of known compounds using near infrared spectroscopy.

Opsomming

Die doel van die studie is om tegnieke te ondersoek wat peste binne sitrus vrugte na oes kan opspoor. Die mikrofokus X-straaleenheid by NECSA is voorberei en gekalibreer om die beste gehalte beelde moontlik te lewer. Tungsten was die mees effektiewe teiken, en Aluminium die beste filter. Die optimum energievlak was 70 KV en 40 W. Met mikrofokus tomografie (μ CT) was beelde met 500 projeksies en 4-sekonde blootstellingsperiode geneem. VKM skade op Delta Valencia lemoene kon nie twee dae na besmetting met behulp van X-straal radiografie opgespoor word nie, aangesien die larwes steeds in die skil of albedo teenwoordig was. Mikrofokus radiografie het 60% van besmetting opgespoor na 10 dae, 77% na 7 dae, en 40% 3 dae na besmetting. Mikrofokus tomografie het 100% van besmetting opgespoor. Radiogramme is geneem kort voor besmetting, en dan weer 1, 2 en 3 dae daarna. Die vrugte is elke keer in dieselfde posisie geplaas. Dit is gedoen in 'n poging om 'n verskil in Gray-waardes te wys tussen gesonde en besmette vrugte. Die waardes is beïnvloed deurdat vrugte nie in identiese posisies geplaas is voor en na besmetting nie. Beeld algoritmes word deur samewerking tussen CRI, NECSA, Maf-Roda (VSA) en die Universiteit van Valencia ontwikkel om automaties μ CT beelde as besmette of gesonde vrugte te identifiseer. 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 en Tastetech (NZ) word voortgesit om chemiese stowwe deur middel van naby-infrarooi spektroskopie op te spoor.

3.2.7 FINAL REPORT: Characterisation of nematode symbiotic bacteria and the *in vitro* liquid culture of *Heterorhabditis zealandica* and *Steinernema yirgalemense*

Project 984 (March 2010 - March 2013) by T. Ferreira, A.P. Malan (SU) and S.D. Moore (CRI)

Summary

Entomopathogenic nematodes, in combination with their associated bacterial symbionts, have proven to be effective against numerous insect pests. In this project, the associated symbiotic bacteria of three EPN species were isolated, and the potential of two nematode species to be mass cultured in liquid media was evaluated. Bacteria species from *Heterorhabditis noenieputensis*, *Steinernema khoisanae* and *Heterorhabditis zealandica*, which were found to be new species, have been described as such. Using *in vitro* mass culture techniques, it was illustrated that *H. zealandica* and its *Photorhabdus* symbiont, as well as *Steinernema yirgalemense* and its *Xenorhabdus* symbiont, can be successfully cultured in a liquid medium. However, the number of nematodes per ml of medium was found to be much higher for *S. yirgalemense*, with 77 000 IJs/ml (13 days), in comparison to the 41 000 IJs/ml (15 days) obtained for *H. zealandica*. The final aim of the project was to determine when *Xenorhabdus* reached the stationary phase, when grown in a 20-L fermenter, as said moment in time would be most suited for adding the infective juveniles (IJs) of *S. yirgalemense* for mass culture. The step concerned would also be the first taken toward the liquid mass culture of *S. yirgalemense* in industrial-size fermenters. Results from this experiment indicated the optimum amount of time required for adding nematodes to the bacterial culture in the fermenter, and for ensuring the optimum recovery of IJs, as well as a subsequent high yield of nematodes within a minimum amount of processing time. This is the first report of its kind to investigate comprehensively the successful liquid culture of two South African EPN species for the sole purpose of evaluating potential commercialisation. Future research goals should be to increase the percentage IJ recovery directly after inoculation into liquid culture, which would increase the number of nematodes produced per ml medium, which would, therefore, significantly reduce the amount of processing time required.

Opsomming

Entomopatogeniese nematodes, in kombinasie met hul geassosieerde simbiotiese bakterieë, het die potensiaal om as doeltreffende biologiese beheer agente teen sleutel plaaginsekte gebruik te word. In hierdie projek is drie geassosieerde bakterie van verskillende nematode spesies geïsoleer en die potensiaal vir massa produksie van twee nematode spesies in vloeistof medium geëvalueer. Die bakterie spesies geïsoleer vanuit *Heterorhabditis noenieputensis*, *Steinernema khoisanae*, en *Heterorhabditis zealandica*, is geïdentifiseer en beskryf as nuwe spesies. Deur gebruik te maak van *in vitro* massa telings tegnieke in 'n vloeistof medium is *H. zealandica* met sy *Photorhabdus* simbiot en *Steinernema yirgalemense* met sy

Xenorhabdus symbiont suksesvol massa geproduseer. Daar is egter gevind dat die hoeveelheid infektiewe larwes (IJ) per ml medium baie hoër was vir *S. yirgalemense* met 77000 IJ/ml (13 dae) in vergelyking met *H. zealandica* met 41000 IJ/ml (15 dae) in dieselfde medium. Die doelwit van die laaste gedeelte van die projek was om te bepaal op watter stadium *Xenorhabdus* die stasionêre fase bereik wanneer dit in 'n 20-L fermentor gekweek word. Dit sal ook die eerste stap wees in die vloeistof massa teling van *S. yirgalemense* in industriële fermentors. Resultate van hierdie eksperiment dui aan die optimale tydsverloop van bakteriële ontwikkeling voor die nematodes bygevoeg moet word, om sodoende optimale voeding van die nematode te verseker wat dan lei tot gevolglike hoë opbrengs in die minimum moontlike telingstyd. Hierdie is die eerste studie wat die teling van twee Suid-Afrikaanse nematode spesies omvattend in vloeistof medium evalueer. Die hoof doelwit was om die potensiaal van hierdie nematode spesies, met die oog op kommersiële gebruik, te meet. Toekomstige navorsing moet gerig word op die verhoging in die persentasie IJ wat begin met voeding na inokulasie in die vloeistof medium, aangesien dit sal lei tot 'n verhoging in die getal nematodes per ml medium, wat op sy beurt weer die produksie tyd aansienlik sal verminder. Die resultate van hierdie studie kan die basis vorm vir toekomstige studies in hierdie spesifieke navorsingsveld.

Introduction

Entomopathogenic nematodes (EPNs) represent an important part of the spectrum of potential biological control agents against citrus pests. Previous research in South Africa has shown that two local nematodes, *Heterorhabditis zealandica* and *Steinernema yirgalemense*, in particular, have great insecticidal potential (De Waal et al., 2010; De Waal et al., 2011a; De Waal et al., 2011b; Van Niekerk and Malan, 2012; Ferreira and Malan, 2013; De Waal et al., 2013). Therefore, the ability to mass culture these two nematode species in liquid medium, using *in vitro* technology, is an important step toward their application as biocontrol agents on a commercial scale against key insect pests. However, for *in vitro* technology to be successful, the nematode-bacteria interaction needs to be understood. Bacterial symbionts of EPNs need to be isolated and characterised, with the life cycle of the nematode in culture needing to be understood to be able to optimise for maximum nematode yield in liquid culture.

The South African citrus industry regards the use of entomopathogenic nematodes (EPNs) as important in an integrated pest management system for the production of pest- and residue-free fruit for the export market. For this goal to be realised, however, research has to be focused on *in vitro* liquid mass production of nematodes for commercial application. There is a need for new and innovative methods to control agricultural pests, as numerous pest insects have developed resistance against broad-spectrum insecticides. Together with the environmental impact of these insecticides and the safety aspect regarding humans and animals, the need to develop new technologies, including EPNs for pest management, is high.

In this project, firstly the associated symbiotic bacteria of three entomopathogenic nematodes species were isolated and described as new species in South Africa. Secondly, the potential of two nematode species, *H. zealandica* and *S. yirgalemense*, to be successfully mass cultured in liquid medium was evaluated.

Stated objectives

- Isolation of symbiotic bacteria, identification, monoculture and storage
- Monoculture of *Heterorhabditis zealandica* from eggs and storage (this was also done with *Steinernema yirgalemense*)
- Testing of different liquid media (these were only preliminary trials and therefore data were not shown in final PhD dissertation)
- Development of optimum culturing conditions using the Erlenmeyer flask method (this was done with both *H. zealandica* and *S. yirgalemense*)
- Optimum production with bench top liquid fermenter (this was done with the associated symbiotic bacteria *Xenorhabdus* sp. isolated from *S. yirgalemense*)
- Novel formulation methods – objective not reached (should have been a separate study)
- Quality testing of product

Materials and methods

See PhD Dissertation. <http://hdl.handle.net/10019.1/80294>

Results and discussion

See PhD Dissertation. <http://hdl.handle.net/10019.1/80294>

Conclusion

Results from this project illustrated that both indigenous *H. zealandica* and its *Photorhabdus* symbiont, and *S. yirgalemense* with its *Xenorhabdus* symbiont, can be successfully mass cultured in liquid. When importing nematodes from other countries, two factors act as major obstacles. The first is the time that it takes for the nematodes to reach South Africa, combined with the temperature at which they have to be kept while in transit. The second problem is the cost that is associated with importing nematodes from another country, which, due to such importing not being economically feasible in the long run, is, therefore, only a short-term solution. Consequently, our own endemic species need to be mass cultured and produced as biopesticides in South Africa.

Future research

Future research should be aimed at optimising the bacterial growth in the medium. Different mediums could be investigated, as well as methods for enhancing stronger food signals. The next step will be the effective culturing of nematodes in industrial size fermenters and the formulation of nematodes for a longer shelf life.

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3.2.8 FINAL REPORT: FCM infestation of lemons in the field

Project 1014 (Apr 2002 – Mar 2013) by Sean Moore and Wayne Kirkman (CRI)

Summary

This study was conducted to determine the level of FCM infestation of lemons in orchards. The justification for the study was the occurrence of lemon orchards within areas treated with SIT for FCM in the Western and Eastern Cape. Sterile moths are not released in lemon orchards and it is therefore possible that infestation of lemons within such an area can compromise the SIT programme by being a reservoir for and hence a continual source of inoculum of wild moths. Between 2009 and 2011, FCM infestation of hanging and fallen mature fruit and fallen marble-sized lemons, was found to range between 0.1% and 2.18% fruit infested. Although it can already be concluded that untreated lemon orchards can harbour significant levels of FCM adults and larval infestation, and can pose a threat for adjacent orchards consisting of more FCM-susceptible cultivars and can compromise the efficacy of an SIT programme, it was decided to continue this study for one more season. This study was conducted in winter, as this is when yellow lemons are harvested for export and can therefore potentially provide an FCM inoculum for more FCM susceptible orchards in spring. Over a period of a month in three lemon orchards, no infested marble-sized fruit and hanging yellow fruit were recorded. However, 1.25% of fallen mature fruit were infested with FCM.

Opsomming

Hierdie studie is uitgevoer om die vlak van VKM besmetting in suurlemoen boorde te bepaal. Die regverdiging vir die studie is die voorkoms van suurlemoen boorde binne areas in die Wes- en Oos-Kaap wat met SIT vir VKM behandel word. Steriele motte word nie in suurlemoen boorde vrygelaat nie en dit is daarom moontlik dat besmetting van suurlemoene binne so 'n streek 'n dreiging vir die doeltreffendheid van die SIT program kan wees. Hulle kan as 'n konstante bron van wilde motte dien. Tussen 2009 en 2011, is tussen 0.1% en 2.18% van volwasse vrugte, op die boom en op die grond, en albaster-grote vrugte, met VKM besmet. Al is die gevolgtrekking van hierdie studie alreeds dat onbehandelde suurlemoen boorde beduidende vlakke van VKM volwassenes en larwe besmetting kan huisves, en dus 'n dreiging inhou vir nabye boorde van meer VKM vatbare kultivars en 'n dreiging vir die doeltreffendheid van 'n SIT program kan wees, is die besluit geneem om hierdie studie vir nog een seisoen uit te voer. Hierdie studie is in die winter uitgevoer, omrede geel suurlemoene op hierdie stadium vir uitvoer gëoes word en dus as 'n moontlike bron van VKM besmetting vir meer VKM-vatbare boorde in die lente kan dien. Oor 'n tydperk van 'n maand in drie suurlemoene boorde is geen besmette albaster-grote vrugte en hangende geel vrugte gekry nie. Daar is nietemin VKM in 1.25% van gevalde ryp vrugte gekry.

Introduction

The sterile insect technique (SIT) is currently being commercially employed in the Western Cape (Citrusdal area) for control of false codling moth (FCM). Within a couple of years the entire Citrusdal Valley should be covered (approximately 6500 ha). Thereafter, the intention is to roll the programme out to other citrus producing areas in South Africa.

As SIT is an area-wide means of controlling the target pest, all cultivars and varieties within a treated area will be included in the programme with uniform intensity releases of sterile individuals throughout. One problem is that the programme is expensive. This cost is easily justified for citrus varieties which are highly

susceptible to FCM. However, grower resistance is experienced where release costs for non-susceptible cultivars, such as lemons are the same as for highly susceptible cultivars, such as navel oranges and mandarin varieties.

Previous studies have shown most lemon fruit stages to virtually be non-hosts to FCM (Moore et al 2005; Moore & Kirkman, 2006). However, over-ripe fruit and very small fruit (pre-juice production) have been shown to be able to accommodate significant levels of infestation in the laboratory (Moore & Kirkman, 2008). Therefore, orchard surveys focus on these two stages of fruit development.

Data accumulated over a couple of seasons indicated that moth activity, measured by catches of male moths in pheromone traps, is similar in lemons as in orchards of susceptible cultivars (Moore et al 2005; unpublished data, Sundays River Citrus Company). Fruit infestation data collected over two years showed that this activity is not simply indicative of male moths using these orchards as a staging post for pursuing females in nearby orchards of more susceptible cultivars, but that FCM does indeed breed within lemon orchards. In some instances over 2% of mature (yellow) lemons were found to be infested. Marble-sized lemons also showed some infestation.

Objectives

1. Establishment of natural infestation levels of lemons by FCM.
2. Adequate data collection to assist with decision-making on sterile FCM release intensity required in lemon orchards within an SIT programme.

1. Materials and methods

Three large lemon orchards which have previously been used for this study have again been selected on farms in the Sundays River Valley (Table 3.2.8.1). Twenty trees in a row will be marked as data trees in each orchard. Monitoring will be initiated in winter, at the time that well-coloured fruit is normally harvested for export. Two categories of fruit which had dropped from these trees (very ripe – yellow – fruit and marble-sized fruit) will be collected and taken back to the laboratory for assessment. Simultaneously, around 30 to 50 yellow fruit will be picked from other (non-data) trees. In the laboratory, fruit will be carefully inspected for any signs of FCM infestation. This will include peeling and dissecting fruit. Fruit will be recorded as FCM infested if any FCM larvae or their frass are found in the fruit.

Table 3.2.8.1. Lemon orchards in Sundays River Valley in which FCM will be monitored during winter 2012.

Farm	Orchard No.	Lemon variety	Rootstock	Year planted	Tree spacing (rows x trees) (m)	No of trees in orchard
Penhill	18	Eureka	Rough lemon	1995	6 x 3	660
Lone Tree	32	Eureka	Rough lemon	1999	6 x 3	880
Halaron	31	Eureka	Volckameriana	1996	6 x 3	666

Results and discussion

Objective / Milestone	Achievement
A. Fruit collection and inspection	Completed

No mature fruit on trees was found to be infested with FCM (Table 3.2.8.2). However, up to almost 3% of fallen mature fruit were found to be infested with FCM. No small fruit were infested. A couple of years ago, a similar survey conducted in summer revealed a slightly higher percentage of fruit infestation (Moore & Kirkman, 2011).

Table 3.2.8.2. FCM infestation of lemon fruit in three orchards in Sundays River Valley, monitored from 5 July to 3 August 2012.

Farm	Fruit maturity	Fruit picked from trees or fallen	Total fruit inspected over 4 weeks	FCM infested fruit	
				No.	%
Penhill	Mature	Picked	200	0	0
		Fallen	38	1	2.63

	Small	Fallen	22	0	0
Lone Tree	Mature	Picked	141	0	0
		Fallen	88	0	0
	Small	Fallen	6	0	0
Halaron	Mature	Picked	199	0	0
		Fallen	34	1	2.94
	Small	Fallen	5	0	0
Total	Mature	Picked	540	0	0
		Fallen	160	2	1.25
	Small	Fallen	33	0	0

Conclusions

Even though FCM activity might be reduced in winter, there is still a possibility that some very ripe lemons might be infested. Therefore, precautions around packing and exporting of yellow lemons should remain in place throughout the year.

Technology Transfer

During grower presentations on FCM, the recommendation to conduct orchard sanitation in lemons is made.

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3.2.9 PROGRESS REPORT: Late season releases of *Trichogrammatoidea cryptophlebiae* for suppression of FCM

Project 1021 (Apr 2012 – Mar 2015) by Sean Moore, Wayne Kirkman (CRI) & Wayne Mommsen (Du Roi IPM)

Summary

Studies on the effectiveness of the FCM egg parasitoid, *Trichogrammatoidea cryptophlebiae*, were conducted a number of years ago, showing that FCM infestation could be reduced by up to 61%. However, this was a result of early season parasitoid releases. Parasitism by naturally occurring *T. cryptophlebiae* generally builds up from December and reaches a peak in January or February. Any releases at and shortly before this time gave negligible benefit over and above that of the naturally occurring parasitoids. Currently such early releases are near impossible in the industry, due to the application of a series of pesticides for control of thrips and other pests and diseases during the first half of the season. *Trichogrammatoidea cryptophlebiae* is very sensitive to many of these pesticides. Where a chemical orientated pest control programme is followed, an FCM parasitoid vacuum is often created, exacerbating this increase in FCM infestation shortly before harvest. This study aims to determine whether mid to late season releases would still be of benefit in reducing FCM infestation, even if the effect is only seen shortly before harvest. Parasitoids were released at four sites – all Navel oranges – at 100 000 parasitoids per hectare, as monthly releases of 25 000 parasitoids from January to April. Thereafter, FCM presence (moths, eggs and larval infestation of fruit) and egg parasitism were evaluated weekly in release and comparable control blocks. At three out of the four trial sites, FCM levels were too low to obtain meaningful results. At the fourth site, FCM levels were very high. However, so too was natural parasitism, thus obscuring any impact which the

released parasitoids might have had. Evaluation of FCM and parasitoid levels will continue until harvest at all four sites.

Opsomming

Studies oor die doeltreffendheid van die VKM eier parasiet, *Trichogrammatoidea cryptophlebiae*, is 'n paar jaar gelede uitgevoer en het gewys dat VKM besmetting met tot 61% verminder kon word. Hierdie is egter as gevolg van vroeë seisoen loslatings. Parasitisme deur *T. cryptophlebiae* wat natuurlik voorkom, bou gewoonlik van omtrent Desember op en bereik 'n piek in getalle in Januarie of Februarie. Enige loslating op hierdie stadium en kort voor hierdie stadium het weglaatbaar min voordeel gegee oor dié van die parasiete wat natuurlik voorgekom het. Tans is sulke vroeë loslatings amper onmoontlik in die bedryf as gevolg van toediening van 'n reeks plaagdoders vir beheer van blaaspootjie en ander plae en siektes gedurende die eerste helfte van die seisoen. *Trichogrammatoidea cryptophlebiae* is baie sensitief vir baie van hierdie plaagdoders. Waar 'n chemies-gëoriënteerde plaagbestrydingsprogram uitgevoer word, veroorsaak dit gereeld 'n VKM-parasiet-vakuum, wat 'n styging in VKM besmetting kort voor oes kan veroorsaak. Die doel van hierdie studie is om te bepaal of mid tot laat seisoen loslatings nogsteeds VKM besmetting sal verminder, selfs as die effek eers kort voor oes gesien word. Parasiete is by vier persele losgelaat – alles Nawellemoene – teen 100 000 parasiete per hektaar. Maandeliks van Januarie tot April is 25 000 parasiete per hektaar losgelaat. VKM teenwoordigheid (motte, eiers en larwe besmetting van vrugte) en eier parasitisme is daarna weekliks in loslatings blokke en vergelykbare onbehandelde kontrole blokke geëvalueer. By drie uit die vier proefpersele is VKM vlakke te laag om waardevolle resultate te kry. By die vierde perseel is VKM vlakke baie hoog. Natuurlike parasitisme was egter ook hoog, en het dus enige impak wat die losgelate parasiet kon gehad het verduister. Evaluasie van VKM en parasiet vlakke sal by al vier persele tot oestyd voortgesit word.

3.2.10 PROGRESS REPORT: Suppression of false codling moth (*Thaumatotibia leucotreta*) larvae with a combination of suboptimal temperature and ionising radiation

Project IBB02/12 (April 2012 – March 2013) by Hendrik and Marsheille Hofmeyr (Ibbsbüren Navorsing)

Summary

The advantage of combining treatment types that could be applied at suboptimal levels for phytosanitary security is discussed. Various experiments consisting of cold treatments of 0.9°C and 2.5°C for periods of 10 to 18 days with and without the addition of 40 Gy to 70 Gy of ionizing radiation, were evaluated against mature larvae of false codling moth, *Thaumatotibia leucotreta*. Cold treatment *per se*, caused high larval mortality, but had little influence on the reproductivity of resultant moths. The ionizing radiation treatments had little effect on larval mortality but enhanced moth sterility. A combination of 2.5°C for 14 to 18 days combined with 60 Gy ionizing radiation is recommended for assessment at probit-9 level for commercial application.

Opsomming

Die voordeel van behandelingstipes wat gesamentlik teen suboptimale vlakke vir fitosanitêre beveiliging aangewend kan word, word bespreek. Verskeie proewe is uitgevoer met kouebehandelings van 0.9°C en 2.5°C vir tydperke van 10 tot 18 dae met en sonder 40 Gy tot 70 Gy dosisse van ioniserende straling teen volwasse larwes van valskodlingmot, *Thaumatotibia leucotreta*. Kouebehandeling het opsigself groot, maar nie totale, larwemortaliteit bewerkstellig, maar min uitwerking op die steriliteit van oorlewende motte gehad. Die stralingbehandelings het min uitwerking op larwegetalle gehad maar motsteriliteit bevorder. 'n Kombinasie van 2.5°C vir 14 tot 18 dae met 60 Gy ioniserende straling word voorgestel as 'n behandeling wat vir kommersiële toepassing op probit-9 vlak getoets behoort te word.

3.2.11 FINAL REPORT: Identifying volatile emissions associated with false codling moth infestation of citrus fruit

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

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 false codling moth in citrus fruit at different levels of infestation in order to reduce phytosanitary risk. The main objectives were to establish a comprehensive range of the various volatiles emitted by citrus fruit, to determine the differences in volatile emission by infested and non-infested citrus fruit, to determine differences in volatile emission by fruit infested at various intervals before testing and to establish differences in volatile emission by fruit as per life-stage of the larvae infesting the fruit (time after infestation). The differences in volatile emission by citrus fruit of different cultivars and varieties were also determined. The ultimate outcome being that this will lead to the development of a detection system in the future which will immediately show infested fruit while on a packing line. Forcibly infested (Cara Cara Navel and early Valencia) and naturally infested (Lane Late Navel and Midnight Valencia) fruit were used. Fruit were harvested at intervals after induced infestation to coincide with different stages of larval development: 0, 5, 10, 18 and 25 days after infestation. At each interval non-infested control fruit were also collected. Collection and trapping of headspace volatiles from the intact fruit was done using Solid Phase Micro-extraction (SPME). Gas chromatography/Mass spectrophotometry (GC/MS) analysis was done in order to analyze the volatile compounds from the head space of the infected and uninfected fruit. Following chemical collection and analysis, each fruit sampled was cut open and examined to determine the larval instar and number of larvae in the fruit. Compounds released and detected were indicative of infestation and were not insect produced but naturally produced fruit volatiles emitted at higher levels as a result of the insect within the fruit. Five major volatile compounds of interest were released by the infested oranges. These major volatile compounds include D-limonene, 3,7-dimethyl-1,3,6-octatriene, (E)-4,8-dimethyl-1,3,7-nonatriene, caryophyllene and naphthalene. Limonene was one of the most abundant volatile compounds released by the infested citrus fruit. Naphthalene, which is possibly produced due to larval feeding and development within the fruit maintained higher concentrations than controls throughout the infestation within the fruit. Naphthalene would be a good indicator of false codling moth infestation, however, not primarily for early infestation detection. A significantly higher concentration of D-limonene, 3,7-dimethyl-1,3,6-octatriene, (E)-4,8-dimethyl-1,3,7-nonatriene and naphthalene was detected using the SEP over the SPME technique. The application of an SPME procedure and the utilization of this method for detection of volatiles present in the headspace of intact infested fruit are evaluated and the possible volatile compounds diagnostic of *Thaumatotibia leucotreta* infestation of orange fruit and differences in volatile compound response in different orange varieties is discussed. The full report on this project can be found in an MSc thesis (Rachel van der Walt) available from NMMU or Sean Moore. Unfortunately there is no URL available. The project was scheduled to continue as a PhD study in 2013. However, the student unfortunately decided to withdraw, thus making this a final report. However, the study will be resumed in 2014 under a different project and by another researcher.

Opsomming

Valskodlingmot is n bekende plaag van ekonomiese belang vir baie verbou 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 priotiteit 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 hoof doelstellings was om 'n omvattende reeks van verskeie vlugtige stowwe wat deur sitrusvrugte vrygestel is te bepaal; om verskille in vlugtigestowwe vrystelling deur besmette en nie-besmette vrugte te bepaal; en om verskille in vlugtigestowwe vrystelling op elke lewensstadium (tydsduur na besmetting) te bepaal. Verskille in vrystelling van vlugtige stowwe deur sitrusvrugte van verskeie varieteite is ook bepaal. Die uiteindelijke beoogde uitkoms is die ontwikkeling van 'n opsporingsstelsel in die toekoms wat besmette vrugte op die paklyn sal kan uitwys. Albei vrugte wat VKM gedwing is om te besmet (Cara Cara Navel en vroë Valencia) en natuurlik besmette vrugte (Lane Late Navel en Midnight Valencia) is gebruik. Vrugte is geoes teen intervalle na geïnduseerde besmetting wat met die verskillende larwe stadiums ooreengekom het: 0, 5, 10, 18 en 25 dae na besmetting. Op elke stadium is nie-besmette kontrole vrugte ook versamel. Versameling en betrapping van hoofruim vlugtige stowwe van die heel vrugte is met Soliede Fase Mikro-ekstraksie (SPME) uitgevoer. Gaskromotografie/Massaspektrofotometrie (GC/MS) analiese is gedoen om die vlugtige stowwe van die hoofruim van die besmette en gesonde vrugte te ontleed. Na chemiese versameling en analise is elke vrug oopgesny en ondersoek om larwe stadium en getal larwes te bepaal. Vlugtige stowwe wat vrygelaat en opgespoor is, is aanduidend van besmetting en is nie van die insek afkomstig nie maar is natuurlike vlugtige stowwe van die vrug wat teen hoër vlakke losgelaat is as gevolg van die insek se teenwoordigheid in die vrug. Vyf hoof vlugtige stowwe van belang is deur die lomoene vrygelaat. Hierdie vlugtige stowwe sluit in D-limonien, 3,7-dimetiel-1,3,6-oktatrieen, (E)-4,8-dimetiel-1,3,7-nonatrieen, kariofileen and naftaleen. Limonien is een van die mees menigvuldige vlugtige stowwe wat deur die sitrusvrugte vrygestel is. Naftaleen, wat waarskynlik as gevolg van larwe voeding en ontwikkeling binne die vrug geproduseer word, het aanhoudend hoër konsentrasies as gesonde vrugte gehad. Naftaleen sou 'n goeie aanwyser van valskodlingmot

besmetting wees, maar egter nie om vroë besmetting aan te wys nie. 'n Beduidend hoër konsentrasie D-limonien, 3,7-dimetiel-1,3,6-oktatrieen, (E)-4,8-dimetiel-1,3,7-nonatrieen and naftaleen is met die SEP tegniek opgespoor in vergelyking met SPME. Die toediening van 'n SPME prosedure en die gebruik van hierdie metode om vlugtige stowwe in die hoofraam van ongeskonde besmette vrugte op te spoor is evalueer en vlugtige stowwe wat moontlik *Thaumatococcus danianus* besmetting van lemoene kan diagnoseer en verskille in vlugtige stowwe respons in verskillende lemoen varieteite word bespreek. Die volle verslag vir hierdie projek is die MSc tesis van Rachel van der Walt, wat by NMMU of Sean Moore gekry kan word. Ongelukkig is dit nog nie op die internet beskikbaar nie. Daar is beplan om in 2013 met die projek as 'n PhD voort te gaan maar die student het ongelukkig besluit om te onttrek. Daroom is hierdie nou 'n finale verslag. Die studie sal egter volgende jaar onder 'n nuwe projek en deur 'n ander navorser hervat word.

Technology Transfer

An oral presentation was made at the biennial Citrus Symposium in August 2012. Additionally, brief feedback on this research was made in the various regions during the September 2012 grower workshop series.

Table 3.2.11.1. Concentration ($\mu\text{g/ml}$) of the volatile compounds detected in False codling moth larvae infested Valencia and Navel oranges using the SPME-GC/MS detection technique.

Compound	Early Navels		Late Navels				Late Valencias	
	Control	Force Infested	Control	Naturally infested	Control	Force Infested	Control	Naturally infested
Acetaldehyde	0-0.01	0.01-0.06	0-0.01	0.01-0.06	0-0.01	0-0.01	0-0.01	0.06-0.13
2- propanol [#]	0-0.01	0.06-0.13	0.01-0.06	0.01-0.25	0.01-0.06	0.01-0.06	0.01-.25	0.06-0.13
2-ethyl-1-hexanol [#]	0-0.01	0.01-0.25	0-0.01	0-0.01	0-0.01	0-0.01	0-0.01	0-0.01
D-limonene [^]	0.08-2.78	0.15-3.50+	0.17-5.86	0.23-1.29	0.8-6.83	0.2-7.19	0.54-2.83	1.41-28.61+
1,3,6 Octatriene 3,7 dimethyl (Z) [^]	0.09-0.93	0.07-1.2+	0.02-0.34	0.09-2.65	0.07-0.25	0.02-1.15*	0.24-3.26	1.22-16.56
1,6 Octadiene-3-ol 3,7 dimethyl [#]	0-0.01	0.01-0.5	0-0.13	0-0.25	0-0.13	0.01-0.06	0-0.01	0.01-0.06
Hexanoic acid [#]	0.01-0.06	0.25-1>	0.01-0.06	0.01-0.06	0.01-0.06	0.06-0.13	0.01-0.06	0.01-0.06
(E)- 4,8 Dimethyl 1,3,7 nonatriene [#]	0-0.13	0.06-0.25	0.01-0.25	0.01-0.25*	0.01-0.05	0.06-0.25	0.01-0.06	0.01-0.25*
1-Undecanol [#]	0-0.01	0.01-0.25	0.01-0.06	0.01-0.06	0.01-0.06	0-0.01	0.01-0.06	0-0.01
Hexyl butanoate [#]	0-0.01	0-0.5*	0-0.01	0.06-1>*	0.01-0.06	0.13-0.5*	0-0.01	0.01-0.5*
Octanoic acid [#]	0-0.01	0-0.06	0-0.01	0.06-0.13	0-0.01	0.06-0.13	0-0.01	0-0.13
Dodecane [#]	0-0.01	0-0.06	0.06-0.13	0.25-1>	0.06-0.13	0.25-1>	0-0.01	0-0.01
2,6 Dihydroxyacetophenone bis (trimethylsilyl) ether [#]	0-0.01	0-0.06	0-0.01	0-0.01	0-0.01	0-0.01	0-0.01	0.01-0.06
Caryophyllene [#]	0.06-1	0.25-1	0.06-1	0.13-1	0.25-14	0.25-1*	0.01-0.06	0.06-0.5
Alloaromadendrene [#]	0.01-0.06	0-0.06	0	0	0	0	0.01-0.06	0.06-0.25
Humulene [#]	0-0.06	0.01-0.5	0	0	0	0	0-0.01	0.01-0.06
alpha.-Panasinsen [#]	0.06-0.25	0.01-1	0.01-0.06	0.06-0.5*	0.01-0.06	0.01-0.25	0.01-0.06	0.01-0.06
Naphthalene [^]	0.05-0.14	0.01-1.28*	0.22-1.14	0-0.51	0.05-0.13	0.02-0.13*	0.123-0.785	0.266-6.30*
2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene [#]	0.25-0.5	0.5-1	0.25-0.5	0.25-1>	0.25-0.5	0.25-1>	0.25-0.5	0.25-0.5
Propanoic acid [#]	0.5-1	1>	0	0	0	0	0.01-0.06	0.13-0.25

Shaded rows indicate major volatile compounds found in the majority of samples. (^)= Quantified using commercially available standards; (#) = NIST mass spectral library (80> confidence level); (*) statistically significant different from control at $p < 0.05$; All concentrations in $\mu\text{g/ml}$.

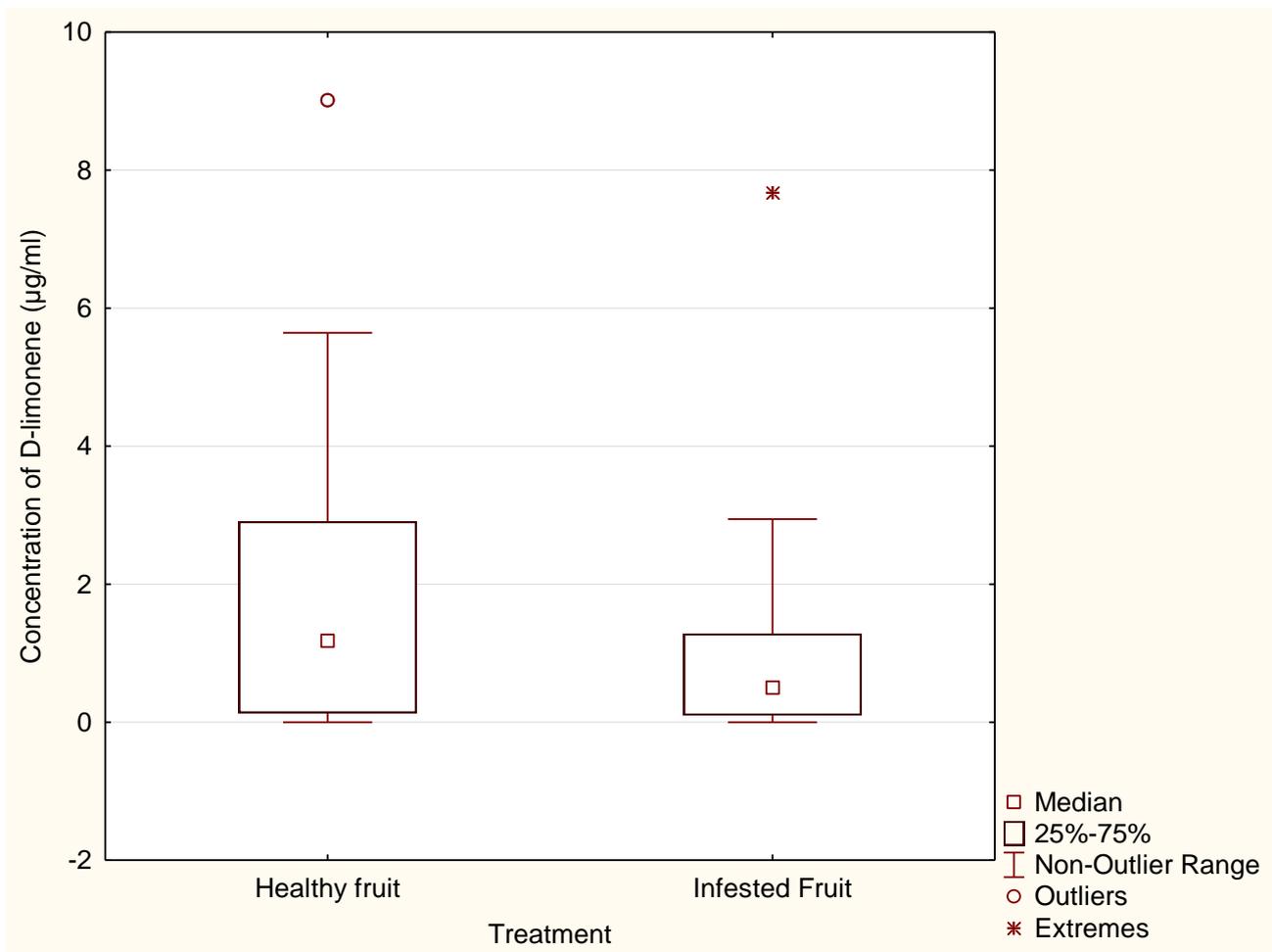


Figure 3.2.11.1. Box plot showing the median concentration of D-limonene released from the healthy and infested Cara cara Navel oranges for the experimental period between 18 and 25 days after infestation.

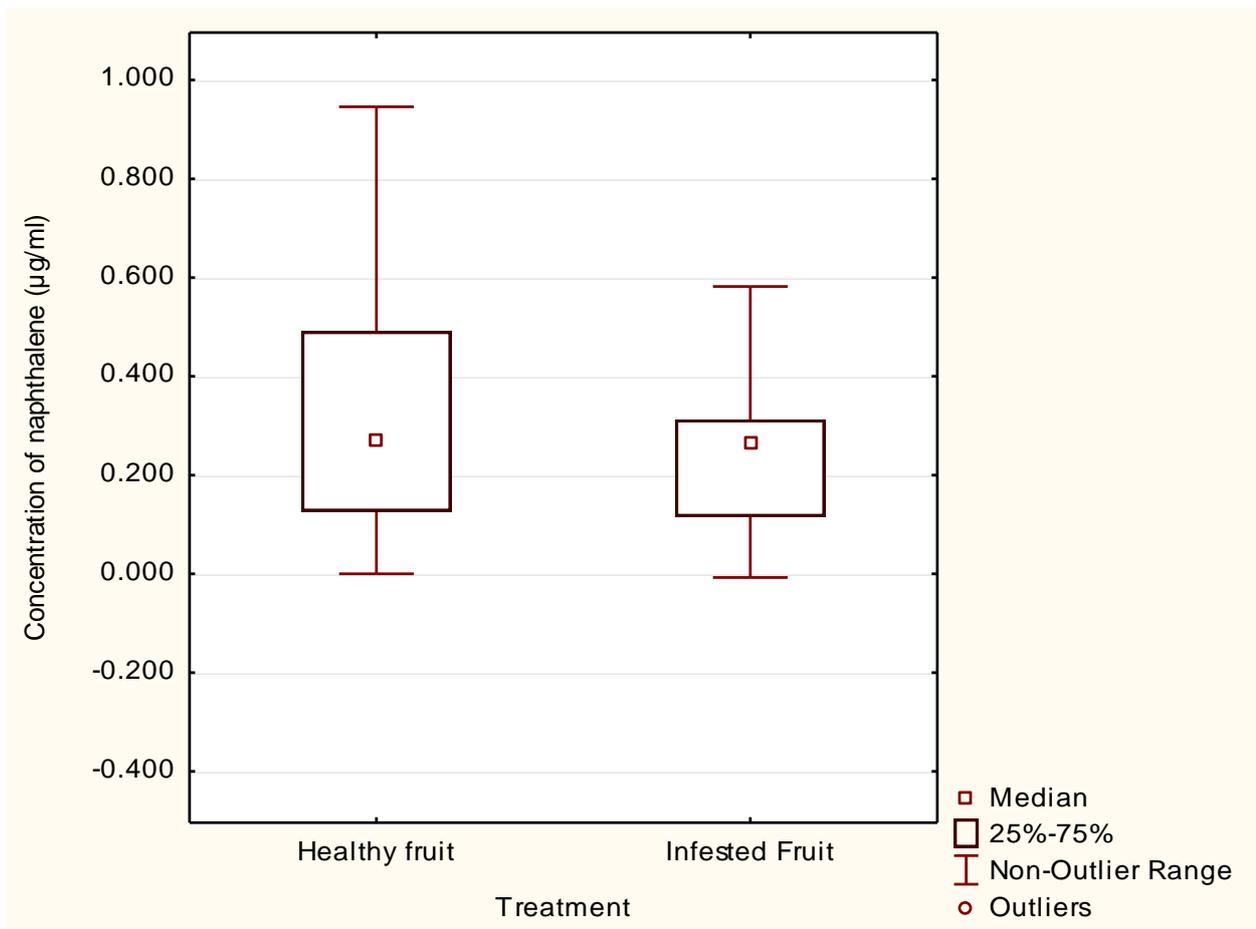


Figure 3.2.11.2. Box plot showing the median concentration of naphthalene released from the healthy and infested Cara Cara Navel oranges for the experimental period between 18 and 25 days after infestation.

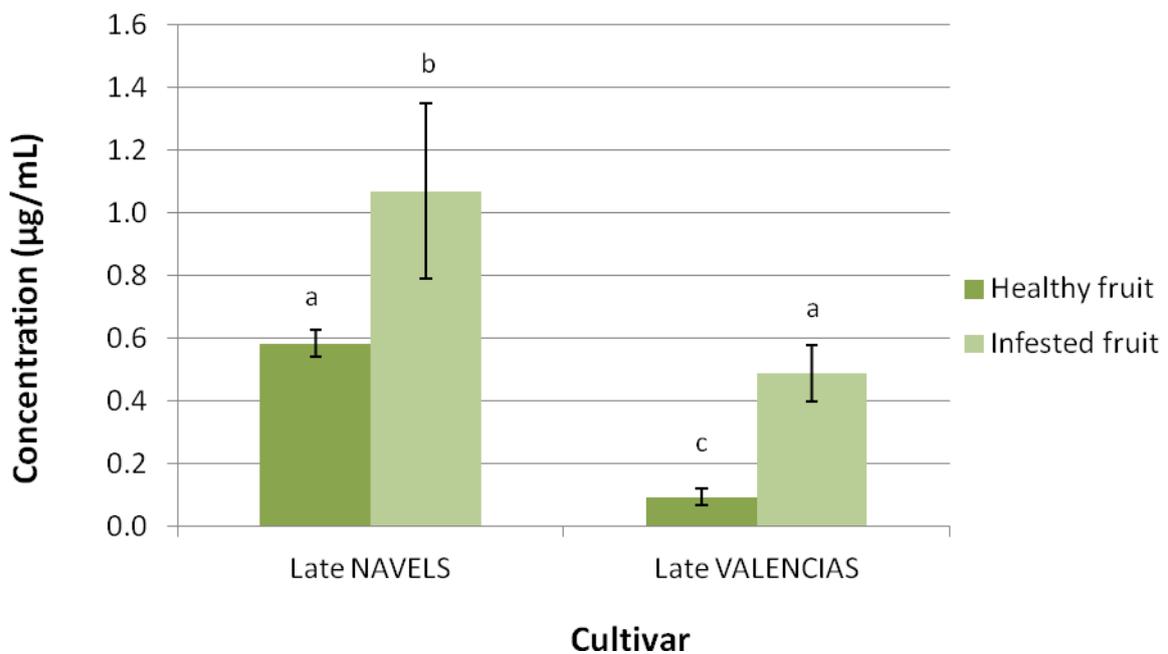


Figure 3.2.11.3. Effect of False codling moth larvae infestation of Lane late Navel and Midnight Valencia oranges on the concentration ($\mu\text{g}/\text{mL}$) of naphthalene after natural infestation within the orchard. Bars with the same letter do not differ significantly by Mann-Whitney test ($p = 0.05$)

3.2.12 **PROGRESS REPORT: The use of entomopathogenic fungi to control the soil-dwelling life stages of false codling moth**

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

Summary

The control of FCM is essential in the citrus industry due to the financial loss which can occur largely as a result of the phytosanitary status associated with FCM. Entomopathogenic fungi (EPF) can potentially serve as an additional biological control option for the control of FCM, specifically the soil-dwelling life stages (late fifth instar, pre-pupae and pupae). Goble *et al.* (2010, 2011) isolated 62 fungal isolates, from soil samples collected from citrus orchards within the Eastern Cape Province of South Africa. Twelve were identified as showing good control potential (Goble *et al.*, 2011). Further laboratory analysis revealed three of these isolates (*M. anisopliae* G 11 3 L6, *M. anisopliae* FCM Ar 23 B3, and *B. bassiana* G Ar 17 B3, as having the most potential against the soil-dwelling life stages of FCM (Coombes, 2013). In general, a dose-dependent relationship was found with an increase in mortality associated with an increase in conidial concentration, with LC₅₀ values of 6.26×10^5 , 1.92×10^6 and 1.98×10^5 conidia/mL calculated for isolates G 11 3 L6, FCM Ar 23 B3, and G Ar 17 B3. Likewise, an increase in mortality was observed with an increase in the amount of time (in days) the larvae were exposed to the fungus. At a fungal concentration of 1×10^7 conidia/mL, it was estimated that late fifth instar larvae are required to be exposed to the fungal isolates for a period of nine days to ensure 90% mortality as a result of fungal infection. Results also indicated the better performance of these isolates over two commercial isolates tested and their ability to persist whilst still remaining infective towards FCM 5th instar larvae over a six month period in a citrus orchard. Whether this is true persistence however remains to be determined (Coombes, 2013). Currently, these three isolates are being evaluated as to their performance in the field. No data is yet available as field trials are only to be initiated in September/October 2013.

Opsomming

Die beheer van VKM in die sitrusbedryf is noodsaaklik vanweë die finansiële verlies wat hoofsaaklik as gevolg van die fitosanitêre status geassosieer met VKM kan gebeur. Entomopathogeniese swamme (EPF) kan moontlik as 'n addisionele biologiese beheer opsie teen VKM, spesifiek die grond-woning lewensfasies (laat vyfde instar, pre-papies en papies), dien. Goble *et al.* (2010, 2011) het 62 swam isolate geïsoleer, van grond-steekproewe wat vanuit sitrusboorde in die Oos-Kaap Provinsie van Suid – Afrika versamel was. Twaalf was geïdentifiseer as isolate wat die beste potensieël beheer aantoon (Goble *et al.*, 2011). Verdere laboratorium-ontleding het ontbloot dat drie isolate (*M. anisopliae* G 11 3 L6, *M. anisopliae* FCM Ar 23 B3, en *B. Bassiana* G Ar 17 B3) as die meeste potensiaal teen grond-woning lewensfase van VKM (Coombes, 2013) het. Oor die algemeen, is 'n dosis-afhankende verhouding tussen 'n toename in mortaliteit en swam konsentrasie waargeneem, met LC₅₀ waardes van $6,26 \times 10^5$, $1,92 \times 10^6$ en $1,98 \times 10^5$ spore/mL bereken vir isolate G 11 3 L6, FCM Ar 23 B3, en G Ar 17 B3 onderskeidelik. Net so, is 'n verhoging in mortaliteit waargeneem met 'n verhoging in tydsduur (dae) wat larwes aan die swam blootgestel is. Swam nawerkingsproewe het getoon dat hierdie drie isolate die vermoë het om oor 'n ses maande tydperk nog infeksie in vyfde instar VKM larwes te veroorsaak. Wanneer 'n swam konsentrasie van 1×10^7 spore/mL gebruik is, was dit geskat dat die laat vyfde instar larwe, blootgestel moet word aan die swam isolate vir 'n periode van nege dae om 'n 90% mortaliteit te verseker as 'n resultaat van die swaminfeksie. Resultate toon ook beter prestasie van die isolate aan oor twee kommersiële isolate wat getoets was en hulle vermoë om te volhard terwyl ander nog besmetlik teenoor VKM vyfde instar larwes oor 'n tydperk van ses maande in 'n sitrusboord, óf hierdie ware volharding is, moet nog bepaal word (Coombes, 2013). Hierdie drie isolate word tans geëvalueer om hulle uithou vermoë in die veld te bepaal. Geen data is nog beskikbaar nie, want veldproewe word eers in September/Oktobor 2013 geïnisieer.

3.2.13 **FINAL REPORT: Morphology and taxonomy of tortricid moth pests attacking deciduous fruit and citrus**

Project US/ENT-08-A2 (2011/8 – 2012/1) by P. Addison, M. Rentel, H. Geertsema (SU) and J. Brown (USDA, Washington DC)

Summary

Cydia pomonella (codling moth), *Thaumatotibia leucotreta* (false codling moth), *Thaumatotibia batrachopa* (macadamia nut borer), *Grapholita molesta* (oriental fruit moth), *Cryptophlebia peltastica* (litchi moth), *Epichoristodes acerbella* (pear leafroller/carnation worm) and *Lozotaenia capensana* (apple leafroller) are the most economically important tortricids affecting various crops in South Africa. The correct identification of

these species, especially of the larval stage, is of great importance in pest management. Using available literature, augmented by additional morphological studies, an interactive identification key (Lucid key) for larval and adult stages of the seven species was developed. The colour and markings of the head, characteristics of the prothoracic and anal shields, the position of the prespiracular setae (L-group) relative to the spiracle on the prothoracic segment, the position of the spiracle on the eighth abdominal segment and L-group on the ninth abdominal segment, as well as the presence or absence of the anal comb are key characteristics for larval identification. For adult identification, wing pattern and genitalia are the most important features. However, the use of genitalia for moth identification might be difficult for the lay user, as the dissection and mounting of these structures requires certain skills and specialized equipment. Thus, genitalia have not been included in the Lucid Key. Differences in the morphological characteristics of most pupae were so minute that this stage was also not included in the Lucid key. However, the pupae of *E. acerbella* and *L. capensana* are easily distinguished from those of the other species by the presence of a cremaster. This study also included the first morphological description of the pupa of *L. capensana*, which can be distinguished from that of *E. acerbella* by various features of the cremaster, antennae, spiracle shape, number of setae on abdominal segments A5-7, the size of spines on A3-7, and the presence/absence of spines on A9. Variation in the shape of the valvae of *T. leucotreta* was used to determine whether divergence has occurred between populations of *T. leucotreta*. Elliptical Fourier analysis was used to analyze the valvar variation in three different populations. Although some variation in valvar shape was detected among mean population values for certain traits, no clear pattern emerged. Principle component analysis also showed no distinct clustering of valvae shape among populations, providing no evidence for divergence in male genitalia and therefore no morphological evidence of incipient speciation.

Opsomming

Cydia pomonella (kodlingmot), *Thaumatotibia leucotreta* (valskodlingmot), *T. batrachopa* (makadamianeutboorder), *Grapholita molesta* (oosterse vrugtemot), *Cryptophlebia peltastica* (lietsjiemot), *Epichoristodes acerbella* (peerbladroller/angelierrusper) en *Lozotaenia capensana* (appelbladroller) is die mees ekonomies belangrike tortrisiede vir die vrugtebedryf in Suid-Afrika. Die korrekte identifikasie van hierdie spesies, veral die larwale stadium, is van groot belang in plaagbestuur. Deur gebruik te maak van beskikbare literatuur, aangevul deur bykomstige morfologiese studies, is 'n interaktiewe uitkenningssleutel ("Lucid key") vir die larwale en volwasse stadia van die sewe spesies ontwikkel. Die kleur en tekening van die kop, kenmerke van die prothorakale en anale skild, die ligging van die prespirakulêre setae (L-groep) relatief tot die spiraculum op die prothorakale segment, die ligging van die spirakulum op die agste abdominale segment en L-groep op die negende abdominale segment, asook die aan- of afwesigheid van die anale kam is sleutel kenmerke vir larwale uitkenning. Vir die volwassenes is die vlerktekening en genitalia die mees belangrike kenmerke. Die gebruik van die genitalia vir motuutkenning kan egter vir die leek gebruiker moeilik wees omdat die disseksie en montering van hierdie strukture bepaalde vaardighede en gespesialiseerde toerusting vereis. Vir dié rede is die genitalia nie in die Lucid-sleutel ingesluit nie. Verskille in die morfologiese kenmerke van meeste papies is klein en die stadium is gevolglik ook nie in die sleutel ingesluit nie. Die papies van *E. acerbella* en *L. capensana* kan egter maklik van die ander spesies onderskei word deur die aanwesigheid van 'n cremaster. Hierdie studie sluit ook die eerste morfologiese beskrywing van die papie van *L. capensana* in, wat van dié van *E. acerbella* onderskei kan word deur gebruik te maak van kenmerke van die cremaster, antennae, spirakulêre vorm, aantal setae op abdominale segmente A5-7, die grootte van stekels op A3-7, en die aan- of afwesigheid van stekels op A9. Die variasie in die vorm van die valvae van *T. leucotreta* is gebruik om te bepaal of divergensie wel tussen bevolkings van *T. leucotreta* plaasgevind het. Elliptiese Fourier ontleding is gebruik om die valvae se variasie by drie verskillende bevolkings te ontleed. Alhoewel enkele variasie in die vorm van die valvae bespeur is by die gemiddelde bevolkingswaardes vir bepaalde eienskappe, kon geen duidelike patroon bespeur word nie. Hoofkomponentontleding het ook geen duidelike groepering van valvae se vorm tussen bevolkings getoon nie, wat geen bewys lewer van divergensie in die manlike genitalia en dus geen morfologiese bewys van beginnende spesiasie.

Introduction

Tortricidae, commonly known as leafrollers or leaftwisters, are the largest family of microlepidoptera with more than 5000 species (Powell, 1964; Pinhey, 1975; Horak & Brown, 1991). The family includes some of the most economically important pests of agriculture, forest trees, and ornamental plants (Powell, 1964; MacKay 1959; Holloway *et al.*, 1987; Razowski, 2002; Timm, 2005). The family is worldwide in distribution but reaches its greatest species-richness in temperate and tropical regions (Common, 1990; Horak & Brown, 1991; Scoble, 1992). The common name, leafrollers, originates from the larval behaviour of spinning and/or rolling leaves of the host plant upon which they feed and develop (Pinhey, 1975; Timm, 2005). Seven major economically important tortricid species can be found in South Africa, all of which have great impact on the local fruit industry: *Cydia pomonella* (Linnaeus, 1758) (codling moth), *Thaumatotibia leucotreta* (Meyrick,

1913) (false codling moth), *Grapholita molesta* (Busck, 1916) (oriental fruit moth), *Cryptophlebia peltastica* (Meyrick, 1921) (litchi moth), *Thaumatotibia batrachopa* (Meyrick, 1908) (macadamia nut borer), *Epichoristodes acerbella* (Walker, 1864) (pear leafroller/carnation worm) and *Lozotaenia capensana* (Walker, 1863) (apple leafroller) (Table 3.2.13.1). The larvae of all these species feed on a range of cultivated crops causing extensive damage and losses to the fruit industry (Powell, 1964; Timm, 2005).

Correct identification, especially of the immature stages, is important because misidentifications can lead to ineffective pest management. Current keys for tortricid species in South Africa are unsatisfactory because they are mostly incomplete. McGeoch & Krüger (1994) developed a key for identifying moth larvae associated with *Ravenelia* galls on *Acacia karroo*. One important tortricid larval species, *C. peltastica*, found on *Acacia karroo* galls, was included in their study. Krüger (1998) subsequently developed a key for identifying adult moths associated with *Ravenelia* galls on *Acacia karroo*, which included larvae of two economically important tortricid species, *C. peltastica* and *T. leucotreta*. Yet, in both McGeoch & Krüger (1994) and Krüger (1998), no description is provided for either *C. peltastica* or *T. leucotreta*. Timm *et al.* (2007, 2008) produced a dichotomous key to distinguish among six economically important tortricid larvae and their pupae present in South Africa, but for some of these species, especially *L. capensana*, a complete description for the immature life stages is lacking.

Species identification is the basis of traditional taxonomy, also known as alpha taxonomy, which relies on subjective visual evaluations (Mutanen & Pretorius 2007). Traditional taxonomy, which also included the describing of species based on morphology, is facing a serious challenge in that there is a lack of time, funding, and expertise (taxonomists have become a dying breed), and that relevant information available is often inaccessible (Walters & Winterton, 2007). This principle could be assisted by using more integrative taxonomy. Integrative taxonomy combines the use of traditional taxonomy together with multiple disciplines and modern identification techniques such as DNA barcoding, interactive identification keys, and morphometrics.

Walter & Winterton (2007) discussed how searches on “identification keys” and “insect keys” increased dramatically based on the number of “hits” on Google over a one-year period from March 2005 to March 2006. The search “Identification keys” increased over the one year period by almost 100 000 hits and “insect keys” by 19 000 hits.

Dichotomous keys have been, and are still used for identification purposes (Osborne, 1963). In dichotomous keys, each “question” has a couplet with two possible contrasting characters (Osborne, 1963; Amante & Norton, 2003; Walters & Winterton, 2007). Depending on the answer chosen, the user is either redirected to another couplet or to an endpoint providing an identity (Amante & Norton, 2003; Walters & Winterton, 2007). Dichotomous keys frequently present one major problem, the unanswerable couplet, for which a user is not able to decide on one statement, and hence, is unable to continue with the key (Amante & Norton, 2003; Walters & Winterton, 2007). Matrix keys, such as LUCID keys, are more interactive and enable the user to select more than one character to examine or to skip characters which are not conspicuous to them, and still reach a possible identification (Amante & Norton, 2003; Walters & Winterton, 2007).

Morphometrics is the “measurement and analysis of a form” (Daly, 1985) and was traditionally based on size, ratios, and linear measurements (Daly, 1985; Mutanen & Pretorius, 2007). Geometric morphometrics has become more and more popular; enabling users to quantify shapes (Mutanen & Pretorius, 2007).

Timm *et al.* (2010) found no evidence of specific host races in a population of *T. leucotreta* population, but evidence was presented for population structure on a fine-scale, indicating that populations of different geographic origins were genetically distinct. Timm *et al.* (2010) suggested that the reason for this divergence could be limited dispersal. This begs the question of whether speciation within *T. leucotreta* has occurred. To answer this question, the male genitalia were studied and analyzed using shape morphometrics. Powell (1964) mentioned that the “male genitalia in tortricids form the basis for classifications,” and that genitalia alone can be used to determine the identity of a species. Various other authors have proven the importance and taxonomic value of genitalia (Horak, 1984). The shape and size of the genitalia play an important role in identification, and by using geometric morphometrics one can identify quantitative evidence of the difference between different species.

Aim and Objectives:

The aim of the present study was to establish an easy identification system for tortricid moth pests of fruit, including the construction of interactive taxonomic keys of all life stages with associated morphological information.

- i) To collate and analyse all available literature on seven major species of economic importance and to compile more comprehensive morphological descriptions;
- ii) to develop an interactive key based on LUCID software using morphological data (published and own study) of local and potential invasive tortricid moth species for use by fruit industry stakeholders; and
- iii) to determine the species status of *T. leucotreta*, using male genitalia and shape morphometrics.

Materials and methods

Development of a key

Literature was consulted for general morphological descriptions of each species of pest tortricid (as indicated in the proposal), for adults, larvae and pupae with complementary morphological studies to fill the shortcomings. It was advisable to include possible future invasive species in the key. Thus the light brown apple moth (LBAM) (*Epiphyas postvittana*) and the European grapevine moth (EGVM) (*Lobesia botrana*) were included.

Insect material of the following was obtained by donation from established laboratory colonies. FCM was obtained from XSIT & Rhodes University, CM from Entomon, LM from River Bioscience and OFM from Embrapa Grape and Wine, Bento Gonçalves, Brazil and Applied Entomology in the Institute of Agricultural Research, Eidgenössische Technische Hochschule Zürich, Switzerland.

Specimens of LM were obtained from Port Jackson galls, PLR from apples and macadamia nut borer (*Thaumatotibia batrachopa*) from macadamia. Adult specimens were either reared from larval stages or collected using light traps, bucket traps and sticky traps.

A diagnostic key was developed using LUCID key 3.5.2 (Lucid, The University of Queensland), based on a key developed by Gilligan & Epstein (2012).

Reciprocal cross-mating trials (FCM)

Cross-mating experiments of four different populations from three provinces were done to determine if viable offspring is produced.

FCM populations from Addo, Nelspruit and Marble Hall were obtained from the laboratory colony at Rhodes University; one population from Citrusdal was received from XSIT. The colonies from Rhodes University were collected in 2008 and no new wild individuals have been added to these colonies since then. By July 2011 the moths from Addo, Nelspruit and Marble Hall were in the 32nd, 30th and 30th generation, respectively (John Opoku-Debrah, personal communication). The Citrusdal colony was completely restarted in 2010 with a new wild population.

These populations comprise the F₀ generation. This generation was left to acclimatise to the new environment and to mate and produce the F₁ generation. Pupae of F₁ were sexed and placed into separate trays (Figure 3.2.13.1) to insure no mating.

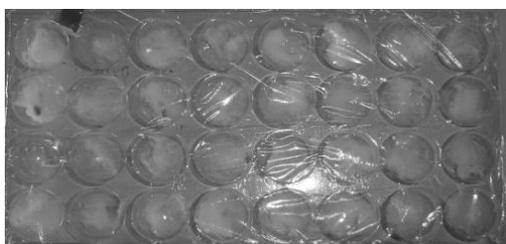


Figure 3.2.13.1. Separation trays used to ensure virgin males and female FCM

One male and one female of each population were placed into a mating arena (treatments) and allowed to mate (Table 3.2.13.1). One male and female from each population were used as controls and two females from each population were also placed together in the mating arena to ensure that females of FCM are not parthenogenic (Table 3.2.13.2).

Standard plastic honey jars (90 mm x 65 mm) were used as the mating arenas for trial 1. For trial 2, holes were made in the lids of the honey jars and covered with fabric gauze.

In trial 1, strips of folded wax paper were added to the arenas to provide a suitable surface area for the female to oviposit.

Table 3.2.13.1. Treatment crossings of different populations for the F₁ generation.

Treatment	Population 1	Population 2
1	Citrusdal ♂	Addo ♀
2	Citrusdal ♀	Addo ♂
3	Citrusdal ♂	Marble Hall ♀
4	Citrusdal ♀	Marble Hall ♂
5	Citrusdal ♂	Nelspruit ♀
6	Citrusdal ♀	Nelspruit ♂
7	Addo ♂	Marble Hall ♀
8	Addo ♀	Marble Hall ♂
9	Addo ♂	Nelspruit ♀
10	Addo ♀	Nelspruit ♂
11	Marble Hall ♂	Nelspruit ♀
12	Marble Hall ♀	Nelspruit ♂

Table 3.2.13.2. Control crossings of different populations for the F₁ generation.

Control	Area 1	Area 2
1	Citrusdal ♂	Citrusdal ♀
2	Addo ♂	Addo ♀
3	Marble Hall ♂	Marble Hall ♀
4	Nelspruit ♂	Nelspruit ♀
5	Citrusdal ♀	Citrusdal ♀
6	Addo ♀	Addo ♀
7	Marble Hall ♀	Marble Hall ♀
8	Nelspruit ♀	Nelspruit ♀

Adults were left for five days to mate and then removed from the arena. The number of eggs laid was recorded and diet, received from Entomon, was added.

The generation emerging from these crosses were regarded as F₂ generation and left to develop and pupate. Males and females were again separated and placed into separation trays again to determine fertility of the F₂ generation. Crosses were as in Tables 3.2.13.3 and 3.2.13.4.

Each cross mating was replicated 10 times for trial 1 and eight times for trial 2.

After adults were removed, the honey jars with eggs were placed into controlled environment chambers at 25°C, approximately 70% RH and 12:12 photoperiod.

In addition to the cross mating trials, the length and width of 30 FCM eggs from each population was measured.

Table 3.2.13.3. Treatment crossings of different populations for the F1 generation.

Treatments	Cross 1: Offspring from treatment in Table 3.2.13.1		Cross 2: Offspring from treatment in Table 3.2.13.1	
1	Citrusdal	Addo ♂	Citrusdal	Addo ♀
2	Citrusdal	Addo ♀	Citrusdal	Addo ♂
3	Citrusdal	Marble Hall ♂	Citrusdal	Marble Hall ♀
4	Citrusdal	Marble Hall ♀	Citrusdal	Marble Hall ♂
5	Citrusdal	Nelspruit ♂	Citrusdal	Nelspruit ♀
6	Citrusdal	Nelspruit ♀	Citrusdal	Nelspruit ♂
7	Addo	Marble Hall ♂	Addo	Marble Hall ♀
8	Addo	Marble Hall ♀	Addo	Marble Hall ♂
9	Addo	Nelspruit ♂	Addo	Nelspruit ♀
10	Addo	Nelspruit ♀	Addo	Nelspruit ♂
11	Marble Hall	Nelspruit ♂	Marble Hall	Nelspruit ♀
12	Marble Hall	Nelspruit ♀	Marble Hall	Nelspruit ♂

Table 3.2.13.4. Control crossings of different populations for the F2 generation.

Control	Cross 1: Offspring from control in Table 3.2.13.2		Cross 2: Offspring from control Table 3.2.13.2	
1	Citrusdal	Citrusdal ♂	Citrusdal	Citrusdal ♀
2	Addo	Addo ♂	Addo	Addo ♀
3	Marble Hall	Marble Hall ♂	Marble Hall	Marble Hall ♀
4	Nelspruit	Nelspruit ♂	Nelspruit	Nelspruit ♀
5	Citrusdal	Citrusdal ♀	Citrusdal	Citrusdal ♀
6	Addo	Addo ♀	Addo	Addo ♀
7	Marble Hall	Marble Hall ♀	Marble Hall	Marble Hall ♀
8	Nelspruit	Nelspruit ♀	Nelspruit ♀	Nelspruit ♀

Morphometric study

Abdomens of FCM males were placed into 10% KOH and left submerged for 24 hours. The genitalia were then removed from the abdomen and cleaned, using a number 00 brush. The valvae of the male genitalia were removed and positioned under glass pieces and left for 24 hours in xylol to harden, after which slides were prepared using Euparal (Bioquip Products, Inc) as a mounting medium. Special care was given to mount each specimen identically so that variation in genitalia shape could be determined. Genitalia were photographed using a Leica DM 2000 automontage microscope with a Leica DFC 295 fixed digital camera and Leica Application Suite (LAS) v.4.0.0. Using Adobe Photoshop Element v.9.0.0 (Adobe System Incorporated), the valvae were each outlined separately, aligned in a single file, and a black and white bitmap image was created for analysis using SHAPE (Fig. 3.2.13.2) (Iwata & Ukai, 2002).

SHAPE was used to determine the contour shapes of the left and right valvae by calculating Elliptic Fourier descriptors (EFDs), which were then converted to principal components for a principal components analysis. SHAPE analysis follows the procedure outlined in Gilligan & Wenzel (2008). A total of 30 harmonics were included. To determine the proportion of variance explained by each principle component axis, the eigenvalue for each axis was assessed. Principle component scores from axes 1 (PC1) to 5 (PC5) were used for further analysis as these accounted for more than 80% of the total variation in both valvae, and the PCs for each of the populations overlap, indicating that there is no significant variation in valvae shape. The mean PC scores were calculated and plotted together to determine the overall variance among populations. To quantify variation among the three populations, a one-way ANOVA was calculated for the principle

components (PC1 – PC5) of both the left and right valvae (Table 3.2.13.9). A Bonferonni adjustment for multiple comparisons was applied by dividing the significance level (0.05) by the number of comparisons (five) to obtain an adjusted significance level of 0.01. Further analyses to determine intraspecific variance in both valvae were conducted in SAS Enterprise guide and Excel, Microsoft Office 2010.



Figure 3.2.13.2: Outlines of left and right valva of *T. leucotreta* used for shape analysis: Specimens arranged by populations: Row 1: Citrusdal (14, 15, 16, 18, 19, 22, 23); Row 2: Citrusdal (5, 7, 9, 10, 11, 12, 13); Row 3: Addo (9, 10, 11, 12, 13, 14, 17); Row 4: Addo (2, 3, 4, 5, 6, 7, 8); Row 5: Mbombela (12, 13, 14, 15, 16, 18, 19); Row 6: Mbombela (2, 6, 7, 8, 9, 10, 11).

Results and discussion

- Task table

Objective / Milestone	Achievement
A. To establish an easy referencing system for tortricid moth pests of fruit, including the construction of taxonomic keys of all life stages and associated morphological and biological information.	
A.1. Collect and combine all morphological descriptions found in the literature of all seven South African species and the two possible invasive species./ April 2012	Completed
A.2. Compare description to specimens and fill the gaps found in literature./ May 2012	Completed, no larval specimens for ALR were obtained.
A.3. Construction of LUCID Key/ June 2012	Completed
B. To determine if FCM adults from different population areas are able to mate and produce viable offspring and if speciation has taken place in FCM.	
B.1. Establishing four population colonies/ June 2011	Colony was established by June 2011, but completely collapsed in January 2012 due to fungal infection
B.2. Cross-mating trials between populations/ December 2011	Two failed cross-mating trials due to fungal infection and escaping larvae skewing the results.
C. To determine the sensitivity of geometric morphometrics to distinguish between different FCM populations.	
C.1. Prepare genitalia specimens and take photographs of genitalia/ March 2012	Completed
C.2. Do SHAPE Analysis of the genitalia/ July 2012	Completed

A. Development of a Key

Table 3.2.13.5 indicates a summary of descriptions of the three different life stages found in the literature. The life stages not marked with x indicates that no literature description was found for that specific life stage, such as for CM, for which no description of the pupae and adult was found. Comparing and combining all descriptions is currently underway and should be completed by April 2012.

Table 3.2.13.5. Indication of life stage descriptions found in the literature.

	Larvae	Pupae	Adult
FCM	x	x	x
CM	x	x	x
OFM	x	x	x
LM	x	x	x
MNB	x	x	x
PLR	x	x	x
ALR			
LBAM	x	x	x
EGVM	x	x	x

Larval diagnostic characteristics:

A few larval morphological characteristics are distinct and diagnostic for specific species, and are summarized below.

General: Setal pinacula are usually concolourous with or darker than the body, however, in *E. acerbella* the pinacula are lighter than the body colour. In *T. batrachopa* the pinacula are very distinct and visible with the naked eye, giving the larvae a spotted appearance. The prothoracic and anal shields of *C. pomonella* have characteristic patterns of spots, and those of *E. acerbella* lack markings. Crochets are triordinal in *T. leucotreta* and *T. batrachopa*, biordinal in *C. peltastica*, and uniordinal in *C. pomonella*, *G. molesta* and *E. acerbella* (in the latter, weakly biordinal).

Head: The head in the studied specimens are yellow brown to dark brown, except for that of *E. acerbella*, which is yellow to olive green. *C. pomonella* has a distinctive pattern on the head, giving it a more mottled appearance than in the other species. *T. batrachopa* has no darker pigmentation at the stemmatal region. *E. acerbella* has pigmentation around stemmata II-IV, and the other stemmata are inconspicuous. Stemmata are equal in size except in *G. molesta* and *C. peltastica*, where stemma I is larger. Stemma II is closer to I than to III, except for *C. pomonella* where the three are sometimes equidistant. In *T. batrachopa* they are equidistant, and in *E. acerbella* stemma II is closer to III than to I. Stemma VI is closer to III than to V except in *G. molesta* where it is also sometimes equidistant, and in *E. acerbella* where it is closer to V than III, almost immediately adjacent. O^1 is equidistant to II and III, except in *C. pomonella*, where it is sometimes closer to III. In *G. molesta* and *C. peltastica* O^1 is always closer to III than to II. A^2 is closer to A^1 and A^3 in *T. leucotreta*, *C. peltastica*, and *T. batrachopa*. A^2 is equidistant to A^1 and A^3 in *C. pomonella*, *G. molesta*, and *E. acerbella*. The spinneret is usually rounded distally except in *C. pomonella* where it is tapered. The spinneret at least 8x as long as wide in *T. leucotreta* and *G. molesta*, but less than 7x as long as wide in *C. pomonella*. It is usually about 7x as long as wide in *C. peltastica*, *T. batrachopa*, *E. acerbella*.

Thorax: The pre-spiracular group (L-group) pinaculum is unmodified in *C. pomonella*, *G. molesta* and *E. acerbella*, but in *Thaumatotibia* and *Cryptophlebia* it extends below the spiracle. Distinct micro setae are easily observed on T2-T3 only in *T. batrachopa*. V^1 s are fused to T2-T3 coxae only in *G. molesta*.

Abdomen: The spiracle on A8 is situated on the posterior two-thirds of the segment except on *C. pomonella* and *E. acerbella* where it is situated on the anterior third. SD^2 usually shares a pinacula with SD^1 , but it is sometimes on a separate pinacula in *G. molesta*. In *T. batrachopa* SD^2 shares a pinacula with SD^1 only on A1, and they are on separate pinacula on A2-A8. D^1 and SD^1 share a common pinaculum on A9 except in *E. acerbella* where they are on separate pinacula. The D^2 setae share a pinaculum, forming a dorsal "saddle" on A9 in all but *E. acerbella* where they are on separate pinacula. L-group on A9 is trisetose and on same pinaculum in all but *C. pomonella* where L^1 and L^2 share a pinaculum and L^3 is on a separated pinaculum. Distance between V^1 s A9 compared to A8 is usually farther apart, except in *G. molesta* where they sometimes are equidistant, always equidistant in *C. pomonella*, and closer together in *E. acerbella*. The anal comb is present in all but *C. pomonella* and *C. peltastica*.

From the above it is clear that distinct and unique suites of characters exist that can be applied in the development of a diagnostic key.

A complete summary of adult characters extracted from literature (white boxes) and own morphological study (shaded boxes) is provided (Appendix 1).

Diagnostic characteristics for pupae

A clear distinction exists between species that belong to Olethreutinae (*T. leucotreta*, *C. pomonella*, *G. molesta*, *C. peltastica*, and *T. batrachopa*) and those that belong to Tortricinae (*E. acerbella* and *L. capensana*), and these features are discussed below.

Epichoristodes acerbella and *L. capensana* both have an elongated cremaster, whereas the other five species lack a distinct cremaster. *L. capensana* and *E. acerbella* can be distinguished from each other by the following: the cremaster of *L. capensana* is elongate and longer than wide (as in *E. acerbella*) but narrower and more rounded. The antenna of *L. capensana* extends beyond the mesocoxa to the tip of the mesotarsus compared to *E. acerbella* where they extend beyond the mesocoxa by a length greater than that of the mesocoxa. The spiracles in *E. acerbella* are oblong, arranged along a distinct darker lateral line from A1 to A7, darker lateral line absent in *L. capensana*. *L. capensana* has seven setae on A5-A6, six setae on A7, five setae on A8, and three on A9, whereas *E. acerbella* has seven setae on A5-A7 and five setae on A8 and A9. The posterior row of dorsal spines on A3-A7 is smaller than on the anterior row in *E. acerbella*, and larger than anterior row in *L. capensana*. Spines on A8 in *E. acerbella* are smaller than on A7 but larger in *L. capensana*. *L. capensana* also has some small irregular spines on A9; *E. acerbella* spines on A9 absent.

Although the five species of Olethreutinae do not have diagnostic pupal characteristics that easily distinguish them from each other, certain small differences are present. The males in *T. leucotreta* and *C. pomonella* show signs of sexual dimorphism, the antennae are thickened and more prominent, extending beneath the mesocoxa reaching almost the mesotarsus, while in females the antennae extend beyond the mesocoxa by the length of the mesocoxa (*T. leucotreta*) or slightly longer (*C. pomonella*). Males of *T. leucotreta* also have a double row of spines on A8, whereas in all the other species and females of *T. leucotreta* possess a single row of spines on A8. The outer clypeal setae of *T. leucotreta* and *C. pomonella* is also longer than the inner setae when compared to *G. molesta*, and *C. peltastica*, where the inner pair is longer and in *T. batrachopa* where they are almost of equal length. The maxilla of *G. molesta* and *T. batrachopa* is about 2 times the length of the labial palpus, whereas in the other three species the maxilla is 1.5 times the length of the labial palpus. *Thaumatotibia leucotreta*, *C. pomonella* and *G. molesta*, each have seven setae on A5-A6, 6 setae on A7, four setae on A8 and three setae on A9. *C. peltastica* has seven setae on A5-A7, five setae on A8 and three setae on A9. *Thaumatotibia batrachopa* has seven setae on A5-A7, four setae on A8 and five setae on A9. Spine size increases for all species from A2-A10.

A summary of pupal characters abstracted from literature (white boxes) and own morphological study (grey boxes) is given in Appendix 1.

Adult diagnostic characteristics

Thaumatotibia leucotreta: Chaetosema dark. Antennae one row of scales per segment. Forewing with a distinct "question-mark" along the termen, a semi-circle along the costal margin and a white discal spot situated between these two pattern elements. Males can be easily distinguished by the distinctive scent gland (darker spot) on the hindwing and dense black tufts on the hind tibia. Forewing length: females 9-10 mm, males 6-8 mm (Gilligan *et al.*, 2011).

Cydia pomonella: Chaetosema pale. Antennae one row of scales per segment. Forewing, greyish colouration with a striped grey-white appearance. Forewing with a striped grey-white appearance, dark brown/ metallic gold bronze ocellus. Apical half medium brown. Males with prominent darker long hairs covering the Cu vein. Forewing length: 7-8 mm (Pinhey, 1975).

Grapholita molesta: Chaetosema light to medium brown. Antennae one row of scales per segment. Forewing dark grey brown to black. Row of darker spots starting at apex down to tornus along termen, forming a slight dotted semi-circle. White discal spot present. Fringe dark with white tips. Hindwing medium brown with medium length hairs on fringe. Forewing length: 4-7 mm (Chapman & Lienk, 1971), distinctly smaller than the other known tortricid pests in South Africa.

Cryptophlebia peltastica: Chaetosema light to medium brown. Antennae one row of scales per segment. Forewing with clear distinct dark brown black triangle (tornal spot) along the dorsum with two light bronze thin bands crossing the tornal spot horizontally. Prominent irregular "bands" or spots crossing diagonal from costal fold to termen. White discal spot present. Fringe darker grey. Forewing length 5-10 mm.

Thaumatotibia batrachopa: Chaetosema pale. Antennae one row of scales per segment. Forewing with light brown down-pointed acute terminal spot; olive green basal and tornal spot, white discal spot present. Sexual dimorphic. Males with scalloped posterior margin and whitish cilia on 1A+2B, prominent anal tuft, longer than in other species; hairy abdomen and hind tibia with denser tufts as in *T. leucotreta*. Prominent anal tuft in males, longer than in other species. Forewing length: 8-10 mm (Meyrick, 1908).

Epichoristodes acerbella: Chaetosema paler. Antennae two rows of scales per segment. Forewing with light to medium yellow to orange colouration sometimes with small brown spots all over. Dorsal area darker in colouration, sometimes with a clear median fascia. Elongated shape, pointed at the apex, rounded at tornus and termen giving the wing a pointed look. Long fringe at anal angle of hindwing. Fore femora with darker scales. Forewing length: 4-7 mm, elongated, pointed apically, rounded at tornus and termen giving the wing a pointed appearance.

Lozotaenia capensana: Chaetosema pale. Antennae two rows of scales per segment. Forewing rectangular-shaped and ranging from darker reddish to brown, to yellow, brown orange to brown. With or without various different markings or patterns. Forelegs covered with darker scales, mid legs with slightly darker scales, hind legs with light brown scales. Mid and hind apical spurs with dark scales. Forewing length: 8–12 mm, rectangular.

A. Reciprocal Cross-mating trials (FCM)

Two trials where conducted for the cross-mating experiment.

Trial 1:

Trial 1 failed shortly after the start of the experiment as the humidity level in the arenas was too high, resulting in fungal infection in the arenas.

Some larvae of replicates 1, 5, 6, survived to pupation, as these arenas were not infected.

The average number of eggs laid by females in the treatment was 253.73 eggs (Table 3.2.13.6). For the male-female control, females laid an average of 293.4 eggs (Table 3.2.13.7) and for female-female control the average number of eggs laid was 82.875 (Table 3.2.13.8). However, all of the eggs laid by the latter cross were infertile producing no offspring.

Table 3.2.13.6. Average number of eggs laid by the F1 generation from 10 replicates of the treatments.

Treatment	Populations 1	Populations 2	Average
1	Citrusdal ♂	Addo ♀	270.7
2	Citrusdal ♀	Addo ♂	272.9
3	Citrusdal ♂	Marble Hall ♀	339.5
4	Citrusdal ♀	Marble Hall ♂	224.3
5	Citrusdal ♂	Nelspruit ♀	193.2
6	Citrusdal ♀	Nelspruit ♂	277.2
7	Addo ♂	Marble Hall ♀	267.5
8	Addo ♀	Marble Hall ♂	216.6
9	Addo ♂	Nelspruit ♀	247.5
10	Addo ♀	Nelspruit ♂	309.6
11	Marble Hall ♂	Nelspruit ♀	181.8
12	Marble Hall ♀	Nelspruit ♂	244
		Average	253.73

Table 3.2.13.7. Average number of eggs laid by the F1 generation from ten replicates of the male-female control.

Control	Population 1	Population 2	Average
1	Citrusdal♂	Citrusdal♀	335.8
2	Addo♂	Addo♀	297.3
3	Marble Hall♂	Marble Hall♀	263.8
4	Nelspruit♂	Nelspruit♀	276.7
		Average	293.4

Table 3.2.13.8. Average number of eggs laid by the F1 generation from 10 replicates of the female-female control.

Control	Population 1	Population 2	Average
5	Citrusdal♀	Citrusdal♀	118.3
6	Addo♀	Addo♀	75.3
7	Marble Hall♀	Marble Hall♀	63
8	Nelspruit♀	Nelspruit♀	74.9
		Average	82.875

Table 3.2.13.9. Average length and width (in mm) of FCM eggs from different populations.

	Mean length	Mean width
Citrusdal	0.78	0.65
Addo	0.81	0.71
Marble Hall	0.76	0.63
Nelspruit	0.77	0.60
Average	0.78	0.65

The average length of FCM eggs was 0.78 mm and width, 0.65 mm (Table 3.2.13.9). The standard deviation for length was 0.0219105 and width, 0.0451474. This is an indication that there is little variation in egg size between populations.

Trial 2:

Due to constant colony collapse of the Marble Hall colony, it was decided to exclude Marble Hall from Trial 2.

Due to high humidity content in Trial 1 mating arena, the setup was changed by drilling holes into the lids of the honey jars. To prevent larvae from escaping, the lids were covered on the inside using fabric gauze.

However, final instar larvae ate through this gauze and escaped. This caused the results for number of larvae reaching pupation to be skewed and also resulted in failure in completing this trial. A stronger fabric would need to be used in further trials.

The average number of eggs laid by females in the treatment was 132.9 eggs (Table 3.2.13.10). For the male-female control, females laid an average of 118.33 eggs (Table 3.2.13.11) and for female-female control the average number of eggs laid was 70.29 (Table 3.2.13.12). However, all of the eggs laid by these female-female crossings were infertile and produced no offspring.

The average number of larvae that continued up until pupation from the eight replicates was 20.5 (Table 3.2.13.13). However, this number could be skewed as at least 20% of the total number of larvae escaped from the jars by eating their way through the gauze.

Comparing the number eggs laid between Trial 1 and 2, it can be seen that there is a large decrease in the number of eggs laid. This decrease could have been influenced by the malfunctioning of the thermostat in the breeding rooms, causing an increase in the temperature of up to 32°C for three days, during which the F₁ generation moths were left in the mating arena to lay eggs. This high temperature could have influenced the fecundity of the female moths. Additionally, the pupae used as F₁ for Trial 2 were the last of that generation and thus only eight replicates could be done.

The last major problem was that the original colony received from Rhodes kept collapsing due to fungal infections. Additionally, as the colonies from Rhodes being almost 35 generations old by January 2012, with no new material added, inbreeding could have seriously weakened the vigour of the populations.

Table 3.2.13.6. Average number of eggs laid by F₁ generation from eight replicates from the treatments.

Treatment	Population 1	Population 2	Average
1	Citrusdal ♂	Addo ♀	155
2	Citrusdal ♀	Addo ♂	163.29
3	Citrusdal ♂	Nelspruit ♀	188.57
4	Citrusdal ♀	Nelspruit ♂	50.43
5	Addo ♂	Nelspruit ♀	141.71
6	Addo ♀	Nelspruit ♂	92.43
		Average	132.9

Table 3.2.13.7. Average number of eggs laid by F₁ generation from ten replicates from the male female control.

Control	Population 1	Population 2	Average
1	Citrusdal ♂	Citrusdal ♀	110.57
2	Addo ♂	Addo ♀	134.57
3	Nelspruit ♂	Nelspruit ♀	109.86
		Average	118.33

Table 3.2.13.8. Average number of eggs laid by F₁ generation from ten replicates from the female female control.

Control	Population 1	Population 2	Average
4	Citrusdal ♀	Citrusdal ♀	23.57
5	Addo ♀	Addo ♀	107.86
6	Nelspruit ♀	Nelspruit ♀	79.43
		Average	70.29

Table 3.2.13.9. Average number of F₁ generation pupae that developed from eight replicates from the treatments.

Cross	Population 1	Population 2	Average
1	Citrusdal ♂	Addo ♀	29.5
2	Citrusdal ♀	Addo ♂	33.75
3	Citrusdal ♂	Nelspruit ♀	17.875
4	Citrusdal ♀	Nelspruit ♂	17.375
5	Addo ♂	Nelspruit ♀	14

6	Addo ♀	Nelspruit ♂	10.5
		Average	20.5

B. Morphometric study (FCM)

2. Principle Component Analysis

Principle component 1 (PC1) and Principle component 2 (PC2) together accounted for 53.5% and 58.4% of the total variation for the left and right valvae, respectively (Figs. 3.2.13.3-5). Combined, PC1 to PC5 accounted for more than 84.9% of variation in the left valvae and 82.3% in the right valvae. Reconstructed shape contours presented in Figs. 3.2.13.2 and 3.2.13.3 allowed for visual examination of the variation of the valvae shape. For the left valvae (Fig. 3.2.13.3), PC1 and PC4 represent the overall size, PC2 represents the overall width, PC3 represents the curvature of the costal margin, and PC5 represents the width at the proximal end. For the right valvae (Fig. 3.2.13.4), PC1 represents the overall size, PC2 represents the width at the proximal end, PC3 represents the overall width, PC4 represents the curvature of the costal margin, and PC5 represents the width at the proximal end and curvature of the costal margin. Because more than 50% of the variation was accounted for by PC1 and PC2, and because other PCs accounted for only minor (>15%) variation, only these two axes were used to provide a visual representation of the overlap in variation among the populations (Polihronakis, 2006). As shown in Fig. 3.2.13.5, PC scores across the three *T. leucotreta* populations overlap on both PC axes, with high levels of intraspecific variation and no distinct grouping formed by any single population. The means for each population are displayed in Fig.3.2.13.6.

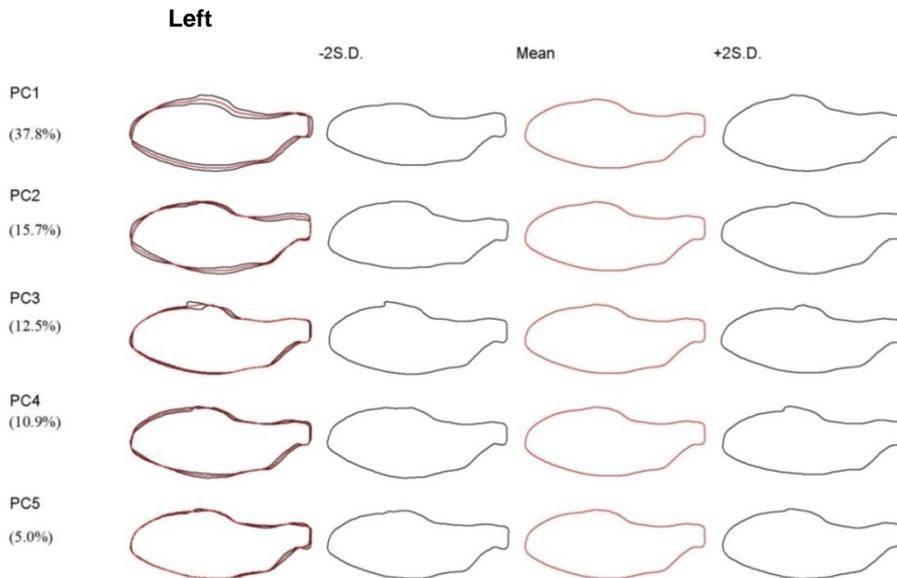


Figure 3.2.13.3. Reconstructed shape contours of the principle components analysis and corresponding percentages for left valva. Superimposed outlines on the left are combined on the mean and ± 2 standard deviations. Mean is outlined in red.

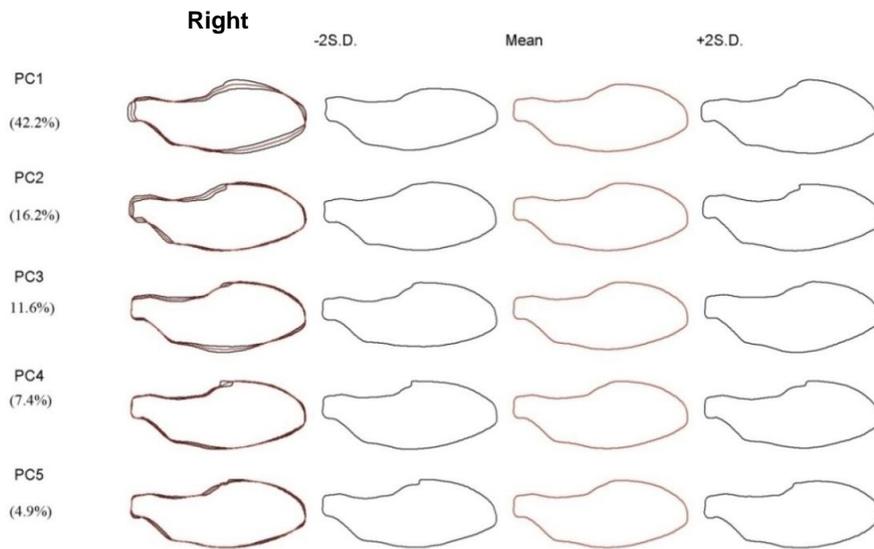


Figure 3.2.13.4. Reconstructed shape contours of principle components analysis and corresponding percentages for right valva. Superimposed outlines on the left are combined on the mean and ± 2 standard deviations. Mean is outlined in red.

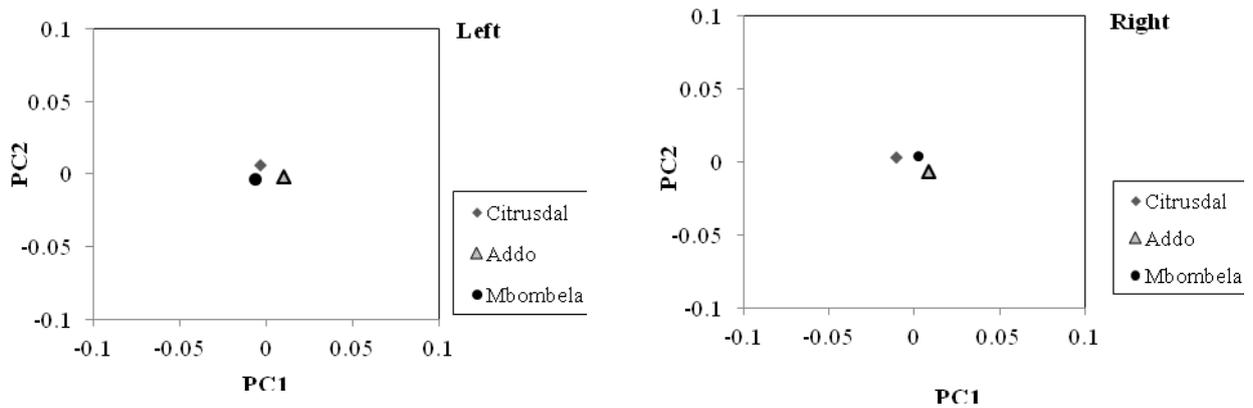


Figure 3.2.13.5. Plot PC1 vs. PC 2 for both left and right valvae. Three *T. leucotreta* populations from different locations were compared.

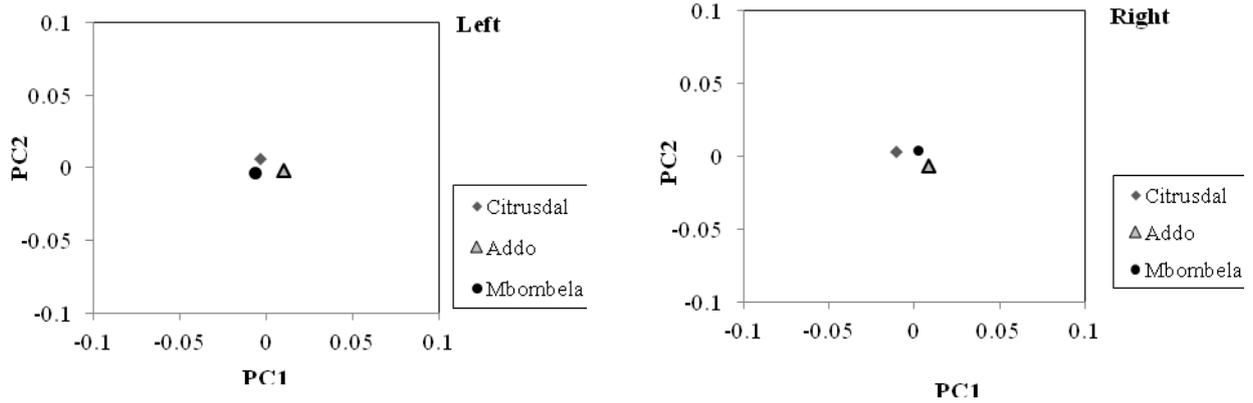


Figure 3.2.13.6. Mean of PC1 vs. mean of PC 2 for each population, indicating the centre of distribution.

Analysis of Variance

To determine if there was a statistically significant difference among PCs in each population, standard errors of the mean for left and right valvae were plotted in Fig. 3.2.13.7. In both, the left and the right valvae, the PCs for each of the populations overlap, indicating that there is no significant variation in valvar shape. For the left valvae, significant variance was demonstrated in PC4 (df = 2; $P= 0.0069$) and PC 5 (df = 2; $P= <0.0001$) (Table 3.2.13.12); however, together these two PCs contribute only 15% of the total variation (Figs 3.2.13.6-7), compared to PC1 and PC2 which account for more than 50% of the total variation. For the right valvae, none of the PC scores showed significant variation (Table 3.2.13.12).

Table 3.2.13.10. Analysis of variance of *T. leucotreta* male valvae. Values in bold indicate statistical significance ($p \leq 0.01$; $df= 2$).

Population	Nr	PC1			PC2			PC3			PC4			PC 5		
		Mean	SD	SE mean	Mean	SD	SE mean									
Left valve																
Addo	14	0.0100	0.0214	0.0057	-0.0021	0.0152	0.0041	0.0047	0.0152	0.0041	-0.0077	0.0115	0.0031	-0.0049	0.0065	0.0017
Citrusdal	14	-0.0037	0.0135	0.0036	0.0063	0.0132	0.0035	-0.0014	0.0140	0.0038	0.0080	0.0116	0.0031	-0.0030	0.0076	0.0020
Mbombela	14	0.0063	0.0353	0.0094	-0.0042	0.0196	0.0052	-0.0033	0.0145	0.0039	-0.0002	0.0138	0.0037	0.0079	0.0082	0.0022
		Df	F ratio	P	df	F ratio	P									
Variance		2	1.70	0.1962	2	1.63	0.2099	2	1.15	0.3267	2	5.67	0.0069	2	12	<0.0001
Right valve																
Addo	14	0.0084	0.0290	0.0077	-0.0065	0.0139	0.0037	0.0027	0.0156	0.0042	0.0019	0.0139	0.0037	0.0014	0.0086	0.0023
Citrusdal	14	-0.011	0.0194	0.0052	0.0030	0.0167	0.0045	0.0040	0.0128	0.0034	-0.0019	0.0101	0.0027	0.0027	0.0080	0.0021
Mbombela	14	0.0023	0.0261	0.0070	0.0035	0.0163	0.0043	-0.0067	0.0099	0.0026	0.00004	0.0079	0.0021	-0.0040	0.0088	0.0024
		Df	F ratio	P	df	F ratio	P									
Variance		2	2.10	0.1356	2	1.83	0.1732	2	2.84	0.0706	2	0.42	0.6609	2	2.47	0.0977

Based on the non-overlap between error bars in Fig. 3.2.13.7, it is evident that the Citrusdal population differs from the Addo population using PC1 and PC2 for both the left and right valvae. For the left valve, the Addo population differs significantly from the other populations for PC3. All three populations differed significantly from each other for PC4 and Mbombela from the other two populations for PC5, however, it should be noted that PC3-PC5 together contributed less than 30% to the variation in shape. For the right valvae, PC3 and PC5 of Mbombela differed significantly from the other two populations. PC4 showed no significant difference for any population. Again it should be noted that PC3-PC5 accounts for less than 25% of the variation in the shape of the valvae. Comparing these to Fig. 3.2.13.6, it is evident that no clear clustering or pattern is formed, thus only limited variation exists.

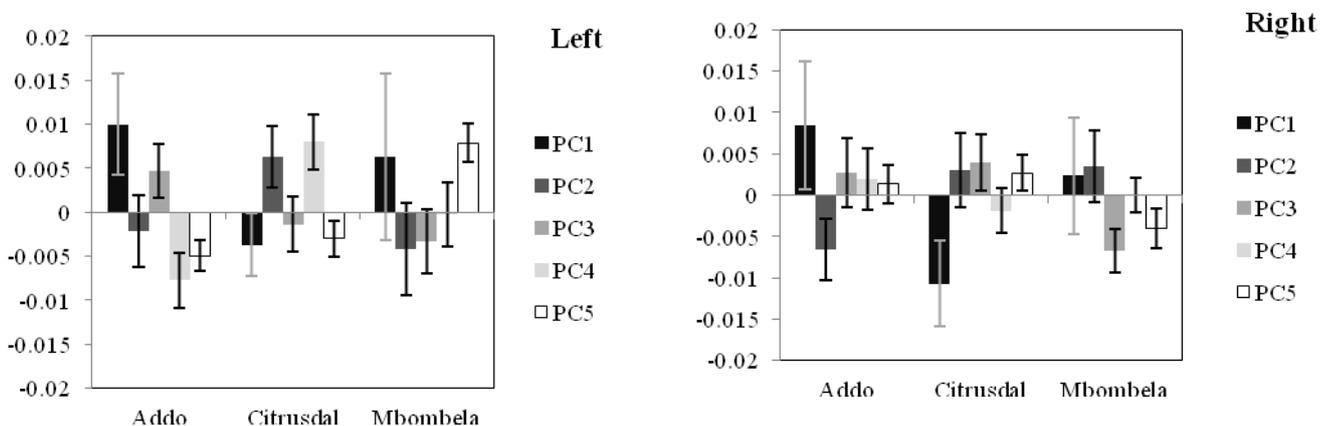


Figure 3.2.13.7. Means (\pm SE) for left and right valvae to determine significant differences in PC values between populations.

3. Discussion and conclusion

The principle component scores significantly differed for only two out of 10 axes. However, these two axes (PC4 and PC5 for the left valvae) contributed only 15% of the variation, insufficient to be described in standard morphological terms. By examining the mean variation in PC axes, certain significant differences were determined among populations, but no clear pattern emerged. The PC score plots showed overlap across all populations with no clustering that would allow distinction across the groups. Without any distinct morphometric differences among populations for both the left and right valvae, it is unlikely that male genitalia can be used to determine slight population differences, let alone if speciation events have occurred in *T. leucotreta*. In a study conducted by Gilligan & Wenzel (2008) on four populations of *Hystrichophora stygiana* (Dyar), high variation in valvar shape was demonstrated within a single population. Similarly, a study on the butterfly *Maniola jurtina* found extreme variability in the valvae of individuals from different populations (Goulson, 1993). Male genitalia are influenced by sexual selection and can evolve at a faster rate than many other traits, thus, genital traits are not always as strongly constrained (Lorkovic, 1953 in Goulson, 1993; Eberhard, 1985). The same argument could be used to explain the limited variation in valvae observed in this study. Although Timm *et al.* (2010) found evidence that populations of *T. leucotreta* from different geographic locations were genetically distinct; no evidence for population-level divergence was found in the morphology of the valvae of the male genitalia. If genitalia diverge and evolve more rapidly than any other body part (Eberhard, 2010a, 2010b), variation among populations would have provided morphological evidence that speciation has occurred. This may be an indication that *T. leucotreta* has undergone slight genetic and morphological divergence but not enough to be recognized as clearly separate lineages.

These results are of significance for alternative pest management practices such as sterile insect technique (SIT) and monitoring systems using sex pheromones. SIT relies on the mating of sterile males from the rearing facility in Citrusdal with wild females in areas throughout the Western Cape Province. If the male genitalia of the wild population used for SIT differ from those of a different wild population in a different geographic location, the SIT programme could fail due to mating incompatibility. For the present, it can be concluded that *T. leucotreta* of different populations are able to mate with each other and therefore without major implications on alternative pest management programs. This could, however, not be fully quantified in this study due to rearing problems. One Masters student was trained, and graduated during March 2013.

Conclusion

The primary goal of this study was to establish a user-friendly tool to aid the identification of the seven major tortricid pests in the South African fruit industry: *C. pomonella*, *T. leucotreta*, *G. molesta*, *C. peltastica*, *T. batrachopa*, *E. acerbella* and *L. capensana*. The specific objectives were to 1) collate and analyse all available literature on the aforementioned seven species and to supplement this information with new morphological analyses to establish more comprehensive descriptions; 2) develop an interactive key based on LUCID software based on morphological data of local and potential invasive tortricid moth species for use by fruit industry stakeholders; and 3) using shape morphometrics of the male genitalia, determine whether geographically isolated populations of *T. leucotreta* can be distinguished.

From these results it is recommended that *T. leucotreta* populations be monitored over time, and that several factors be taken into account to determine if speciation events take place in future, as suggested by de Queiroz (2007).

Technology Transfer

Oral presentations were made at the biennial Citrus Symposium in August 2012 and the Congress of the Entomological Society of southern Africa in July 2013.

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APPENDIX 1

Important morphological characteristics to identify and distinguish between the six economically important tortricid larvae (White boxes as in literature. Dark grey and highlight boxes are morphological characteristics that were studied and added).

<u>General</u>	<i>T. leucotreta</i>	<i>C. pomonella</i>	<i>G. molesta</i>	<i>C. peltastica</i>	<i>T. batrachopa</i>	<i>E. acerbella</i>
Size	12-25 mm length	15-19mm length	10-15mm	20mm	13-23 mm length	17-21
Shape	Slender elongated	Slender elongated	Elongated	Elongated	Elongated	Elongated
Colour	Cream to light red, orange	Body colour to light brown	Whitish, light brown or more often reddish	Whitish brown to light pink to light red,	Cream to grey green	Light green with stripes: dark green on dorsal-yellow green on lateral midlines
Integument structure	Rugose					
Setal pinacula	Easily observed	Moderate in size	Moderately large	Easily observed, large,.	Moderately large	Conspicuous
Setal pinacula colour	Darker than body colour	Body colour or darker greyish	Body coloured	Darker than body colour	Medium to dark greyish brown	Lighter than body colour
Setal length	Moderately long	Moderately long, spine-like appearance	Short, spine-like	Moderately long	Moderately long	Moderately long
Spinulation of integument	Easily apparent	Easily observed	Slender, darker than body colour	Slender, darker than body colour	Easily observed	Easily observed
<u>Head</u>	<i>T. leucotreta</i>	<i>C. pomonella</i>	<i>G. molesta</i>	<i>C. peltastica</i>	<i>T. batrachopa</i>	<i>E. acerbella</i>
Head colour	Yellowish brown to dark brown	Yellow brown	Yellow brown to black	Light to dark brown	Yellow brown	Yellow brown to olive green
Head pigmentation	Darker pigmentation at ocellar area and postgenal juncture	Dark brown pattern & blackish pigment on ocellar areas and at postgenal juncture.	Often overlaid with darker pattern; dark pigmentation on ocellar area & at postgenal juncture.	Darker pigmentation at ocellar area and postgenal juncture	Darker pigmentation at ocellar area	Darker pigmentation at postgenal juncture
Head shape	Hypognathous					
Aver. width prior pupation	1.31 mm	1.71 mm	0.92-1.11mm	1.7mm	1.5mm	1.04mm
Head position	Vertical angle acute	Vertical angle acute	Vertical angle more or less acute	Vertical angle acute	Vertical angle acute	Vertical angle acute
Adfrontals	Tapering anteriorly, extending to vertical angle	Extending to vertical angle	Extending to vertical angle	Extending to vertical angle, tapering anteriorly	Extending to vertical angle, tapering anterior- & posteriorly	Narrow, extending to vertical angle
Ocellar shape	Rounded	Rounded	Rounded	Rounded	Rounded	Rounded
Stemmata size & shape	Equal in size, irregularly rounded	Large distinct, convex, irregularly rounded except for VI, more oval	Irregularly rounded, I larger than others	II-VI rounded equal size, I elongate, approx. 1.5x diameter of III.	Equal in size, irregularly rounded	Inconspicuous except for III, surrounded by dark pigmentation
Stemma II position	Closer to I than III	Closer to I than III or equidistant	Less than diameter away from stemmata I and III	Closer to I than III, by less than half a diameter of II.	Equidistant from I & III	Closer to III than I.
Stemma III position	Closer to IV than II	Closer to IV than II	Very close to IV	Closer to IV than II	Closer to IV than II	Closer to IV than II
Stemma IV	Closer to III than V	Closer to III than V	Equidistant from III and V, sometimes closer to III	IV closer to III than VI	IV closer to III than VI	Closer to V than III, almost connecting
Stemma V position	Close to IV than VI	Closer to IV than VI	Closer to IV than VI	Closer to IV than VI	Closer to IV than VI	Closer to IV than VI
Stemma V separated from stemma VI	By nearly half diameter of V	By slightly more than half the diameter of V	By nearly diameter of V	By slightly 1.5x diameter of V	By equal diameter of V	By more than a diameter of V

Stemma IV separated from stemma VI	By distance slightly less than diameter of IV	By ½ diameter of IV	By ½ diameter of IV	By distance slightly less than diameter of IV	By distance less than ½ diameter of IV	By distance slightly less than diameter of IV	
O¹ distance from stemma II & III	Equidistant	Equidistant, or slightly closer to III	Closer to stemma III	Closer to stemma III	Equidistant	Equidistant	
O² position	Posterior ventrad to I	More ventral than caudal to O ¹	Ventral to I,	Posterior and in line with I	Posterior ventrad to I	Posterior and in line with I	
A² position to A¹ and A³	Closer to A ¹ than A ³	Equidistant	Equidistant	Closer to A ¹ than A ³	Closer to A ¹ than A ³	Equidistant	
A³ position	Closer to L ¹ than A ²	Closer to A ² than L ¹	Equidistant to A ² and L ¹	Closer to L ¹ than A ²	Closer to L ¹ than A ²	Equidistant to A ² and L ¹	
Lines joining O¹ & A¹	Closer to III than II	Equidistant or closer to III than II	Through anterior ventrad edge of II	Closer to III than II	Through median of III	Closer to II than III	
Lines joining O¹ & L¹	Through median of I	Through median of I	Through median of I	Through median of I.	Through median of I	Through median of I	
Lines joining O¹ & A²	Closer to II than III	Through median of II	Through median or anterior edge of II	Closer to II than III.	Equidistant from II & III	Closer to II than I	
P¹ position	Closer to Adf ² than F ¹	Closer to Adf ² than F ¹	Closer to Adf ² than F ¹	Closer to Adf ² than F ¹	Closer to Adf ² than F ¹	Closer to Adf ² than F ¹	
Mandibles	Five teeth	Five teeth, darker in denticulate region	Five teeth	Five teeth	Five teeth	Five teeth	
Mandibles 1 - 3 teeth	Larger, acuminate, often 2 & 3 flattened	Large and pointed, often 2 & 3 flattened	Large and pointed, second largest	Larger, acuminate, often 2 and 3 flattened	Larger, acuminate, often 2 and 3 flattened	2 and 3 large and pointed	
Mandibles teeth 4	Smaller and flattened	Smaller, often flattened	Smaller	Smaller and flattened	Smaller and flattened	Smaller and flattened	
Mandibles teeth 5	Straight-edged	Bluntly rounded	Smallest and bluntly rounded	Straight-edged	Straight-edged	Straight-edged	
Distal end of spinneret	Rounded,	Tapered,	Rounded,	Rounded,	Rounded,	Rounded	
Spinneret length	8x longer than wide	6-6.5x longer than wide	7-8.5x longer than wide	7x longer than wide	7x longer than wide	7x longer than wide	
Thorax	<i>T. leucotreta</i>	<i>C. pomonella</i>	<i>G. molesta</i>	<i>C. peltastica</i>	<i>T. batrachopa</i>	<i>E. acerbella</i>	
Prothoracic shield colour	Yellowish brown to dark brown	Body coloured to slightly greyish brown	Yellowish brown	Yellow brown	Yellow brown	Yellow or body coloured	
Prothoracic shield pattern	Darker patches	Fully coloured or with posterior shading	Brownish pattern- speckled appearance	Sometimes with green or brown pigment present	Some medium brown pigmentation	Lightly sclerotized with small patches of darker pigmentation.	No pattern
SD¹ position on prothoracic shield	Equidistant to SD ² than XD ²	SD ¹ closer to SD ² than XD ²	Equidistant to SD ² than XD ²	SD ¹ slightly closer to SD ² than XD ²	SD ¹ equidistant from XD ² and SD ²	Equidistant to SD ² than XD ²	
D¹ position on prothoracic shield	Slightly posterior to D ² , slightly below level of XD ¹	Slightly posterior to D ² , above level of XD ¹	Slightly posterior to D ² , in line with XD ¹	Posterior to XD ¹ and anterior to D ²	Slightly posterior to D ² , slightly below level of XD ¹	Slightly posterior to D ² , above the level of XD ¹	
Spiracle shape	Circular, large	More oval than circular	Circular	Oval/Circular	Very prominent, circular	Circular,	
Spiracle colour	Medium to dark brown	Light to medium brown	Dark brown -black	Dark brown	Dark brown	Dark brown	
L group pinaculum on T1	Enlarged, extending below spiracle	Not extending below spiracle	Not extending below spiracle	Extending beyond spiracle	Extending beyond spiracle	Not extending below spiracle	
L¹ distance & angle from L² & L³	Equidistant, straight line	Equidistant, straight line	Equidistant or closer to L ² , below a straight line joining L ² and L ³ ,	Equidistant & in straight-line	Straight line	Equidistant, straight line	
L-group length	L ¹ longest, L ² shortest, L ³ 1/3 to a 1/4 x shorter than L ¹	L ² twice as long as L ³ , L ¹ longer	L ¹ longest,	L ¹ longest, L ³ shortest	L ¹ longest, L ³ shortest	L ¹ longest, L ³ shortest	
D¹ position to D² on T2-3	Dorsal						
V's position on T2 & T3	Separated from coxae	Separated from coxae	Fused to coxae	Separated from coxae	Separated from coxae	Separated from coxae	
Thoracic claws	Small, curved, medium	Short, light brown	Pale, curved, slender	Medium brown, curved	Medium brown, curved	Curved	

	brown					
Abdomen	<i>T. leucotreta</i>	<i>C. pomonella</i>	<i>G. molesta</i>	<i>C. peltastica</i>	<i>T. batrachopa</i>	<i>E. acerbella</i>
Spiracle shape & size	Oval, small, seldom larger than setal bases	Small, elliptical, larger than setal base.	Moderate	Small, circular, seldom larger than setal base	Small, circular, seldom larger than setal base	Circular, smaller than setal base
SD¹ distance from spiracle	1.5x spiracle diameter	2-3x its diameter	1.5-3x SD ¹ diameter except on A8	2.5x its diameter from spiracle	1.5x spiracle diameter	0.5x spiracle diameter
Position of spiracle on A8	Posterior third of segment	Anterior two-thirds	Posterior third of segment	Posterior third of segment	Posterior third of segment	Anterior two-thirds
SD¹ and SD² position	On same pinaculum	On same pinaculum	On same pinaculum, sometimes SD ² separated	On A1 same pinaculum, On A2-A8 separate pinacula.	On same pinaculum	On same pinaculum
SD² A1-A8	Highly reduced, appearing absent	Highly reduced, appearing absent	Highly reduced, appearing absent	Highly reduced, appearing absent	Highly reduced, white setal base	Highly reduced, appearing absent
SD¹ position to spiracle on A8	Anteroventral, 3x spiracle diameter	Anterior or slightly anterior ventral, 1.5-2x its diameter	Approx. anterior, 1- 1.5x its diameter	Anteroventrally, 1.5x spiracle diameter	Anteroventrally, 2x spiracle diameter	Anteroventrally, by half spiracle diameter.
L group on A9	Trisetose	Trisetose, or bisetose with L ³ separated on own pinacula	Trisetose	Trisetose	Trisetose	Trisetose
SV group	A7 Bisetose, A9 Unisetose	A1-A2 trisetose, A1-A2, A7-A8 bisetose, A9 unisetose	A9 Unisetose	A9 Bisetose	A8 Bisetose, A9 Unisetose	Bisetose on A8 and A9
SV² position to SV¹ on A1-A2	Ventrad and slightly cephalad	Anterior-ventrad	Anterior-ventrad	Ventrad and slightly cephalad from SV1	Anterior-ventrad	Ventral
SV³ position to SV¹ on A1-A2	Antero-dorsad	Antero-dorsad	Antero-dorsad, or absent	Dorsal	Antero-dorsad	Antero-dorsad
SV group on A1, 2, 7,8, and 9	3:3:2:2:1	3:3:2:2:1 or 3:3:2,1:1:1 or 2:3:2:2:1 or other combinations	2:3,2:2:2:1; 3,2:3:2:2:1; 2:3,2:2:2:2;	3:3:3:2:2	3:3:2:2:1	3:3:3:2:2
On A9 D¹ & SD¹	On same pinaculum					On separate pinaculum
On A9, D²s position	Sharing single pinacula forming a "saddle"					Not sharing pinaculum
On A9 L¹ distance to L² and L³	Equidistant	Equidistant or slightly further apart	Equidistant	Equidistant	Equidistant	Equidistant or slightly closer to L ³
V¹ on A9 compared to A8	Slightly further apart	Equidistant	Equidistant or further apart	Slightly further apart	Slightly further apart	Equidistant or closer together
Anal comb	Present	Absent	Present	Absent	Present	Present
Anal comb colour	Darkly pigmented	Absent	Yellow to dark brown,	Absent	Darkly pigmented	Transparent
Abdomen	<i>T. leucotreta</i>	<i>C. pomonella</i>	<i>G. molesta</i>	<i>C. peltastica</i>	<i>T. batrachopa</i>	<i>E. acerbella</i>
Prong shape	Basal part of prong strongly tapered dorsally, width of base 1/4 length of tooth, upper levels of larger prongs medially at same level	Absent	Equal in length and parallel, two mesal spines sometimes blunt	Absent	Basal part of prong strongly tapered dorsally, width of base nearly 1/4 length of tooth, prongs merging into distinct medial structure	Width of base approx equal to or less than length of prongs. Basal part of each prong tapered dorsally
Number of prongs	2 –10 bluntly dentate prongs	Absent	4-5 prongs	Absent	5-8 prongs	6-9 bluntly dentate prongs
Anal shield colour	Medium brown	Darker than body colour	Brown	Yellow brown	Yellow brown	Yellow or body coloured
Anal shield pattern	Sometimes small brown pigmentation	Speckled or patchy appearance (larger patches)	Sometimes small brown pigmentation	Sometimes small brown pigmentation	Sometimes small brown pigmentation	No pigmentation
Anal shield shape	Tapering posteriorly, posterior margin evenly rounded.	Posteriorly rounded	Posteriorly rounded	Posteriorly rounded	Posteriorly rounded	Rounded and strongly tapered posteriorly
L¹s distance compared to D¹s	Further apart	Further apart, sometimes equidistant	Further apart	Further apart	Equidistant	Closer together

D²s & L¹s setal length	D ² s half as long as L ¹ s	L ¹ long or longer than anal segment, D ² s half of L ¹ s	D ² s half as long as L ¹ s	L ¹ slightly further lateral than D ¹ s	D ² more than half as long as L ¹	D ² s shorter than L ¹ s
D¹s distance	Closer to SD ¹ than each other.	Closer to SD ¹ than each other	Equidistant from SD ¹ and each other	Closer to SD ¹ than each other almost in straight line.	Closer to SD ¹ than each other.	Closer to SD ¹ than each other.
D¹s & SD¹s setal length	D ¹ s slightly shorter than SD ¹	D ¹ s shorter than SD ¹	D ¹ s slightly shorter than SD ¹	D ¹ s shorter than SD ¹	Equally long or D ¹ slightly longer	Equally long
Proleg crochets arrangement	Irregularly triordinal	Unevenly uniordinal	Uniordinal	Biordinal	Triordinal	Unevenly uniordinal, almost biordinal
Crochets number	36-42	28-35	30-40	50-58	34-44	32-54
Anal proleg crochets arrangement	Irregularly triordinal, absent in medial half	Unevenly uniordinal, "in situ" more or less ovoid	Uniordinal, "in situ" sometimes oval or almost circular	Biordinal, absent in medial half.	Triordinal, absent in medial half	Unevenly uniordinal, almost biordinal
Anal crochet numbers	24-32	15-25	19-25	46-54	26-32	28-36

Important and distinct morphological characteristics for identifying and distinguishing between the six economically important tortricid pupae (White boxes as in literature. Dark grey boxes are morphological characteristics that were studied here and added).

General	<i>T. leucotreta</i>	<i>C. pomonella</i>	<i>G. molesta</i>	<i>C. peltastica</i>	<i>T. batrachopa</i>	<i>E. acerbella</i>	<i>L. capensana</i>
Length & width	8-10 mm long, 2-2.5 mm wide	9-13 mm long, 2-2.5 mm wide	4.5-5.5 mm long, 1.8 mm on average wide	8.5-10.5 mm long, 3-3.5 mm wide.	6-8.5 mm long	8-12 mm long, 2-2.5 mm wide	
Colour	Brown	Brown	Yellow brown	Brown	Brown	Yellow brown	Yellow brown
Cremaster	Absent	Absent	Absent	Absent	Absent	Well developed, elongate, longer than wide, tip broadly flattened	Well developed, elongate, longer than wide, tip flattened and rounded
Head	<i>T. leucotreta</i>	<i>C. pomonella</i>	<i>G. molesta</i>	<i>C. peltastica</i>	<i>T. batrachopa</i>	<i>E. acerbella</i>	<i>L. capensana</i>
Frontal region	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown	Yellow to medium brown	Yellow to medium brown
Front	Slight broad mesal ridge	Smoothly rounded	Slight broad mesal ridge	Slight broad mesal ridge	Slight broad mesal ridge	Smoothly rounded or slight broad mesal ridge	Smoothly rounded broad mesal ridge
Frontal setae	One pair of setae						
Eyes	Large and prominent	Darker and prominent	Darker and prominent	Large and prominent	Large and prominent	Large and prominent	Large and prominent
Clypeal setae	2 pairs, outer pair longer	2 pairs, outer pair longer.	2 pairs, inner pair longer	2 pairs, inner pair longer	2 pairs equal in size	2 pairs, outer pair slightly longer	2 pairs, outer pair slightly longer
Maxilla length	1.5x length of labial palpus	1.5x length of labial palpus.	2x length of labial palpus	1.5x length of labial palpus	2x length of labial palpus	2-2.5x length of labial palps	2-2.5x length of labial palps
Structure of Antenna	Sexual dimorphism <i>Males</i> : Thickened, prominent, extending beneath the mesocoxa, almost to tip of mesotarsus. <i>Females</i> : Extending beneath the mesocoxa, by the length of mesocoxa.	Sexual dimorphism. <i>Males</i> : Thickened and prominent, extending to the tip of mesotarsus. <i>Females</i> : Extending beyond mesocoxa by length longer than that of mesocoxa.	Expanded at the cephalic extremity and gradually tapering caudad	Prominent, extending beyond mesocoxa, by length less than half of mesocoxa	Prominent, extending beyond mesocoxa, by length of mesocoxa	Extending beyond mesocoxa, by length greater than mesocoxa. <i>Males</i> : Thickened	Prominent, extending to tip of mesotarsus
Thorax	<i>T. leucotreta</i>	<i>C. pomonella</i>	<i>G. molesta</i>	<i>C. peltastica</i>	<i>T. batrachopa</i>	<i>E. acerbella</i>	<i>L. capensana</i>
Segments	Well delimited dorsally						
Forewings	Forewings almost obscuring hindwings completely						
Protarsus	Extending past procoxa by 1/4 length of mesocoxa	Extending slightly beyond procoxa.	Extending to tip of procoxa	Extending past procoxa by 1/5 length of mesocoxa	Extending past procoxa by 1/3 length of mesocoxa	Extending past procoxa by 4/5 length of mesocoxa	Extending past procoxa by length of mesocoxa
Mesotarsus	Well developed, clearly delimited. Extending beyond hindwing in line with forewing	Well developed, past extending forewings	Well developed, extending to wing tips	Well developed, clearly delimited	Well developed, past Extending forewings	Well developed, clearly delimited	Well developed, extending slightly past wing tips.
Metacoxa	Slightly visible in females, in males extending almost to tip of mesotarsus	Slightly visible					
Pronotum	5x as long as vertex along midline.	4-4.5x as long as vertex along midline	4x as long as vertex along midline	4x as long as vertex along midline	4x as long as vertex along midline	4x as long as vertex along midline	Not in specimen
Pronotum setae	2 pairs, 1 lateral & 1 medial close to mid-dorsal line						
Mesonotalsetae	2 pairs, 1 midway on alar furrow, 1 pair near mid-dorsal line						
Metanotal setae	1 pair, in anterior corner of the "M" shaped hindwings						

Abdomen	<i>T. leucotreta</i>	<i>C. pomonella</i>	<i>G. molesta</i>	<i>C. peltastica</i>	<i>T. batrachopa</i>	<i>E. acerbella</i>	<i>L. capensana</i>
Spiracle	Oblong, prominent	Oval, prominent	Circular	Oblong, indistinct	Oblong, indistinct	Oblong, prominent with a clear darker lateral line crossing along segments	Oval, prominent.
Ventral lateral half of abdomen, setae on A4	4 setae, 1 pair close to spiracle, 2 single setae - 1 medio-anterior and 1 anterior	3 - 4 setae on A4, 1 pair close to spiracle, single setae one medio-anterior to medial line, 1 setae sometimes concealed by wings	4 setae, 1 pair close to spiracle, 2 single setae - 1 medio-anterior and 1 anterior	3 setae, 1 pair close to spiracle and a single setae medio-anterior.	4 setae, 1 pair close to spiracle, 2 single setae, 1 medio-anterior & 1 anterior.	3 setae, pair close to spiracle, single seta close to medial line.	
Ventral lateral half abdomen setae on A5-A7	7 setae on A5-A6, lateral group paired, 1 group of 3, arranged triangular shaped, and 2 single setae; 6 setae on A7, as for A5-A6, but group of 3 setae reduced to a pair			7 setae, lateral group paired, 1 group of 3, arranged triangular shaped, and 2 single setae			7 setae, lateral group paired, 1 group of 3, arranged triangular shaped, and 2 single setae
Ventral setae on A8	4 setae as for A5-A6, except group of three setae absent			5 pair, 2 pairs, one lateral close to spiracle, 1 close to mid-ventral line. Single seta between pairs	4 setae as for A5-A6, except group of three setae absent	5 pair, 2 pairs, one lateral close to spiracle, 1 close to mid-ventral line. Single seta between pairs	5 pair, 2 pairs, one lateral close to spiracle, 1 close to mid-ventral line. Single seta between pairs
Ventral setae on A9	Three setae spaced evenly on medio-lateral line			3 setae, 1 lateral pair and 1 single setae close to medial line.	5 pair, 2 pairs, 1 lateral, 1 close to mid-ventral line. Single seta between pairs		3 single setae at the posterior margin
Genital opening: females	Ventromedially on anterior margin on A9, extending to anterior margin of A10	Slit-like, ventro-medial on A8	Slit-like, ventro-medial on A8	Ventromedially on anterior margin on A9 and extending to anterior margin of A10	Ventromedially on anterior margin on A9 and extending to anterior margin of A10	Slit-like, ventro-medial on A8	Slit-like, ventro-medial on A8
Genital opening: males	Ventromedially on A9 and extending to anterior margin of A10	Slit-like, ventro-medial on A9 with 2 dome-like structure next to genital opening	Slit-like, ventro-medial on A9 with 2 dome-like structure next to genital opening	Ventromedially on A9 and extending to anterior margin of A10	Ventromedially on A9 and extending to anterior margin of A10	Slit-like, ventro-medial on A9	Slit-like, ventro-medial on A9
Anal rise	Thickened, distinctly curled setae situated anteriorly to the outermost ventral spine on A9	4 pairs of thickened, distinctly curled setae. 2 on ventral side, 2 pairs on dorsal side	2 hooked setae on either side of anal rise, third hooked seta latero-caudad	2 pairs of thickened, distinctly curled setae medially adjacent to ventral spines on A10	2 pairs of thickened, distinctly curled setae. Lateral to anal pore 1 pair of spines, anterior spine larger. Posterior to anal pore - 1 medioposterior pair and adjoining laterally 2 pairs of equal length	smooth	
Anal rise spines in males	Anterior pair of setae on anal rise anterior to anal pore	Anterior pair of setae on anal rise anterior to anal pore	Anterior pair of setae on anal rise anterior to anal pore	Anterior to pore	Adjacent to anal pore	Smooth	
Anal rise spines in females	Anterior pair of setae on anal rise usually adjacent to anal opening	Anterior pair of setae on anal rise usually adjacent to anal opening	Anterior pair of setae on anal rise usually adjacent to anal opening	Anterior pair setae situated anterior to pore	Anterior pair setae situated anterior to pore	Smooth	

Dorsal spines on A1	Smooth without spines, single setae close to medial line.						Smooth, posterior margin developed with posterior directed teeth. Single setae close to medial line on anterior edge
Abdomen	<i>T. leucotreta</i>	<i>C. pomonella</i>	<i>G. molesta</i>	<i>C. peltastica</i>	<i>T. batrachopa</i>	<i>E. acerbella</i>	<i>L. capensana</i>
Dorsal spines on A2	Anterior row extending less than halfway across segment, caudal row well developed and extending almost across segment				Anterior row reduced, extending less than halfway across segment posterior row prominent.	Anterior and posterior row of spines slight	Anterior margin with anterior and posteriorly directed teeth. Anterior directed teeth in posterior ridge. Posterior directed teeth small
Number of dorsal spines on A3-A7	Posteriorly directed dorsal spines, anterior row distinct, posterior row indistinct	Posteriorly directed dorsal spines, anterior row distinct, posterior row indistinct. A7 posterior row shorter than A2-A6	Double row; Cephalic spine row 2x large and half as numerous than on caudal row	Posteriorly directed dorsal spines, anterior row prominent, posterior row indistinct	Posteriorly directed dorsal spines, anterior row prominent, posterior row indistinct	Anterior row of dorsal spines readily apparent and posterior row slight	Posteriorly directed dorsal spines, anterior row smaller than posterior row
Dorsal setae A2-A7	3 single setae, 1 on the lateral margin and 1 close to medial line on anterior ridge. 1 seta half way between lateral margin and medial line on posterior ridge.						
Number of dorsal spines between antero-mesad setae	3-7 spines	6-8 spines	6-9 spines	4-12 spines	4-8 spines	5-12 spines	6-8 spines
Dorsal spines on A8	Single row of prominent spines, 9-10 larger irregularly-sized spines in females. Double row of irregularly-sized spines in males, cephalic row with 8-11 larger spines, Posterior row with 6-8 minute spines	11-18 single row of spines. directed posteriorly	One row of spines, 4-8 larger spines and 0-3 smaller additional spines.	Single row of prominent posteriorly directed dorsal spines, 10-13 spines	Single row of prominent posteriorly directed dorsal spines. Approximately 10 large and 3 small spines	Spines reduced, irregular sized. Anterior row with 10-16 spines. Posterior row with 0-9 spines	12-18 anterior spines and 7-9 posterior minute spines
Dorsal setae on A8	One pair of setae on outer lateral margin and single seta close to mid-dorsal line. 1 setae on lateral margins on posterior row of spines					3 single setae, 1 on the lateral margin and 1 close to medial line on anterior ridge. 1 seta half way between lateral margin and medial line on posterior row of spines	
Dorsal spines on A9	5-6 larger irregular medial spines, some with smaller supplementary spines	7-9 larger spines	One row of spines, 5-9 spines	Single row of prominent posteriorly directed dorsal spines. 6-9 spines	Single row of prominent posteriorly directed dorsal spines. Approximately 8 spines	No Spines	Couple of spines small spines, strong
Dorsal spines on A10	2-3 medial small spines. Two larger spines on the lateral margin with a single seta below each spine	7-12 spines, irregular in size	6 spines, one row	Single row of prominent posteriorly directed dorsal spines	Single row of prominent posteriorly directed dorsal spines	No Spines	
Spine sizes	Increasing to A10	Increasing to A10	Increasing to A10	Increasing to A10	Increasing to A10	Increasing to A7 reduced in A8	Increasing to A8
Cremastral setae	Absent	Absent	Absent	Absent	Absent	4 pairs of thickened, distinct curled cremastral setae	4 pairs of thickened, distinct curled cremastral setae

3.2.14 **PROGRESS REPORT: Impact of abbreviated and complete cold-treatment on survival and fitness of FCM larvae**

Project 1039 (April 2012 – March 2014) by Sean Moore, Wayne Kirkman and Vaughan Hattingh (CRI)

Summary

Due to a Pest Risk Assessment (PRA) currently being conducted by the European Plant Protection Organisation (EPPO) on FCM and the impending debilitating outcome of this PRA for export of southern African citrus to Europe, an ad hoc trial on the effect of abbreviated cold treatments on FCM survival was initiated. Findings of the trials can be summarised as follows: FCM larvae (predominantly 1st (L1) and 5th (L5) instar) were exposed to temperatures of 2°C, 3°C and 4°C for periods ranging from 14 to 22 days, both in fruit and in jars containing artificial diet. There was no L1 survival at 2°C, and no L5 survival after 18 days at 2°C in diet. At 3°C and 4°C there was some L1 survival, and minimal L5 survival even after 22 days of cold treatment. In naturally infested fruit there was no L1 or L2 survival after 18 days at 2°C, 2% L3 survived, 8% L4 survived and 12% L5 survived. It now needs to be determined exactly what degree of risk reduction needs to be shown. Thereafter, trials with the selected parameters (indications at this stage are that this could be 2°C for 18 days) must be replicated sufficient times to statistically demonstrate the point.

Opsomming

As gevolg van 'n Plaag Risiko Raming (PRR) wat tans deur die Europese Plantbeskermings Organisasie (EPPO) op VKM uitgevoer word en die dreigende uitkoms van die PRR vir die uitvoer van sitrus van suidelike Afrika Europa toe, is 'n ad hoc proef oor die effek van verkorte koue behandelings op VKM oorlewing gëinisieer. Proef resultate kan soos volg opgesom word: VKM larwes (oorheersend 1de (L1) en 5de (L5) instar) is aan temperature van 2°C, 3°C en 4°C blootgestel vir tydperke van 14 tot 22 dae, albei in vrugte en in vlessies met kunsmatige dieet. Daar is geen L1 oorlewing teen 2°C en geen L5 oorlewing na 18 dae teen 2°C in dieet. Teen 3°C en 4°C was daar 'n lae vlak van L1 oorlewing en 'n baie lae vlak van L5 oorlewing, selfs na 22 dae se kouebehandeling. In natuurlik besmette vrugte is daar geen oorlewing van L1 of L2 na 18 dae teen 2°C, 2% L3 oorlewing, 8% L4 oorlewing en 12% L5 oorlewing. Dit mate van risiko vermindering wat gewys moet word moet nou bepaal word. Daarna moet proewe met die geslekteerde parameters (op hierdie stadium lyk 18 dae teen 2°C belowend) genoegsaam herhaal word om die punt statisties te kan bewys.

3.2.15 **FINAL REPORT: Ovipositional preferences and differences in host susceptibility of navel orange varieties by false codling moth, *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae) in South Africa**

Project 1041 (Feb 2012 – Sep 2012) by Claire Love, Martin Hill (RU) and Sean Moore (CRI)

Summary

False codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) presents a significant threat to the South African citrus industry. In order to limit income loss due to direct larval damage or from fruit rejection due to the phytosanitary status of this pest, additional preharvest control techniques are required for navel oranges, which are known to be susceptible to FCM damage. A number of navel orange varieties have been developed and differences in female FCM ovipositional preference and susceptibility of varieties to larval penetration are known to exist. Navel orange varieties were grouped according to time of maturity (early, mid and late season). Female FCM were subjected to choice and no-choice tests with these varieties, measured by oviposition. Host susceptibility was tested by allowing neonate FCM larvae to penetrate the different Navel varieties. In the early maturing group, Fischer Navels were least preferred for oviposition and the least susceptible to larval penetration. The mid- and late season maturing groupings showed limited differences in oviposition preference, although host susceptibility did appear to be an important factor in assessing the vulnerability of fruit to FCM. Despite being widely planted in South Africa, the mid-season Palmer Navels were highly susceptible to larval penetration, while for the late season varieties, Cambria and Glenora Late were the least susceptible to FCM. As a result of these laboratory trials, it is recommended that farmers increase cultivation of Fischer Navels as the principal early season variety, avoid Palmer Navels in favour of other mid-season maturing varieties and give preference to the late maturing Cambria and Glenora Late varieties, in order to limit FCM damage.

Opsomming

Valskodlingmot (VKM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), is 'n beduidende dreiging vir die Suid-Afrikaanse sitrusbedryf. Om verliese in inkomste te verminder as gevolg van vrugskade

of afkeurings as gevolg van die fitosanitêre status van die plaag, word assisionele voor-oes bestrydingstegnieke vir Nawellemoene benodig. Nawellemoene is bekend as vatbaar vir VKM skade. Verskeie Nawellemoen varieteite is ontwikkel en verskille in eierleggings voorkeur van VKM wyfies en vatbaarheid van die varieteite vir penetrasie van larwes kom voor. Nawellemoen varieteite is volgens rypwording tyd gegroepeer (vroë, mid en laat seisoen). VKM wyfies is onderworpe aan keuse en nie-keuse toetse met hierdie varieteite, gemeet deur eierlegging. Gasheer vatbaarheid is getoets deur penetrasie van pasuitgeborede larwes op verskillende Nawellemoen varieteite. In die vroë rypwordingsgroep is die minste voorkeur in eierlegging aan Fischer Nawels gegee en dié varieteit was ook die minste vatbaar vir larwe penetrasie. Die mid en laat seisoen rypwordingsgroepeerings het beperkte verskille in eierleggingsvoorkeur gewys. Nietemin het dit geblyk dat gasheer vatbaarheid 'n belangrike faktor in die valuering van kwesbaarheid van vrugte vir VKM was. Ondanks die feit dat die mid-seisoen Palmer Nawel in Suid-Afrika wyd geplant is, is hierdie varieteit hoogs vatbaar vir larwe penetrasie. Onder die laat seisoen varieteite is Cambria en Glenora Late die minste vatbaar vir VKM. As gevolg van hierdie laboratoriumproewe, om VKM skade te beperk, word daar aanbeveel dat boere hulle aankweking van Fischer Nawels as die hoof vroë seisoen varieteit vermeerder; dat hulle Palmer Nawels in die guns van ander mid-seisoen varieteite vermy; en dat hulle voorkeur aan Cambria en Glenora Late varieteite in die laat rypwordingsgroep gee.

Introduction

False codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), is native to South Africa and an important economic pest of several citrus cultivars, due to pre-harvest damage caused by larvae feeding on the fruit and post-harvest interception of infested fruit (Newton 1998; Moore 2012). FCM is known to have a wide host range (Venette et al. 2003; Kirkman 2007). However, citrus is a preferred host with Navel oranges (*Citrus sinensis* L. Osbeck) being the cultivar most vulnerable to FCM damage (Newton 1998; Moore 2012). This is problematic as Navels are the second most common export, just behind Valencia oranges, out of the five citrus cultivars grown in South Africa (Anonymous 2012).

Various options are available to citrus farmers for the management and control of FCM, including the use of good orchard sanitation, population monitoring (Hofmeyr and Burger 1994), chemical insecticides, mating disruption, attract and kill technology, sterile insect technique (SIT) (Bloem et al. 2003; Carpenter et al. 2004), biological control through the use of natural enemies (Newton 1988; Newton and Odendaal 1990), and microbial control with the *Cryptophlebia leucotreta* granulovirus (CrleGV) (Newton 1998; Moore et al. 2011; Moore 2012; Moore and Hattingh 2012). Despite this suite of control options being available to farmers, complete elimination of the pest from orchards is almost impossible (Kirkman and Moore 2007).

Consequently, additional techniques are needed to suppress populations to as low a level as possible and further reduce FCM infestation of fruit, in compliance with phytosanitary requirements imposed by some South African export markets (Moore 2012). One such opportunity is to take cognisance of the natural preferences of female false codling moth for particular varieties of navel orange, as well as the susceptibility of these different varieties to penetration by first instar larvae. Newton (1990) showed that when using five different navel orange varieties, distinct preferences were shown by female moths. However, the number of varieties available to growers for commercial use has increased substantially since that time. Exploiting both the natural preference of FCM females for particular navel orange varieties and determining the susceptibility of varieties to penetration by FCM larvae would enable farmers to make astute decisions to improve their management of FCM and decrease the risk of infestation in fruit. This would include selection of varieties for planting (both new and replacement plantings), prioritization and intensification of control efforts in susceptible orchards and selection of non-sensitive markets for export of the more FCM-susceptible varieties.

The aim of the study was therefore to determine which navel orange varieties are preferred by female FCM as oviposition sites and to determine which of these varieties are most susceptible to successful larval penetration and development.

Materials and methods

Navel Orange Varieties

Fifteen navel orange varieties which are all commercially grown in the Eastern Cape Province of South Africa were identified for use in this study through consultation with Citrus Research International (CRI). No experimental varieties were included. All fruit were collected from farmers in the Sundays River Valley, near the town of Addo and received no postharvest treatments. The approximate maturity time of the different navel orange varieties was determined using a CRI navel maturity chart (Fenwick et al. 2011) and the fruit was subsequently placed into either an early, mid or late season maturity grouping. The maturity groupings were identified as follows: early (mid-April to mid-May), mid (mid-May to mid-June) and late (mid-June to

mid-July) (Table 3.2.15.1). Any varieties which did not fall into the maturity time periods were excluded from the study. Only mature or fully 'coloured up' fruit were used in the experiments and this was determined by using a colour maturity chart (Anonymous 1995). After collection the fruit was stored in a cold room at -4°C for a maximum of one month to preserve the fruit and maintain the volatile profile. Internal quality tests to determine fruit peel mass (g), juice %, brix (sugar %), acid content (g per 100 ml citric acid) and the ratio of brix:acid were performed on six fruit of each variety according to standard protocols (Anonymous 1999).

Table 3.2.15.1. Internal quality test results for all 15 navel orange varieties in each maturity grouping showing averages for six oranges of each variety

Variety	Peel Mass (g)	Juice (%)	Brix (Sugar %)	Acid Content (g/100ml citric acid)	Ratio (Brix: Acid)
Early Season					
Newhall	60.8	46.8	10.8	0.97	11.1
Fukumoto	70.4	38.8	12.5	1.16	10.8
Fischer	74.9	48.4	10.8	0.91	11.9
Mid-season					
Palmer	59.6	52.8	11.6	1.13	10.3
Cara Cara	54.5	54.6	11.4	1.09	10.5
Bahianinha	83.7	47.1	10.5	0.69	15.2
Washington	63.6	50.4	12.2	0.93	13.1
Late Season					
Witkrans	60.0	57.1	10.5	0.98	10.7
Powell Summer	70.0	50.0	11.0	1.20	9.20
Autumn Gold	60.0	53.8	13.0	1.12	11.6
Cambria	60.0	53.8	10.4	1.05	9.9
Robyn	60.0	53.8	9.7	0.83	11.7
Summer Gold	60.0	57.1	10.0	0.87	11.5
Lane Late	60.0	53.8	10.8	1.17	9.2
Glenora Late	60.0	57.1	10.9	1.2	9.1

FCM cultures

Egg sheets and late instar FCM larvae or pupae were obtained from a commercial culture held at River Bioscience, Addo, South Africa. Individual pupae were placed into plastic pharmaceutical vials (size no. 20) and allowed to eclose, in order to separate the sexes. Individual adult moths were sexed, male moths being identified through their smaller size as well as the presence of an anal tuft and dense tufts of scales on the hind tibia, both of which female moths lack (Catling and Aschenborn 1978; Newton 1998). Within 24 hours of eclosing, a virgin female and male were paired together in the same vial, which was then stoppered using sugar water moistened cotton wool. The paired adults were allowed to copulate overnight at a temperature of 25°C (\pm 1°C) in a controlled environment (CE) room with a low relative humidity (RH) of between 20 and 45% before use in the trial conducted the following day.

Ovipositional preference of adult female FCM on different navel orange varieties

The ovipositional preference trials were divided into choice and no-choice tests under dark conditions. The trials were all conducted in CE rooms under nocturnal conditions, with the temperature controlled at 25°C (\pm 1.5°C) with low relative humidity ranging from 25 to 45%. Nocturnal conditions were required as FCM adults are active at night (Stofberg 1954). A pilot study had indicated that four hours was sufficient time to allow oviposition to occur on the majority of the fruit. Therefore the oranges were exposed to FCM for this length of time with the number of eggs oviposited being recorded hourly, before being removed from the cage at the end of the four hour period. Eggs are small and pale in colour and the fruit were therefore checked very thoroughly under light conditions in a CE room by the same observer on every occasion to as far as possible reduce counting errors.

Choice trials were conducted using 10 navel oranges of each variety in each particular maturity grouping. Three replicates for each choice test were performed. The navel oranges were labeled and removed from cold storage 24 hours prior to usage to allow them to reach room temperature, before being placed randomly in the sealed containers used for the trials. For the early and mid-season maturity choice trials, plastic tubs (60 x 40 x 40 cm) sealed with netting, were used. For the late maturity grouping, larger mesh cages were used (90 x 60 x 60 cm) due to the larger number of varieties in this grouping (Table 3.2.15.1). In this case,

one orange of each variety was used, with thirty replicates being performed. Sixteen pairs of moths per ten oranges were used and adjusted proportionately when the number of oranges used was altered. Once the moths had been released into the cage it was sealed to prevent their escape. For the no-choice trials, ten oranges of the same variety were placed into plastic tubs (60 x 40 x 40 cm) and paired moths released into the tub before being covered with netting. This was replicated three times for each variety. Once the trial was completed, the number of eggs oviposited onto the fruit was recorded through careful examination of the fruit.

Host susceptibility of different navel orange varieties to FCM larvae

Egg sheets containing high numbers of FCM eggs were placed into glass jars, sealed with a mesh lid to allow airflow, and kept at 25°C. The eggs turned brown in colour shortly before hatching. All oranges were removed from cold storage and labeled one day prior to the experiment being run, to allow warming to room temperature. Once hatching had occurred, first instar neonate larvae were carefully removed from the jar using a 000 paintbrush and placed onto surface of the navel orange. Two neonate larvae were placed onto each orange. Each fruit was placed on its side and the larvae were positioned equidistant between the navel and peduncle ends of the fruit. Once placed in this position the larvae were inspected for signs of movement to indicate that both were still present and alive. Once the larvae had been placed onto the fruit it was kept at 25°C in a CE room for 20 days to allow larval penetration and development to occur.

Oranges were then inspected for the presence of external penetration marks and the presence of any mould on the fruit was recorded as a measure of percentage cover. Each fruit was then dissected by cutting the orange into thin slices and searching for evidence of larvae or larval damage. The presence of internal penetration marks, number of larvae and developmental instar were recorded and analysed. This was replicated three times per variety using 20 fruit per replicate.

Statistical analysis

All statistical analyses were performed using Statistica Version 10 (Statsoft Inc.). The ovipositional preference data were found to not be normal and as a result non-parametric statistical tests were used. For all analysis of oviposition preferences between different navel orange varieties, a Kruskal-Wallis test was used to determine significance and a multiple comparison of mean ranks test was used to determine where the significant differences were. For analysis of host susceptibility, a Chi-squared test was used to determine if a relationship existed between the susceptibility of different navel orange varieties and FCM larval penetration (Fowler et al. 2005).

Results

Oviposition preferences on early maturing navel orange varieties

The early maturing grouping contained three different navel varieties which were Newhall, Fukumoto and Fischer. The median amount of oviposition by female FCM under choice conditions showed that the moths had a significantly higher preference for the Newhall and Fukumoto navel orange varieties, although there was no difference between these two ($H = 19.440$, $p = 0.0001$). Fischer navel oranges were the least preferred variety in the early season maturity grouping (Fig. 3.2.15.1a). A similar trend was seen for the no-choice tests where Fischer navel oranges were once again significantly less preferred to the Newhall and Fukumoto varieties ($H = 15.430$, $p = 0.004$) (Fig. 3.2.15.1b).

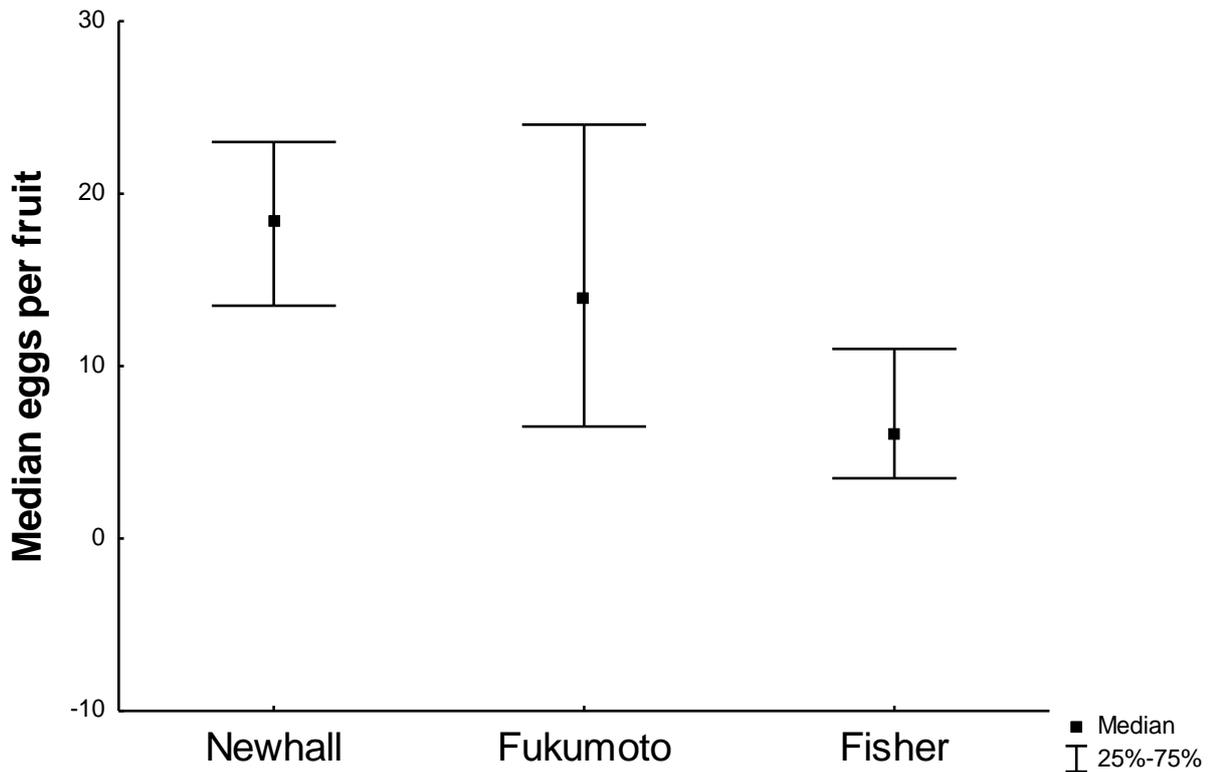


Figure 3.2.15.1a. Oviposition preference of female *T. leucotreta* adults on three different early maturing navel orange varieties in choice trials (n = 84). Different letters denote significant differences (multiple comparison of mean ranks, p < 0.05).

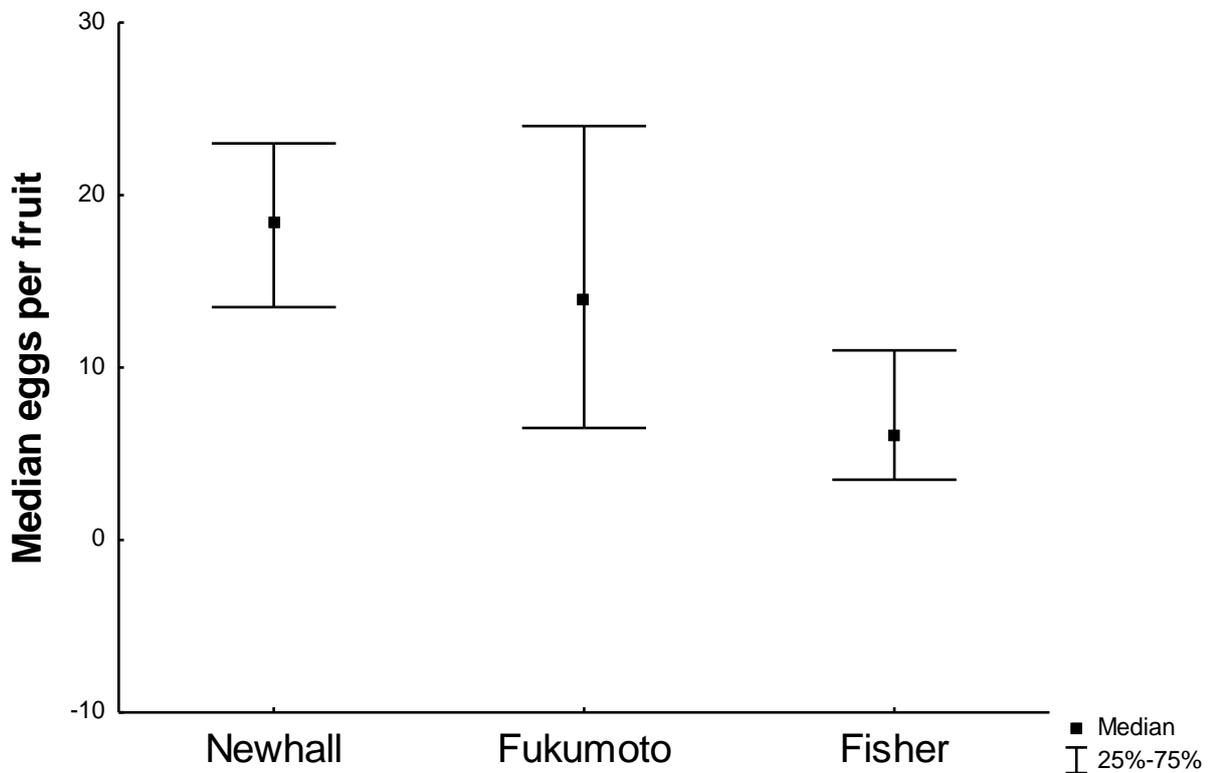


Figure 3.2.15.1b. Oviposition preference of female *T. leucotreta* adults on three different early maturing navel orange varieties in no-choice trials (n = 90). Different letters denote significant differences (multiple comparison of mean ranks, p < 0.05).

Susceptibility of early maturing navel orange varieties to larval penetration

Host susceptibility trials with the early season maturity grouping showed that Fukumoto was the variety most susceptible to penetration and damage by first instar FCM larvae, followed by Newhall and Fischer; Fischer

being significantly less susceptible than both Fukumoto and Newhall ($\chi^2 = 16.441$, $p > 0.0003$), with a total of only five larvae being found during dissection (Fig. 3.2.15.2). The internal quality tests for the different navel orange varieties suggest that a possible reason for this low penetration rate is due to Fischer navels having the largest peel mass of the three early season maturing varieties making successful penetration more difficult. Despite Fukumoto navels having the highest average acid content, this variety recorded the highest larval penetration and development, although the high brix of this variety may have positively influenced development (Table 3.2.15.1).

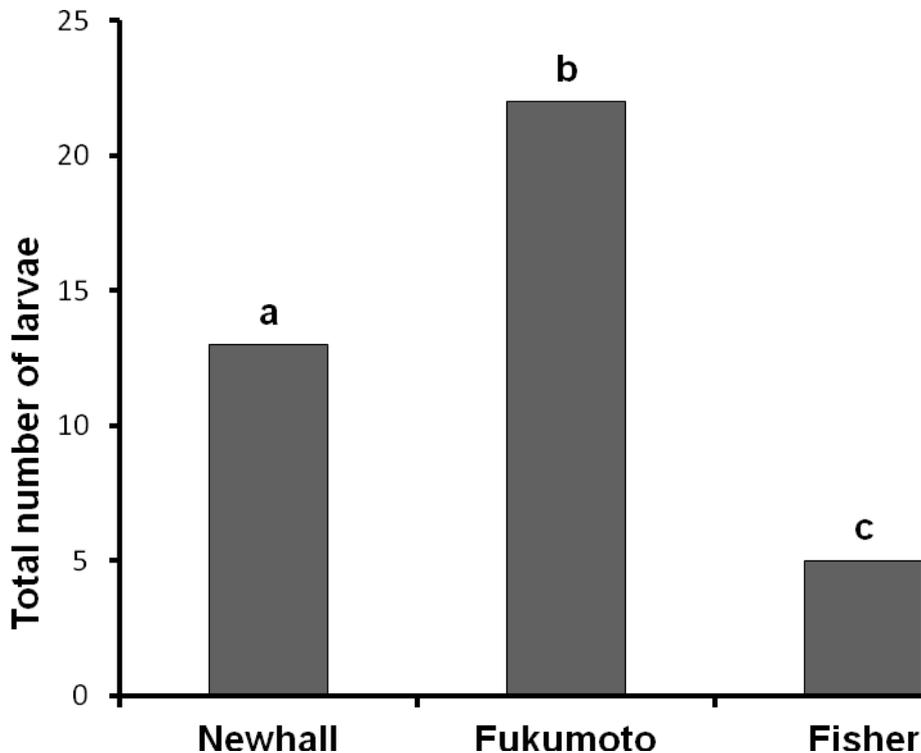


Figure 3.2.15.2. Susceptibility of three different early maturing navel orange varieties to penetration and development of *T. leucotreta* larvae (n = 180). Different letters denote significant differences (multiple comparison of mean ranks, $p < 0.05$).

Oviposition preferences on mid-season maturing navel orange varieties

Four different varieties (Cara Cara, Washington, Bahianinha and Palmer) comprised the mid-season maturing grouping. In the choice tests for the mid-season maturing varieties there were no significant differences in the median amount of oviposition between any of the four different varieties ($H = 3.811$, $p = 0.2826$) (Fig. 3.2.15.3a). The no-choice tests showed slightly more variation in oviposition preferences, with Washington and Bahianinha navel orange varieties being significantly more preferred than Cara Cara navels, however, no difference existed between Washington and Bahianinha ($H = 20.684$, $p = 0.001$). Preference for Palmer navel oranges did not differ significantly from that of the other three varieties (Fig. 3.2.15.3b).

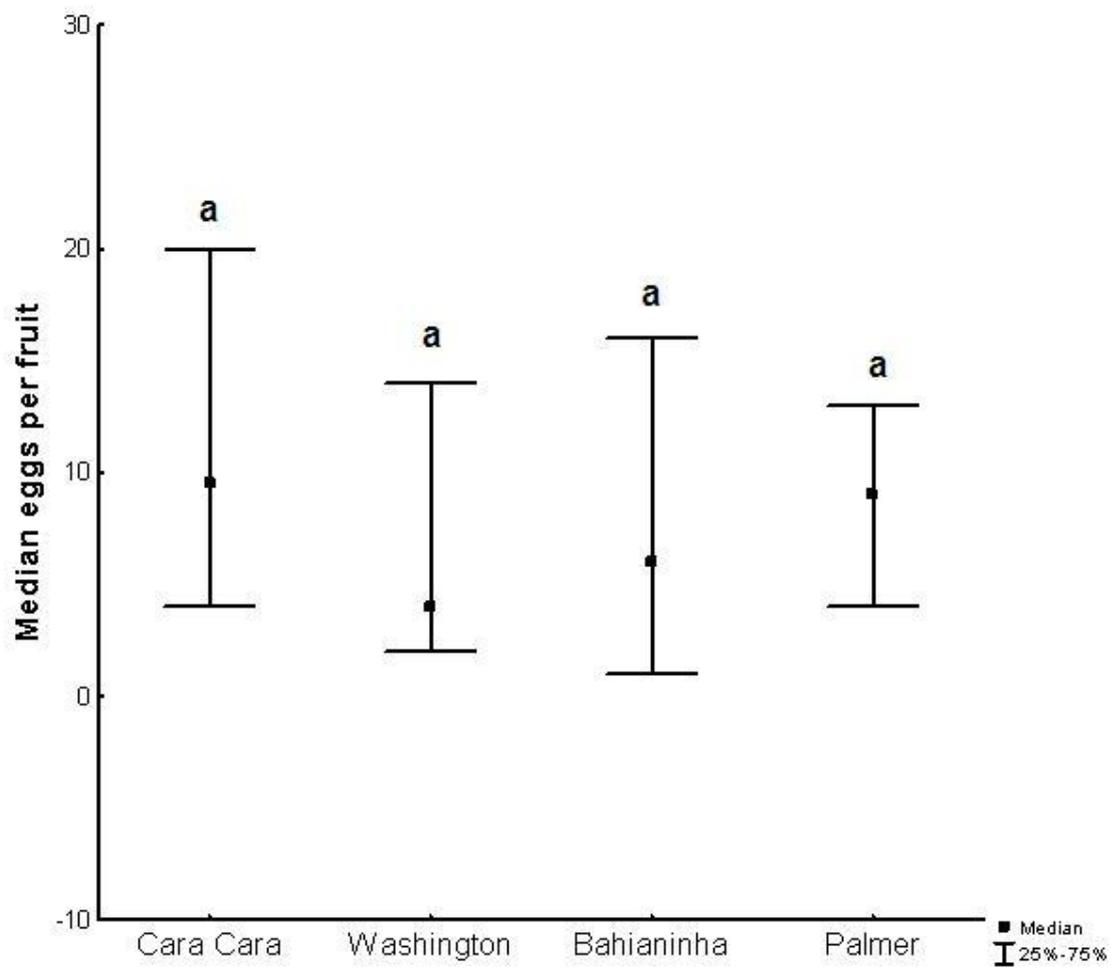


Figure 3.2.15.3a. Oviposition preference of female *T. leucotreta* adults on four different mid-season maturing navel orange varieties in choice trials (n = 120). Different letters denote significant differences (multiple comparison of mean ranks, $p < 0.05$).

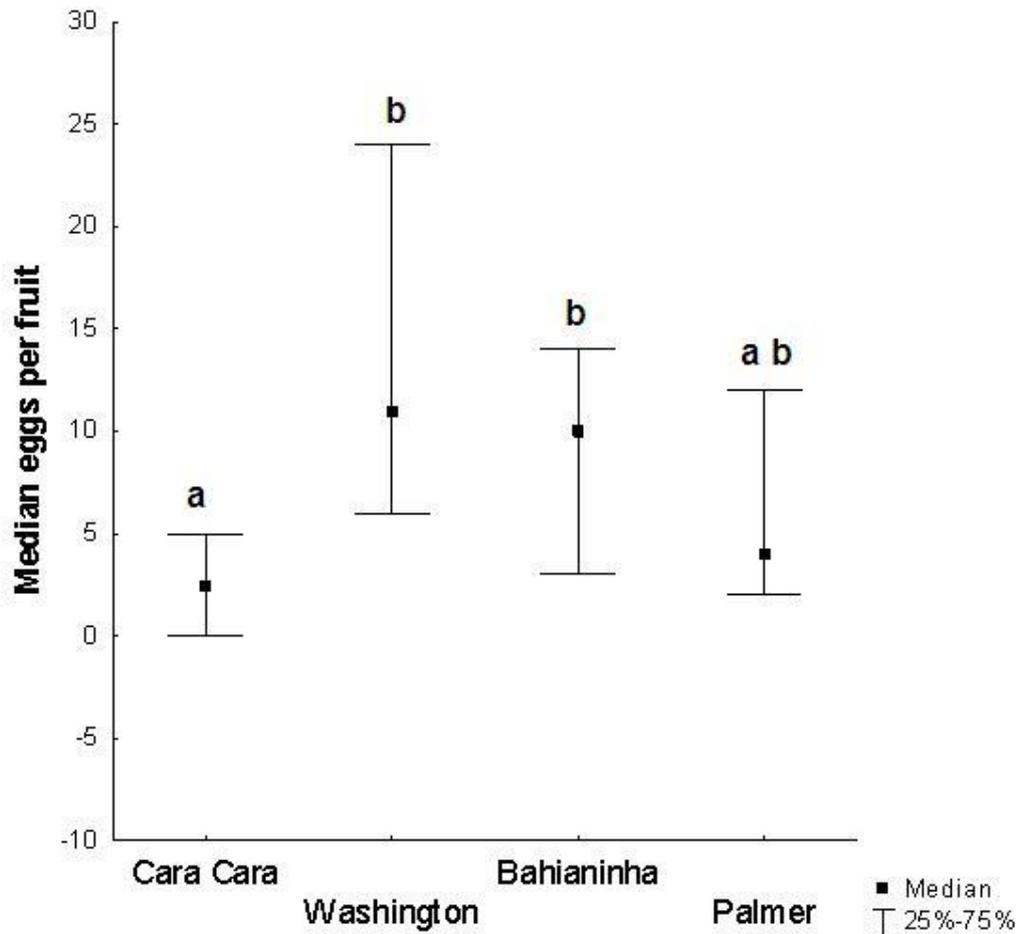


Figure 3.2.15.3b. Oviposition preference of female *T. leucotreta* adults on four different mid-season maturing navel orange varieties in no choice trials (n = 120). Different letters denote significant differences (multiple comparison of mean ranks, p < 0.05).

Susceptibility of mid-season maturing navel orange varieties to larval penetration

Palmer navel oranges were infested with a significantly higher total number of FCM larvae than the other three varieties in the grouping, indicating far higher levels of successful penetration and damage ($\chi^2 = 57.801$, p < 0.00001). The 45 larvae found in this variety was also the highest number of all 15 varieties used in the study. The Bahianinha variety contained substantially fewer FCM larvae than Palmer navels; however, this was still significantly more than was found in either Cara Cara or Washington navel orange varieties (Fig. 3.2.15.4). Palmer navel oranges had the second thinnest peel mass in this grouping, but, unexpectedly, Cara Cara navel oranges had the smallest peel mass in this grouping, but the lowest amount of successful penetration and development (Table 3.2.15.1).

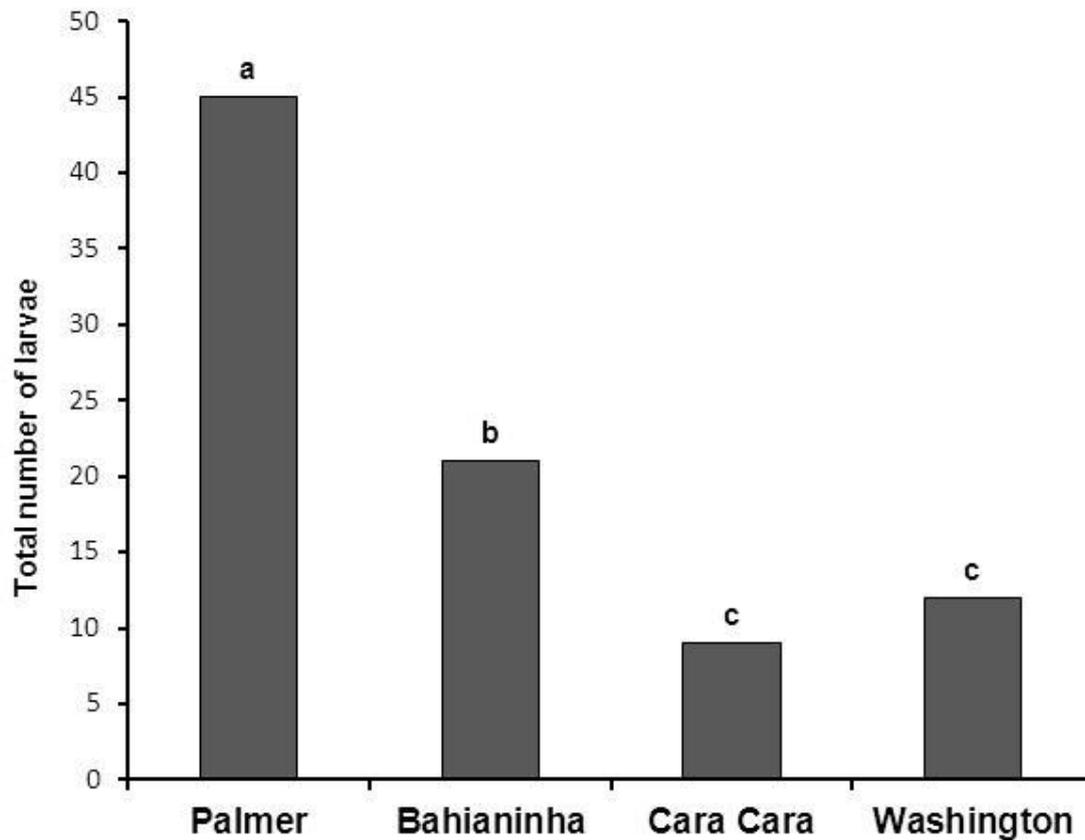


Figure 3.2.15.4. Susceptibility of four different mid-season maturing navel orange varieties to penetration and development of *T. leucotreta* larvae (n = 240). Different letters denote significant differences (multiple comparison of mean ranks, $p < 0.05$).

Oviposition preferences on late maturing navel orange varieties

The late season maturity grouping contained the largest number of navel orange varieties, eight in total (WK = Witkrans, PS = Powell Summer, AG = Autumn Gold, CM = Cambria, R = Robyn, SG = Summer Gold, LL = Lane Late, GO = Glenora Late) (Table 3.2.15.1). The choice tests for these varieties revealed that female FCM showed no particular preference for any of the varieties ($H = 3.154$, $p = 0.8704$) (Fig. 3.2.15.5a). When the results of the no-choice tests were compared for all of these varieties, some differences in median oviposition preference were found. Powell Summer, Cambria and Glenora Late navel oranges were significantly preferred for oviposition over Lane Late navels ($H = 31.928$, $p < 0.0001$). There were, however, no significant differences in oviposition on fruit amongst any of the other varieties (Fig. 3.2.15.5b).

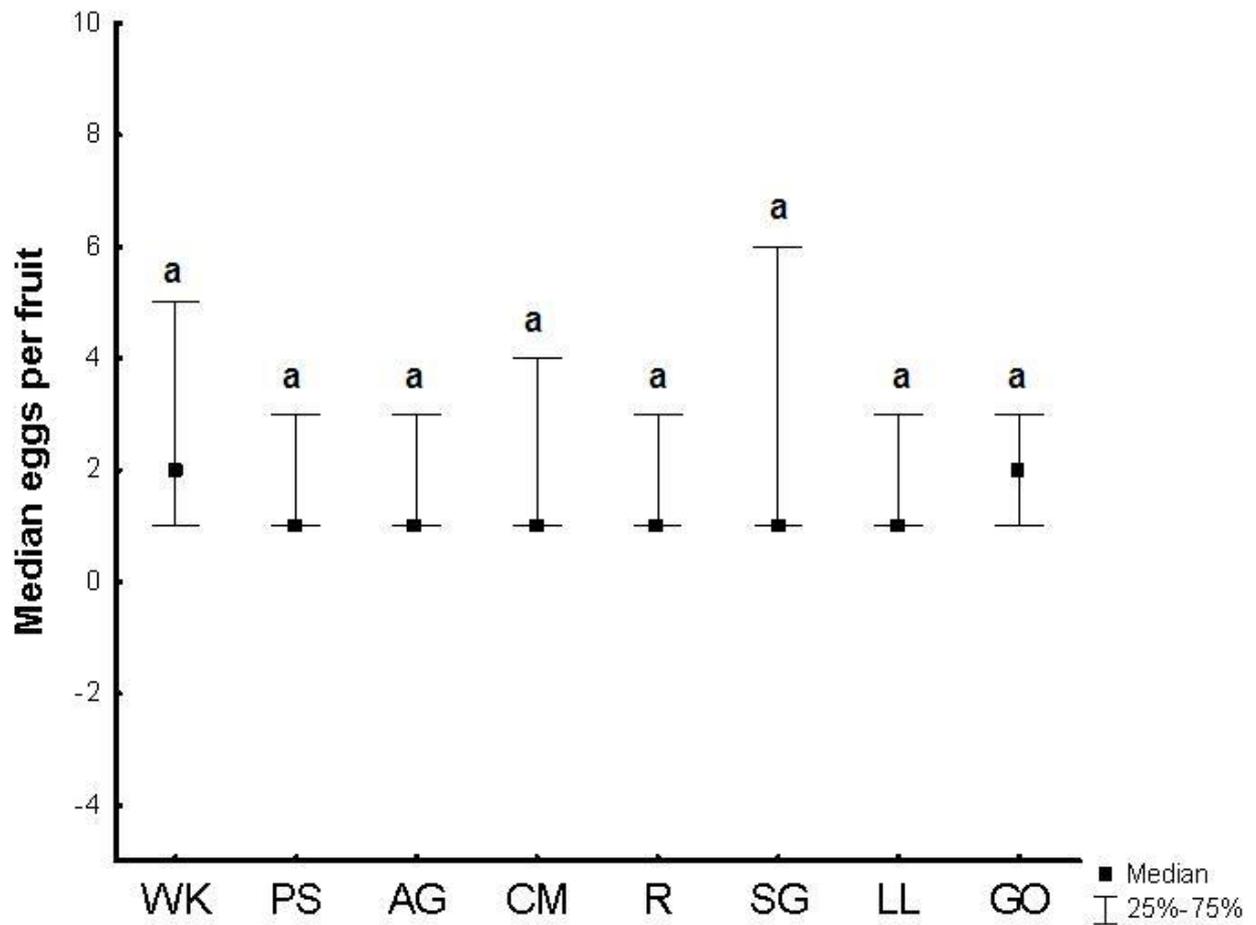


Figure 3.2.15.5a. Oviposition preference of female *T. leucotreta* females on eight different late maturing navel orange varieties in choice trials (n = 240). Different letters denote significant differences (multiple comparison of mean ranks, $p < 0.05$).

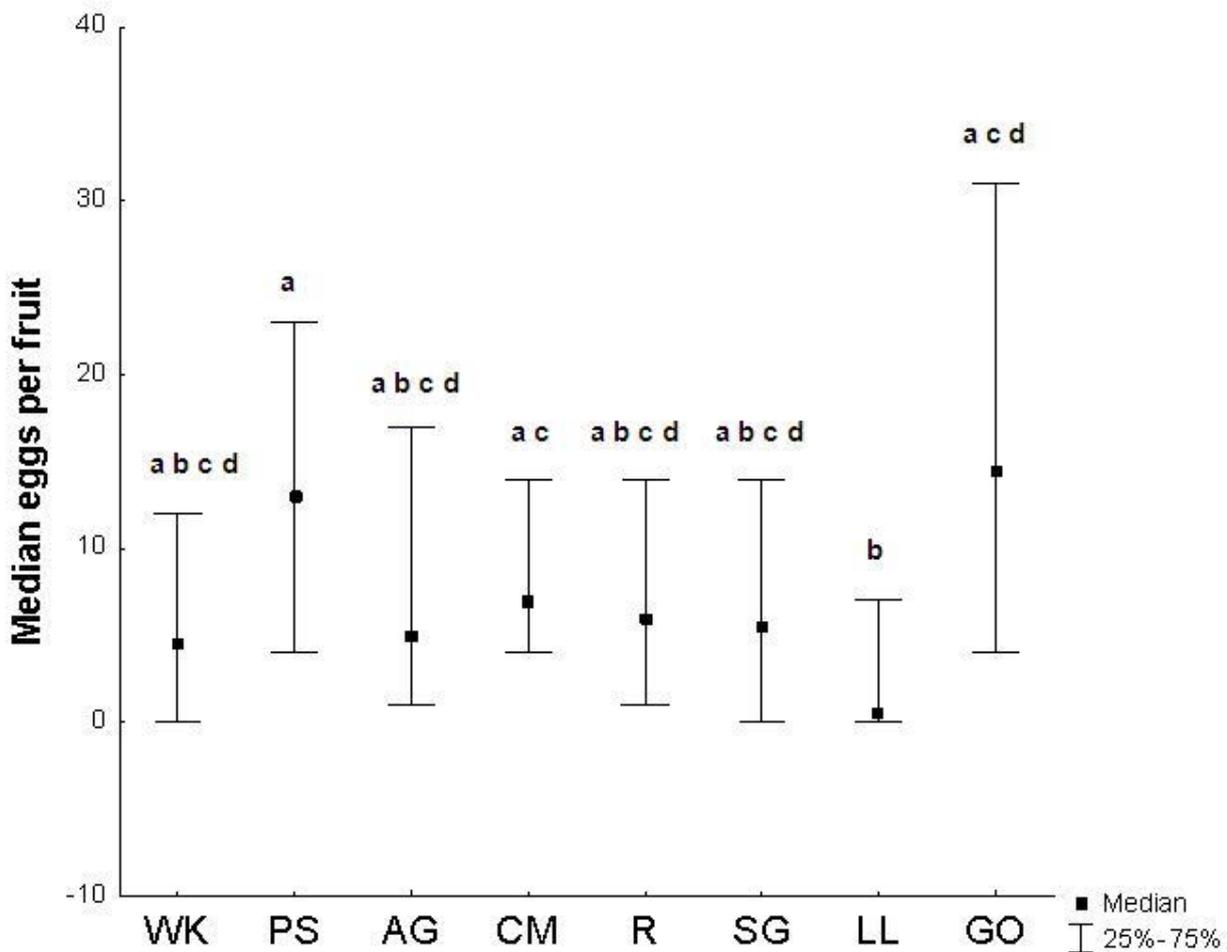


Figure 3.2.15.5b. Oviposition preference of female *T. leucotreta* adults on eight different late season maturing navel orange varieties in no choice trials (n = 240). Different letters denote significant differences (multiple comparison of mean ranks, p < 0.05).

Susceptibility of late maturing navel orange varieties to larval penetration

Overall, the total larval numbers for the late maturing navels were far lower than those for the other two maturity groupings. The host susceptibility trials showed that Cambria, Glenora Late and Witkrans navel orange varieties showed significantly less larval penetration and damage than the other five late season maturing varieties ($\chi^2 = 354.052$, p < 0.0001). Powell Summer and Lane Late varieties had the highest total numbers of larvae penetrating, but these were not significantly different to those amounts found in Autumn Gold, Robyn or Summer Gold varieties (Fig. 3.2.15.6). Higher resistance of fruit to penetration as a result of thicker peel mass was not a factor here, as seven of the eight varieties were found to have the same average peel mass, except for Powell Summer which had a larger peel mass (Table 3.2.15.1). Cambria and Glenora Late had high acid contents, but both Powell Summer (most successful larval penetration) and Glenora Late (second least successful larval penetration) had the same acid content, which was the highest of the group. However, the higher brix of the Powell Summer navels may have contributed to the substantially greater larval penetration in this variety (Table 3.2.15.1).

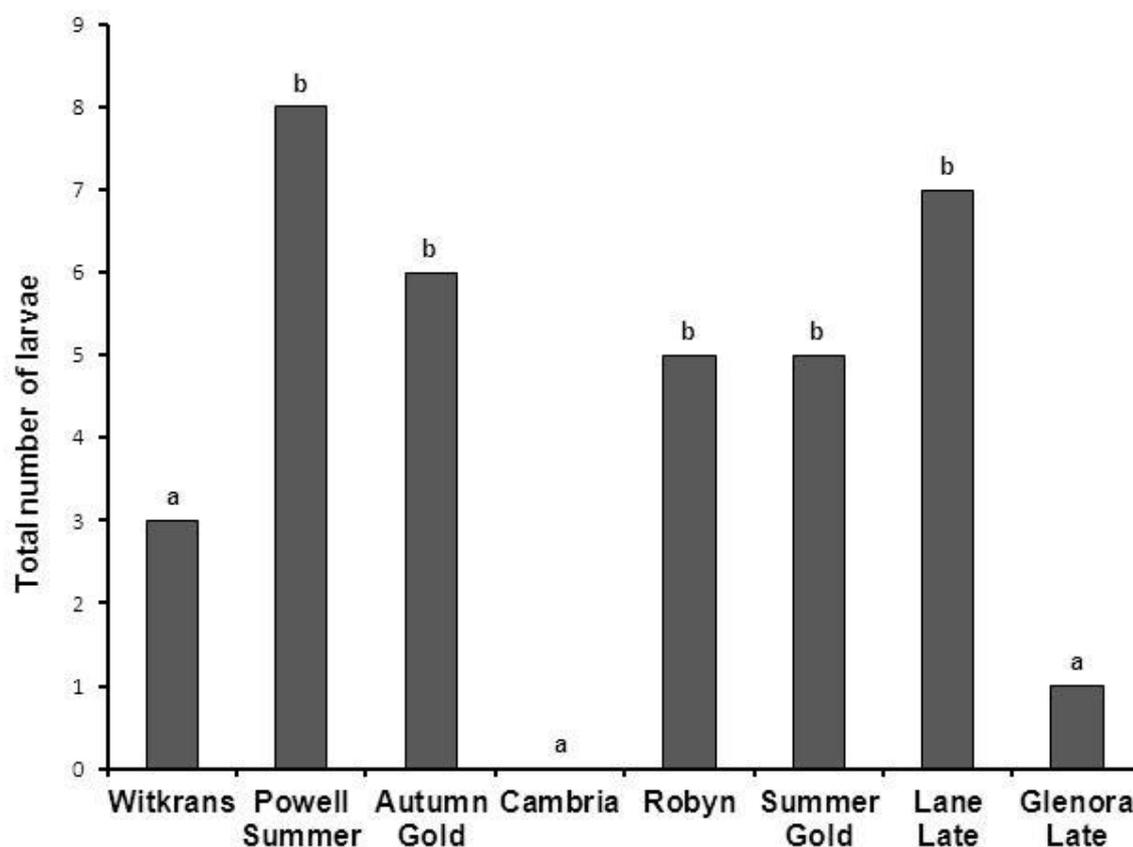


Figure 3.2.15.6. Susceptibility of four different late maturing navel orange varieties to penetration and development of *T. leucotreta* larvae (n = 480). Different letters denote significant differences (multiple comparison of mean ranks, $p < 0.05$).

Discussion

The use of oviposition preferences and host susceptibility to manage pests has been well documented in agriculture. *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) has been noted as showing a preference for ovipositing onto flowering tobacco, maize and sunflower plants when compared to other host species (Thompson and Pellmyr 1991; Firempong and Zalucki 1990; Jallow et al. 2004). Differences in host plant susceptibility through altered pest performance have been found to occur in the case of the cereal leafminer, *Syringopais temperatella* Led. (Lepidoptera: Scythrididae), on various wheat and barley cultivars (Al-Zyhoud 2012). Differences in life history and fecundity of *H. armigera* were identified on different varieties of soybean (Naseri et al. 2009). The use of resistant rice varieties is considered a useful management technique to reduce damage to the crop by the stem-boring pest, *Scirpophaga incertulatus* Walker (Lepidoptera: Pyralidae) (Sarwar et al. 2012). The consideration of a pest insect's oviposition preference and host plants' relative susceptibility are therefore well-established in agricultural pest control.

Specifically on citrus, previous work by Newton (1990) had established that certain varieties were preferred for oviposition by adult female FCM. However, this study only made use of five sweet navel orange selections. Since this time, the number of navel orange varieties grown commercially and principally for export has increased substantially in order to meet the demands of various markets and to ensure a continual supply for the northern hemisphere over the warmer months when large amounts of citrus fruit are not produced in this hemisphere (Mather 1999; Urquhart 1999; Ndou and Obi 2011; Anonymous 2011). Oviposition by FCM is substantially higher on navel oranges than on other citrus cultivars (Newton 1998), however at the varietal level only a limited amount of significant oviposition preference was found to occur in this laboratory study. It is known that the chemical properties of host plants have a significant influence on oviposition by female phytophagous insects (Renwick 1989; Thompson and Pellmyr 1991). Ioannu et al. (2012) found that the essential oils produced by sweet oranges strongly influenced the number of eggs laid by female Mediterranean fruitfly, *Ceratitidis capitata* Weidemann (Diptera: Tephritidae). The emission of volatiles has also been strongly implicated in influencing which plants phytophagous insects orientate to and land on (Renwick 1989). Either or both of these factors may be influencing oviposition preference in the case of FCM.

Sweet navel orange varieties may be very closely related, as shown by a study in India where the genetic diversity of 22 different cultivars of *C. sinensis* was assessed and found to be very low, despite being highly diverse in morphology (Malik et al. 2012). It is possible that the difference in the attractiveness of the fruit is not substantial enough to show any statistical differences in oviposition preference when female moths were allowed a choice, but may have influenced females when no varietal choice was allowed, as was seen for certain varieties in the no choice trials. Furthermore, the relationship between preference and host susceptibility was not always clear as noted for varieties such as Lane Late and Glenora Late. The host susceptibility showed far more substantial differences in the vulnerability of the fruit than the oviposition trials. This and the sometimes unclear relationship between these two factors may be partially explained by the influence of volatile emissions and essential oils on oviposition, while the susceptibility of the fruit is largely governed by the physical and internal chemical properties of the fruit. The variability in these properties of the fruit can be seen in the differences in internal quality and the highly diverse morphology found by Malik et al. (2012).

The most notable preference for oviposition was found to occur in the early maturing grouping where Fischer navel oranges were found to be significantly less preferred, than the other two early maturity varieties, Newhall and Fukumoto, in both the choice and the no-choice trials. The host susceptibility trials also revealed that the Fischer variety had significantly less successful penetration and development by larval FCM, indicating that this variety can be considered relatively resistant to FCM damage. This is an important finding for the South African citrus industry, as while Fischer navels have been available to growers for some time, as an early season variety, few growers currently make use of it, unlike in California (Andrew Lee pers. comm.).

Within the mid-season maturing navels, the Palmer variety was clearly the most susceptible to FCM larval penetration, which is confirmed by the findings of Newton (1990), where a field trial comparing Bahianinha and Palmer navels showed that the crop losses from FCM damage on the Palmers were substantially higher than the losses on Bahianinha navels. The implications of this are of great consequence to navel orange farmers, as the Palmer is currently the most planted navel orange variety in South Africa, with the varietal mutation having been discovered in 1950 (Anonymous 2012). Some growers have begun moving away from growing Palmers due to the extended growing season and other problems with the cultivar, but it remains popular with many other growers (Fenwick et al. 2011). Citrus farmers should consider reducing their planting of Palmer navels in favour of one of the other mid-season varieties, preferably the Bahianinha navel, which was far less susceptible to penetration by FCM larvae in the laboratory trials and has also been found to be so in the field (Newton 1990). Although less preferred in the no choice trials and less susceptible in this laboratory study, the Cara Cara is a speciality or niche variety, due to the red pigmentation of the fruit's flesh (Fenwick et al. 2011) and may not be suitable for all markets. The Washington navel is harvested slightly later and although apparently less susceptible in the laboratory trials, susceptibility does not appear to differ as markedly from the Palmer in the field.

Based on host susceptibility of the late maturing group, planting of Cambria and Glenora Late navels should be favoured, while Powell Summer and Lane Late navel production should be decreased. Cambria is a popular variety of navel for export, with almost a million cartons being exported from South Africa in 2012, indicating that increasing production of this variety would not result in problems for exporting growers (Anonymous 2012). Substantially less fruit of the Powell Summer and Lane Late navel varieties is exported and this should be maintained. Additionally, these more susceptible varieties should be exported to markets which are less stringent against FCM (Anonymous 2012). The export of Glenora Late is currently very low (Anonymous 2012), showing the potential for growth for the variety in overseas export markets, considering its relatively low susceptibility to FCM infestation.

Any new navel orange varieties – whether mutations or by breeding – should take into account the preferences of FCM females and the susceptibility of the cultivars themselves to larval penetration. One example of this is the development of the experimental 99 navel variety which is apparently less susceptible to FCM damage than other navel orange varieties (Fenwick et al. 2011). The identification and selection for planting of navel orange varieties which are less susceptible to FCM, strongly supports an IPM programme, by reducing the level of intervention (especially chemical) required to combat the pest. A general reduction in chemical intervention is also desirable as Maximum Residue Levels (MRLs) of insecticides permitted on fruit are lowered and the banning of certain active ingredients in chemical insecticides is on the increase in many export markets (Anonymous 2011; Ndou and Obi 2011).

Conclusion

Based on this laboratory study, new plantings should focus on favouring the Fischer variety in the early maturing grouping and Bahianinha as the principle mid-season maturing variety with both Cambria and

Glenora Late being suggested for later in the season. An orchard replacement programme may also be initiated by growers in order to make use of more resistant varieties when replacing old or highly susceptible trees. The implementation of replanting programmes and changes to varieties grown has been recommended to Spanish citrus growers, although this was largely due to changes in market demands (Poole 2000). Improved varietal selection may then be used to assist with FCM control, particularly in orchards in areas where the moth poses a greater threat. A further option would be to export more susceptible varieties to markets which are less sensitive to the presence of FCM. As the phytosanitary regulations of export markets become stricter and the limitations on insecticidal usage increase, there is increasing pressure on growers to seek alternative and effective IPM-compatible control options. As such, the use of management strategies which are able to exploit the natural behaviour or limitations of the pest through oviposition preference and host susceptibility is expected to provide a useful contribution to the management of FCM in South African citrus orchards.

Acknowledgments

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Future Research

No future research is planned or suggested on this topic. However, whenever a new citrus variety is considered for planting it should first be tested for its relative susceptibility to pests, particularly those which have phytosanitary status, such as FCM.

Technology Transfer

Preliminary findings were presented in poster form at the biennial Citrus Research Symposium in August 2012. An oral presentation on the final results was made at the Congress of the Entomological Society of southern Africa in July 2013. A manuscript has been submitted for publication in a peer-reviewed journal.

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

Project 1042 (Sep 2011 – Mar 2015) by Sean Moore (CRI), Ralf-Udo Ehlers (e-nema), Aruna Manrakhan, Wayne Kirkman, John-Henry Daneel (CRI), Jean de Waal (Dow) and Rynhardt Nel (Goedehoop Sitrus)

Summary

A total of five field trials were conducted with entomopathogenic nematodes (EPNs) in citrus orchards during the 2012/13 season: two in the Eastern Cape, two in the Western Cape and one in Mpumalanga. The trials were designed to test the efficacy of soil application with commercial formulations of *Heterorhabditis*

bacteriophora and *Steinernema feltiae* for control of false codling moth and fruit flies. EPNs were applied at 10 or 20 infective juveniles (IJs) per cm², either through the irrigation system, using microsprinklers or with the use of a spray machine, aiming the bottom three nozzles on either side of the machine to spray underneath the tree canopy. Applications were made in autumn, winter or spring. Survival and persistence of EPNs in the soil were monitored using caged sentinel larvae (5th instar FCM) at various intervals after application. Population suppression of FCM was measured using a pheromone trap in the dead centre of each treatment orchard. Damage reduction was evaluated by inspecting fallen fruit from data trees at weekly intervals from January until harvest. Survival of EPNs was variable but generally for a longer duration than expected (up to 5 months after application), particularly where good soil moisture was maintained. Generally, *S. feltiae* survived the winter better than did *H. bacteriophora*; the higher rate of application was sometimes better than the lower rate. In four out of the five trials, FCM trap catches were lower in the treatments than in the untreated control. The success rate measured by fruit infestation was similar, with up to 81% reduction in one case. Only one trial showed notable reduction in the fruit fly population relative to the untreated control.

Opsomming

In totaal is vier veldproewe met entomopatogeniese nematodes (EPNs) in sitrusboorde gedurende die 2012/13 seisoen uitgevoer: twee in die Oos-Kaap, twee in die Wes-Kaap en een in Mpumalanga. Proewe is ontwerp om die effektiwiteit van grond toediening met 'n kommersiele formulering van *Heterorhabditis bacteriophora* en *Steinernema feltiae* vir die beheer van valskodlingmot en vrugtevlieë te toets. EPNs is teen 10 of 20 nematodes (IJs) per cm² toegedien, of deur die besproeiingsstelsel met die gebruik van mikrobeproeiers of met die gebruik van 'n spuitmasjien met die onderste drie neuse aan albei kante gemik onder die boom kappie. Toedienings is in die herfs, winter of lente gemaak. Oorlewing en nawerking van EPNs in die grond is met die gebruik van gehokte VKM 5de instar larwes gedoen op verskillende tye na toediening. Populasie onderdrukking van VKM is gemeet deur gebruik van 'n feromon lokval in die middel van elke behandelings boord. Skade vermindering is evalueer deur die ondersoek van gevalde vrugte van data bome op 'n weeklikse basis van Januarie tot oestyd. Oorlewing van EPNs was wisselvallig maar is oor die algemeen langer as wat verwag is (tot 5 maande na toediening), veral waar die grond nat gehou is. Oor die algemeen het *S. feltiae* beter as *H. bacteriophora* die winter oorleef; die hoer toedienings konsentrasie was soms beter as die laer dosis. In vier uit die vyf proewe is VKM lokval vangstes laer in die behandelings boorde as in die onbehandelde kontrole. Sukses gemeet deur vrugbesmetting het omtrent dieselfde tendens getoon, met tot 81% vermindering in een geval. In net een proef was daar 'n beduidende afname in die vrugtevlieë populasie in vergelyking met die onbehandelde kontrole.

3.2.17 PROGRESS REPORT: Gene expression analysis of *Thaumatotibia leucotreta* as result of different isolates of *Cryptophlebia leucotreta* granulosus virus

Project 1049 (2012/01 – 2014/12) by A.E. Timm and J. Ridgeway (RU)

Summary

It is known that *Thaumatotibia leucotreta* responds differently to different isolates of *Cryptophlebia leucotreta* granulosus virus (CrleGV). This project looks at the differences in *T. leucotreta* response to CrleGV at genomic level to determine the degree of genetic differences elicited by different viral isolates and at different temperatures. Since RNA isolation is one of the first steps in any genomic study, we evaluated five different protocols for extracting RNA from *T. leucotreta*: RNeasy (Qiagen), SV total RNA isolation system (Promega), Trizol (Promega), a cetyl trimethyl ammonium bromide (CTAB) protocol and the so-called one-step method. Extraction procedures were evaluated for robustness by including individuals of *Plutella xylostella*, *Tenebrio molitor* and *Thanaophilus micans*. RNA quality and quantity were evaluated using gel electrophoresis and NanoDrop spectrophotometer readings. The RNeasy kit provided RNA of consistently high quality and quantity, whereas all other protocols often produced RNA that was degraded, of a low quantity, or inconsistent. The RNA produced using all five protocols was converted to cDNA and used in a PCR reaction. All the cDNA produced could be amplified successfully in a PCR reaction. This cDNA is being used to optimise genomic protocols, such as identifying suitable housekeeping genes for qPCR analysis. While genomic protocols are being optimised, we are commencing biological trials to evaluate the effect of granulosus virus on *T. leucotreta* at different temperatures.

Opsomming

Dit is bekend dat *Thaumatotibia leucotreta* se reaksie teenoor die *Cryptophlebia leucotreta* granulosus virus (CrleGV), verskil vir die verskillende isolate. Hierdie projek kyk na die verskille in *T. leucotreta* se reaksie teenoor CrleGV op genoom-vlak om die vlak van genetiese verskille wat deur verskillende virale isolate en verskillende temperature ontlok is te bepaal. Omdat RNA isolasie een van die eerste stappe in enige

genomiese studie is, het ons vyf verskillende protokolle vir RNA isolasie van *T. leucotreta* geëvalueer: RNeasy (Qiagen), SV totale RNA isolasie stelsel (Promega), Trizol (Promega), 'n cetyl ethyl ammonium bromide (CTAB) protokol en die sogenaamde een-stap-metode. Isolasie prosedures is evalueer vir robuustheid deur die insluiting van individue van *Plutella xylostella*, *Tenebrio molitor* en *Thanatophilus micans*. RNA kwaliteit en kwantiteit is evalueer met behulp van gel elektroforese en Nanodrop spektrofotometer lesings. Ons het bepaal dat die RNeasy kit RNA van konsekvent hoë kwaliteit en kwantiteit verskaf, terwyl al die ander protokolle dikwels RNA van swak kwaliteit en lae kwantiteit produseer het. Die geïsoleerde RNA van al vyf protokolle is omgeskakel na cDNA en in PCR gebruik. Al die cDNA wat produseer was kon suksesvol in PCR gebruik word. Hierdie cDNA word tans gebruik om genomiese protokolle, soos die identifisering van geskikte huishouding genes vir qPCR analise, te optimaliseer. Terwyl genomiese protokolle optimaliseer word, het ons met biologiese proewe begin om te evalueer wat die effek van granulovirus op *T. leucotreta* by verskillende temperature is.

3.2.18 FINAL REPORT: A comparison of late-season FCM control options

Ad Hoc project (April 2012 – March 2013) by Wayne Kirkman and Sean Moore (CRI)

Summary

A field trial was conducted on Navel oranges in the Eastern Cape to compare the efficacy of all registered FCM control spray options applied late in the season. An experimental formulation of Cryptogran was also included. Most products gave inadequate control of FCM. However, FCM infestation in the orchard was high and orchard sanitation was inadequate, therefore this must be regarded as a worst case scenario. This highlights the importance of good FCM management from early in the season. Runner and Cryptogran were the only two products to significantly reduce FCM infestation. Powder formulations of molasses and Cryptogran appeared to be suitable substitutes for the registered formulations, and would increase ease of use for the growers. However, further testing is required.

Opsomming

'n Veldproef is op Nawellemoene in die Oos-Kaap uitgevoer om die werking van alle geregistreerde VKM spuitmiddels laat in die seisoen te vergelyk. 'n Eksperimentele formulasie van Cryptogran is ook ingesluit. Meeste produkte het onvoldoende beheer van VKM gegee. VKM besmetting was egter hoog en boord sanitasie was onvoldoende, dus kan hierdie as 'n ergste moontlike scenario beskou word. Dit beklemtoon die belangrikheid van VKM bestuur vanaf vroeg in die seisoen. Runner en Cryptogran is die enigste twee produkte wat VKM besmetting betekenisvol verminder het. Dit het voorgekom dat gepoeierde Cryptogran en melasse geskikte plaasvervangers vir die geregistreerde formulasies is, en sal die gebruik van die produk vergemaklik. Nietemin word verdere proewe benodig.

Introduction

Due to the current status of FCM as a phytosanitary threat, an *ad hoc* trial was conducted to test various late season control options for FCM. Different formulations of molasses and Cryptogran were tested. New and old chemical alternatives for FCM control were tested in a late-season application.

Objectives

To compare the efficacy of various late season control options for FCM.

Materials and methods

A trial was conducted to compare the efficacy of most of the available control options for FCM, in a late season spray trial. Powdered Cryptogran and powdered molasses were included (Table 3.2.18.1). The trial was conducted on Atmar 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 2003. 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 10 May 2012, at an average rate of 19.6 L per tree for all treatments. Dropped fruit from each tree were collected weekly from three weeks after spraying, 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 3.2.18.1. Various treatments applied to an orchard of Lane Late navel orange trees on Atmar Farm in the Sundays River Valley on 10 May 2012.

Treatment no.	Treatment (all doses per 100 L water)
1	Untreated control
2	Cryptogran (10 ml) + molasses (250 ml) + Break-Thru (5 ml)
3	Powdered Cryptogran (0.9 g) + powdered molasses (225 g) + Break-Thru (5 ml)
4	Cryptex (3.3 ml)
5	Coragen (17.5 ml)
6	Delegate (20 g)
7	Triflumuron (20 ml)
8	Meothrin (30 ml)
9	Cypermethrin (25 ml)
10	Runner (60 ml)

Results and discussion

Infestation in only two of the treatments was significantly different from the untreated control (Table 3.2.18.2). These were Cryptogran and Runner. Once again the pyrethroids gave poor control. It was surprising that some products, such as Delegate and Coragen, which had given good results in previous trials, performed poorly. This once again highlights the fact that there is no silver bullet for late-season FCM control. The efficacy of powdered Cryptogran and powdered molasses was similar to that of the current registered combination.

Table 3.2.18.2. FCM infestation for various treatments applied to an orchard of Lane Late navel orange trees on Atmar Farm in the Sundays River Valley on 4 May 2012, evaluated from 4 June to 7 July 2012.

Treatment no.	Treatment (all doses per 100 L water)	Mean no of FCM infestation (fruit/tree/week)	Reduction in infestation (%)
1	Untreated control	1.16a	
2	Cryptogran (10 ml) + molasses (250 ml) + Break-Thru (5 ml)	0.58bc	50.0
3	Powdered Cryptogran (0.9 g) + powdered molasses (225 g) + Break-Thru (5 ml)	0.68abc	41.4
4	Cryptex (3.3 ml)	0.84abc	27.6
5	Coragen (17.5 ml)	0.90abc	22.4
6	Delegate (20 g)	0.78abc	32.8
7	Triflumuron (20 ml)	1.02ab	12.1
8	Meothrin (30 ml)	1.04ab	10.3
9	Cypermethrin (25 ml)	1.00abc	13.8
10	Runner (60 ml)	0.52c	55.2

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

Conclusion

Runner proved to be the most effective product in this trial. Only Runner and Cryptogran reduced FCM infestation significantly. This is the third trial that showed that powdered molasses and dried Cryptogran are suitable alternatives to the currently registered formulations. Cryptogran with powdered molasses did not significantly reduce FCM infestation, but was not significantly less effective than the registered option. Previous trials have shown that powdered molasses is a suitable substitute for molasses. These formulations could improve ease of use for growers, while not compromising efficacy.

This trial showed once again that there is no silver bullet for late-season FCM control, and highlights the importance of good FCM management throughout the season.

Acknowledgments

River Bioscience is thanked for the supply of Cryptogran for trials. Merwe Serfontein is thanked for making his orchards available and for assisting with the management of the trial sites.

Future research

No further research is planned, however, it is possible that similar ad hoc spray trials could be conducted in future seasons if considered necessary.

Technology transfer

None.

References

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3.3 PROGRAMME: FRUIT FLY

Programme coordinator: Aruna Manrakhan (CRI)

3.3.1 Programme summary

Fruit flies remain pests of phytosanitary concern to the citrus industry. The two local pest fruit fly species on citrus are *Ceratitis capitata* (Wiedemann), Mediterranean fruit fly (Medfly) and *Ceratitis rosa* Karsch, Natal fly. Increased detections of the invasive fruit fly pest *Bactrocera invadens* Drew Tsuruta and White in the northern parts of South Africa have also raised enormous concerns among the grower community. The status of *B. invadens* in South Africa has recently been changed from absent/transient to present in specified areas and under official control. The increased importance of *B. invadens* in South Africa is reflected in the number of projects addressing this pest under the CRI fruit fly programme. In the year 2012-2013, there were equal number of projects addressing *B. invadens* and the local *Ceratitis* fruit flies.

Colonies of the *Ceratitis* fruit flies continued to be maintained (3.3.2). Fruit fly materials were used for applied and basic research at CRI and at different universities. At CRI, fruit fly materials were used for research on: (1) a new attract and kill system (Project 915), (2) a GRAS post-harvest fumigant (Project 913) and (3) cold disinfestation treatments.

A new attract and kill system is being developed under Project 915 (3.3.3). The new attract and kill system was evaluated in a citrus orchard between April and May 2012. The new system was compared to standard fruit fly treatments: weekly GF-120 bait sprays and M3 bait stations. Although, no fruit damage due to fruit flies was recorded in blocks treated with the new attract and kill system, fruit fly catches were still higher in blocks under the new treatment compared to blocks under standard treatments. Modifications were made to the new attract and kill system to improve efficacy of control. In laboratory assays, the modified versions were found to be promising and still effective beyond 4 weeks of aging.

The CRI *B. invadens* surveillance network continued to be maintained (3.3.4). Research on field control of *B. invadens* with male annihilation technique and bait was completed (3.3.5). In citrus orchards in Namibia and Kenya, the combined use of male annihilation technique (Invader-B-lok containing methyl eugenol and malathion) and bait sprays or bait stations was found to be effective against *B. invadens*. The sensitivity of *B. invadens* to methyl eugenol was determined in mango and citrus orchards in Tanzania using mark-release-recapture methods (3.3.10). Young 4 day old *B. invadens* males were used in the studies. Results indicated that a density of 4 methyl eugenol baited traps per km² will be able to detect low population of *B. invadens*. A CLIMEX model for determination of potential global distribution of *B. invadens* is being finalised (3.3.6). Trapping results obtained from different climatic regions in Africa demonstrated the adaptation of *B. invadens* to a wide range of climates, being able to even survive in the hot extremes bordering the Sahara desert. Moisture in the form of rain or irrigation is a limiting factor in the abundance and distribution of *B. invadens*.

The use of Polymerase Chain Reaction- Restriction Fragment Length Polymorphism (PCR- RFLP) to distinguish *B. invadens* from local *Ceratitis* fruit fly pests was tested (3.3.9). PCR-RFLP analyses using a primer pair from the 16S ribosomal RNA gene and the restriction enzyme DraI enabled the distinction of *B. invadens* from three *Ceratitis* pest species.

Two projects under the fruit fly programme focussed on post-harvest treatment for fruit flies. Fumigation with the full dose of GRASFUM for 24 h caused 100% fruit fly mortality in oranges but left an off-taste in Shamouti oranges (3.3.7). Further tests will be conducted with GRASFUM. The new fumigant will also be combined with short cold treatments. The determination of the least susceptible immature life stage of Medfly to cold disinfestation at 1°C had to be redone using the Japanese protocol (3.3.8).

Programopsomming

Vrugtevlieë bly 'n plaag van fitosanitêre belang vir die sitrusindustrie. Die twee plaaslike vrugtevlieg spesies wat 'n plaag op sitrus is, is *Ceratitis capitata* (Wiedemann), die Mediterreense vrugtevlieg (Medvlieg) en *Ceratitis rosa* Karsch, die Natalse vrugtevlieg. Die verhoogde toename in opsporings van die indringer vrugtevlieg plaag *Bactrocera invadens* Drew Tsuruta en White in die noordelike dele van Suid-Afrika dra ook by tot groot kommer onder produsente. Die status van *B. invadens* in Suid-Afrika is onlangs verander van afwesig /verbygaande na teenwoordig in sekere gespesifiseerde gebiede wat onder amptelike beheer is. Die toenemende belangrikheid van *B. invadens* in Suid-Afrika word weerspieël in die aantal projekte wat hierdie plaag tans in die CRI vrugtevliegprogram aanspreek. In die jaar van 2012-2013, was daar 'n gelyke aantal projekte tussen *B. invadens* en die plaaslike *Ceratitis* vrugtevlieë.

Die onderhouding van die bestaande vrugtevlieg kolonies word voorgesit. (3.3.2). Die vrugtevlieg materiaal is vir toegepaste en basiese navorsing by CRI en verskeie universiteite gebruik. By CRI, is vrugtevlieg materiaal gebruik vir navorsing oor: (1) 'n nuwe lok-en-doodmaak sisteem (Projek 915), (2) 'n GRAS na-oes berokingsmiddel (Projek 913) en (3) koue disinfestasië behandelings.

'n Nuwe lok-en-doodmaak sisteem word tans ontwikkel onder Projek 915 (3.3.3). Die nuwe sisteem was geëvalueer in 'n sitrusboord tussen April en Mei 2012. Die nuwe sisteem was met die standaard vrugtevlieg behandelings vergelyk: 'n weeklikse GF-120 lokaasbespuiting en die M3-lokaasstasies. Alhoewel daar geen vrugskade as gevolg van vrugtevlieë in die blokke wat behandel was met die nuwe lok-en-doodmaak sisteem nie, was vrugtevlieg vangste steeds hoër in hierdie blokke in vergelyking met standaard behandelde blokke. Veranderinge is gemaak aan die nuwe lok-en-doodmaak sisteem om die doeltreffendheid daarvan te verbeter. Tydens laboratorium toetse, is die veranderde weergawe belowend gevind en was steeds effektief na 4 weke van veroudering.

Die CRI *B. invadens* waarnemingsnetwerk word steeds in stand gehou (3.3.4). Navorsing oor die natuurlike beheer van *B. invadens* deur middel van die manlike uitwissingstegniek en lokaas is voltooi (3.3.5). In sitrusboorde in Namibië en Kenia, is die gesamentlike gebruik van die mannetjie uitwissingstegniek (Invader-B-lok met metiel-eugenol en malathion) en lokaasbespuitings of lokaasstasies gevind om doeltreffend te wees teen *B. invadens*. Die sensitiwiteit van *B. invadens* ten opsigte van metiel-eugenol was bepaal met die merkvangs-en-hervang metode in mango- en sitrusboorde in Tanzanië. Jong 4 dae oue *B. invadens* mannetjies is in die studie gebruik. Resultate het aangedui dat 'n digtheid van 4 metiel-eugenol lokaasvalletjies per km² in staat sal wees om 'n lae bevolking van *B. invadens* op te spoor. 'n CLIMEX-model om die potensiële globale verspreiding van *B. invadens* te bepaal word gefinaliseer (3.3.6). Lokval resultate wat uit verskillende klimaatstreke van Afrika verkry is, toon aan dat *B. invadens* aangepas is by 'n wye reeks klimaatstoestande en is selfs in staat om te oorleef in die warm uiterstes aangrensende die Sahara woestyn. Vog in die vorm van reën of besproeiing is 'n beperkende faktor op die volopheid en verspreiding van *B. invadens*.

Die gebruik van die Polimerase Kettingreaksie-Beperkings Fragment Lengte Polimorfisme (PCR-RFLP) om *B. invadens* van plaaslike *Ceratitis* vrugtevliegplae te onderskei is getoets (3.3.9). PCR-RFLP-ontledings en die gebruik van 'n voorloper paar van die 16S-ribosomale-RNA-gene en die beperkingsensiem Dral het onderskeid tussen *B. invadens* en die drie *Ceratitis* plaag spesies moontlik gemaak.

Twee projekte in die vrugtevliegprogram fokus op die na-oes behandeling van vrugtevlieë. Beroking met 'n volle dosis van GRASFUM vir 24 uur het 100% vrugtevlieg sterftes in lemoene veroorsaak, maar het 'n afsmaak in Shamouti lemoene gelos (3.3.7). Verdere toetse sal met GRASFUM gedoen word. Die nuwe berokingsmiddel sal ook met 'n kort kouebehandeling gekombineer word. Die bepaling van die minste vatbare onvolwasse lewensstadium van die Medvlieg ten opsigte van koue disinfestasië teen 1°C, moes oorgedoen word volgens die Japannese protokol (3.3.8).

3.3.2 PROGRESS REPORT: Fruit fly rearing

Project 407 (1999/2000 – 2013/14) by A Manrakhan, J-H Daneel & R Beck (CRI)

Summary

Three fruit fly species: *Ceratitis capitata* (Medfly), *C. rosa* (Natal fly) and *C. cosyra* (marula fly) are reared on artificial diets at CRI Nelspruit. Fruit fly materials were used for research on: (1) a new attract and kill fruit fly system (Project 915), (2) a GRAS post-harvest fumigant for fruit fly (Project 913) and (3) cold disinfestation treatment for Medfly (Project 1054). Fruit fly pupae were also supplied to University of Stellenbosch, University of Pretoria, University of Kwa-Zulu Natal and Rhodes University for research on fruit fly physiology and control.

Opsomming

Drie vrugtevlieg spesies: *Ceratitis capitata* (Medvlieg), *C. rosa* (Natal vlieg) en *C. cosyra* (maroela vlieg) word op 'n kunsmatige dieet geteel by CRI Nelspruit. Vrugtevlieg materiaal is vir navorsing benodig oor: (1) 'n nuwe lok-en-doodmaak vrugtevliegsisteem (Projek 915), (2) 'n GRAS na-oes berokingsmiddel vir vrugtevlieë (Projek 913) en (3) 'n koue disinfestasië behandeling vir Medvlieg (Projek 1054). Vrugtevlieg papies is ook aan die Universiteit van Stellenbosch, die Universiteit van Pretoria, die Universiteit van Kwa-Zulu Natal en Rhodes Universiteit voorgesien vir navorsing oor vrugtevlieg fisiologie en beheer.

3.3.3 PROGRESS REPORT: A new bait for more effective control of all *Ceratitis* fruit flies

Project 915 (2008/9 – 2013/14) by A Manrakhan, John-Henry Daneel & Rooikie Beck (CRI)

Summary

The main aim of this study was to develop more efficient bait application techniques for fruit fly control. A new bait station made of wax coated bleached pulp paper designed into a cone and containing a mixture of ammonium acetate, trimethylamine, HymLure and malathion was tested in a citrus orchard in Low's Creek from April to May 2012. The new bait station was compared to blocks treated with the M3 bait station, blocks treated with GF-120 and 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. Overall, catches of *C. capitata* males and females were highest in the untreated blocks. Among the treated blocks, *C. capitata* male and female catches were highest in the blocks treated with the new bait stations and lowest in the blocks treated with the M3 bait stations. No fruit fly infestation was recorded in the blocks treated with the new bait stations. Modifications were made to the bait station in order to improve efficacy of control. The modifications made were on the attractant toxicant combinations. Six modified versions of the bait station were tested at different ages (fresh, 2 weeks, 4 weeks, 8 weeks and 12 weeks) in the laboratory. Two of the versions were almost twice as effective against *C. capitata* and *C. rosa* than the 2011-2012 bait station, when tested after 4 weeks of outdoor aging. The two promising 2013 bait station versions are currently being evaluated in citrus orchards at Low's Creek.

Opsomming

Die hoofdoel van hierdie studie was om 'n meer doeltreffende lokaasstoedieningsmetode te ontwikkel vir die beheer van vrugtevlieë 'n Nuwe wasbedekte, gebleikte, pulppapier lokaasstasie wat in 'n keel gevorm is en gevul is met 'n mengsel van ammoniumasetaat, trimethylamine, HymLure en malathion was in 'n sitrusboord by Low's Creek gedurende April en Mei 2012 getoets. Die nuwe lokaasstasie was met M3- en GF-120 behandelde blokke en onbehandelde blokke vergelyk. Die nuwe lokaasstasie was in 'n digtheid van 200 eenhede per hektaar uitgesit, teenoor die 400 eenhede van die M3. Die algehele vangs van *C. capitata* mannetjies en wyfies was die hoogste in die onbehandelde blokke. Tussen die behandelde blokke was *C. capitata* mannetjies en wyfies vangste die hoogste in die blokke wat behandel was met die nuwe lokaasstasies en die laagste in die blokke wat met die M3-lokaasstasies behandel was. Daar was geen vrugtevlieg besmetting in die blokke wat met die nuwe lokaasstasies behandel was aangeteken nie. Die nodige veranderinge is aan die lokaasstasie gemaak om die doeltreffendheid daarvan te verbeter. Die veranderinge was gerig op die lokmiddel en gifstof kombinasies. Ses aangepaste weergawes van die lokaasstasies op verskillende ouderdomme (vars, 2 weke, 4 weke, 8 weke en 12 weke) was in die laboratorium getoets. Twee van die weergawes was byna 2 maal meer effektief teen *C. capitata* en *C. rosa* in vergelyking met die 2011-2012 lokaasstasies, wanneer hulle na 4 weke blootstelling getoets is. Die twee belowendste 2013 lokaasstasie weergawes word tans in sitrusboorde by Low's Creek geëvalueer.

3.3.4 PROGRESS REPORT: Surveillance of *B. invadens* in commercial citrus orchards in South Africa

Project 966 (2009/10 – 2013/14) by Aruna Manrakhan, John-Henry Daneel (CRI) & Rooikie Beck (CRI)

Summary

Monitoring of *B. invadens* population continues across South Africa. New trapping sites were set in the CRI surveys in Limpopo and Mpumalanga: near Levubu, near Giyani, near Nwanedi, around Nelspruit and between Malelane and Komatipoort. The CRI *B. invadens* surveillance network consists of a total of 75 traps, 61 of which are maintained on a monthly basis by CRI personnel. Additionally, *B. invadens* traps were also monitored in southern Zimbabwe and Swaziland by co-workers (Zimbabwe) and CRI personnel (Swaziland). Insect specimens collected during surveys by CRI and by the Department of Agriculture, Forestry and Fisheries (DAFF) were identified and results were updated in the *B. invadens* national surveillance database.

In 2012, *B. invadens* was detected in surveillance traps in 14 areas in Limpopo, Mpumalanga and North West Provinces: near Groblersbrug, in Baltimore, near Mokapane, between Beitbridge and Pontdrift, in Musina, Tshipise, in Louis Trichardt town, in Levubu, in and near Tzaneen, in Hoedspruit, near Hazyview, in Zeerust, in Komatipoort and in and around Tshidnizi village. In all above areas the threshold level for eradication actions, which is 2 positive finds within a distance of 5 km or less, was triggered. All 14 affected areas were quarantined and eradication actions were carried out. In most of the areas, eradication was declared successful since there were no further finds of *B. invadens* for a period of 4 weeks after eradication actions were terminated and for a period of 12 weeks since the last find. However, few areas still remained problematic. Further to this, in the period January to March 2013, *B. invadens* was again detected in several areas in Limpopo, Mpumalanga and North West Provinces. The currently affected local municipalities are Musina, Mutale, Makhado, Thulamela, Greater Giyani, Greater Letaba, Greater Tzaneen, Greater Tubatse, Maruleng, Mogalakwena, Lepelle-Nkumpi, Bushbuckridge, Mbombela, Nkomazi, Umjindi, Ramotshene Moiloa and Rustenburg. *Bactrocera invadens* is now considered to be present only in specified areas in South Africa which are subject to official control. Affected commercial citrus orchards are implementing control actions in order to eradicate or suppress *B. invadens*.

Opsomming

Die monitering van *B. invadens* bevolkings regoor Suid-Afrika gaan voort. Nuwe opspoorareas vir CRI-opnames is in Limpopo en Mpumalanga gestig: naby Levubu, Giyani, Nwanedi, rondom Nelspruit en tussen Malelane en Komatipoort. Die CRI *B. invadens*-waarnemingsnetwerk in Suid-Afrika bestaan uit 'n total van 75 lokvalle waarvan 61 maandeliks deur CRI-personeel onderhou word. Daarby word lokvalle vir die monitoring van *B. invadens* ook in suidelike Zimbabwe en Swaziland deur medewerkers (Zimbabwe) en CRI-personeel (Swaziland) gediens. Insekmonsters wat gedurende die opnames van CRI en deur die Departement van Landbou, Bosbou en Visserie (DAFF) versamel is, was geïdentifiseer en die resultate is in die *B. invadens* nasionale waarnemings databasis opgedateer. Gedurende 2012 is *B. invadens* in waarnemingslokvalle opgespoor in 14 gebiede in die Limpopo, Mpumalanga en Noord-Wes Provinsies: naby Groblersbrug, in Baltimore, naby Mokapane, tussen Beitbridge en Pontdrift, in Musina, Tshipise, in Louis Trichardt dorp, in Levubu, in en naby Tzaneen, in Hoedspruit, naby Hazyview, in Zeerust, in Komatipoort en in en rondom Tshidnizi dorp. In al die bogenoemde areas is die drempelwaarde wat uitwissingstappe regverdig, van 2 positiewe vondse binne 'n afstand van 5 km of minder, oorskry. Al 14 geaffekteerde gebiede is onder kwarantyn geplaas en uitwissingsaksies is uitgevoer. In die meeste van die gebiede, is uitwissing suksesvol verklaar nadat geen verdere vondse van *B. invadens*, vir 'n tydperk van 4 weke na die beëindiging van die uitwissingsoptrede en vir 'n tydperk van 12 weke sedert die laaste vonds, was nie. 'n Paar gebiede is egter nog steeds problematies. Gepaardgaande hiermee is *B. invadens* weer opgespoor gedurende 'n tydperk van Januarie tot Maart 2013 in verskeie gebiede in die Limpopo, Mpumalanga en Noordwes provinsies. Die huidige geaffekteerde plaaslike munisipaliteite is Musina, Mutale, Makhado, Thulamela, Groter Giyani, Groter Letaba, Groter Tzaneen, Groter Tubatse, Maruleng, Mogalakwena, Lepelle Nkumpi-, Bosbokrand, Mbombela, Nkomazi, Umjindi, Ramotshene Moiloa en Rustenburg. *Bactrocera invadens* word slegs in gespesifiseerde gebiede in Suid-Afrika as teenwoordig beskou, wat dan onderhewig is aan amptelike beheer. Beheermaatreëls is in geaffekteerde kommersiële sitrusboorde geïmplementeer om *B. invadens* uit te wis of te onderdruk.

3.3.5 FINAL REPORT: Field control of *Bactrocera invadens* with MAT and bait Project 926 (2008/9 – 2012/3) by T.G. Grout and P.R. Stephen (CRI)

Summary

This report summarizes proactive research that was conducted to ensure that *Bactrocera invadens* could be controlled in citrus orchards once it arrived in South Africa. The research depended on the collaboration of researchers and farmers in tropical Africa where citrus is seldom grown commercially and orchards were usually small. The research included the development of the Invader-B-lok male annihilation method that effectively reduced large populations of *B. invadens* to manageable levels and the combination of this technique with protein bait sprays or M3 bait stations to control the females at commercially-acceptable levels. Combined use of Invader-B-loks with either Hym-Lure and mercaptothion bait sprays, or M3 bait stations provided commercial control of all fruit flies threatening citrus in Namibia where *B. invadens* only is present for half the year. In Kenya, where there are two wet seasons and *B. invadens* is a threat for most of the year, the combination of Invader-B-lok with M3 bait stations or GF120 or Prolure plus mercaptothion also provided commercial control of all citrus fruit flies. Invader-B-lok is now successfully being used in South Africa with bait sprays or bait stations to control *B. invadens*.

Opsomming

Hierdie verslag som die pro-aktiewe navorsing op wat gedoen is om te verseker dat *Bactrocera invadens* in boorde beheer kan word, sou dit in Suid-Afrika vestig. Die navorsing is afhanklik van samewerking met navorsers en produsente in tropiese Afrika waar sitrus selde kommersiël verbou word en boorde gewoonlik klein is. Die navorsing sluit die ontwikkeling van die Invader-B-lok manlike uitwissingstegniek in wat effektief groot populasies van *B. invadens* tot beheerbare vlakke verminder en die kombinasie van hierdie tegniek met proteïen-lokaas of M3 lokvalle om die wyfies op kommersiël aanvaarbare vlakke te beheer. Gesamentlike gebruik van Invader-B-loks met of Hym-Lure en mercaptothion lokaas bespuitings, of M3 lokvalle, verskaf kommersiële beheer van alle vrugtevlieë wat sitrus in Namibië bedreig, waar *B. invadens* slegs vir die helfte van die jaar teenwoordig is. In Kenia, waar daar twee nat seisoene is, en *B. invadens* 'n bedreiging vir die grootste gedeelte van die jaar is, het die kombinasie van Invader-B-lok met M3 lokvalle of GF120 of Prolure plus mercaptothion ook kommersiële beheer van alle sitrus vrugtevlieë verskaf. Invader-B-lok word nou suksesvol in Suid-Afrika met lokaas bespuitings of lokvalle gebruik om *B. invadens* te beheer.

Introduction

Bactrocera invadens Drew, White & Tsuruta (Drew et al. 2005) was found to be present in Kenya in 2003 (Lux et al. 2003) and the next year was recorded in West and Central Africa. Prior to 2004, only fruit flies belonging to several *Ceratitidis* species were known to infest mango in West Africa but mango (*Mangifera indica* L.) became a favoured host for *B. invadens*. Subsequently, many more hosts have been described (Drew et al. 2005, Rwomushana et al. 2008, Goergen et al. 2011) but the dominant hosts appear to be mango, marula (*Sclerocarya birrea* (A. Rich.) Hochst.), guava (*Psidium guajava* L.), and *Citrus* species. In 2008, when this research to develop control techniques for *B. invadens* before it arrived in South Africa was initiated, the pest was not yet near our borders and the first research was done in collaboration with R. Hanna and D. Gnanvossou of the IITA in Benin using mango orchards. It was difficult to find large enough orchards of citrus in tropical Africa for trial purposes and attempts to use a large government citrus farm near Muheza in Tanzania in 2009 failed due to bureaucracy. Trials were subsequently conducted on citrus in Uganda where *B. invadens* populations were high but orchards were only around 2 ha in size. A commercial citrus farm near Tsumeb in Namibia was used for two seasons but the presence of *B. invadens* there was seasonal and numbers of flies were not as high as in the tropics. Finally, a commercial citrus farm was found in Kenya in 2011 where trials were conducted for two seasons.

Due to the success of the male annihilation technique (MAT) against the closely related *B. dorsalis* Hendel (Steiner et al. 1965) we wanted to evaluate this technique against *B. invadens* and determine whether it alone could control the fly or whether bait sprays would also be required.

Stated objectives

To obtain data on the control of *B. invadens* by using the male annihilation technique, protein baits with a toxicant, and a combination of the two.

Materials and methods

Benin with IITA collaboration from R. Hanna and D. Gnanvossou 2008-9

Six mango orchards, two in each of three villages, were selected for the experiment in March 2008. Five orchards were 2 ha in size while the sixth orchard had an area of 3 ha. The orchards were located in Tchaourou (08.98.177N 002.62.003E and 08.92.147N, 002.56.569E); Tchatchou (09.05.707N, 002.33.309E and 09.21.451N, 002.37.297E); and Alafiarou (09.01.443N, 002.41.364E and 09.01.449N, 002.38.861E). Orchard elevations ranged from 301 to 352 masl. All orchards contained mixed plantings of early, mid and late season mango varieties, with Gouverneur, Eldon and Kent being the dominant varieties. Vegetation surrounding the orchards was composed mainly of *Vitellaria paradoxa* C.F. Gaertn., *Annona senegalensis* Pers., *Anacardium occidentale* L. and *Sarcocephalus latifolius* (Smith) Bruce.

In each village, the two orchards were randomly designated to MAT and control treatments. Each MAT orchard had 40 M3 devices (without the usual attractant and toxicant) per lure type at the rate of 20 devices per ha, except for the 3 ha orchard in which 60 devices of each lure were installed. The lures used in the MAT devices (MATDs) were methyl eugenol (ME) for *B. invadens* and terpinyl acetate (TA) for *Ceratitis cosyra* (Walker). Only one type of MATD was hung per tree at about 1.5 m above ground level near the middle of the tree canopy. Devices of the two lures were placed in alternative mango tree rows – 10 m between tree rows and 20 m between MATDs within a tree row.

MATDs were charged with 4 ml solution containing 3 ml of the lure (ME or TA) and 1 ml of undiluted malathion (500 g/L EC) toxicant. ME MATDs were recharged once a month while TA MATDs were recharged at 15-day intervals due to higher volatility of TA. In each of the MAT treatment orchards, five of each lure MATDs (four at the periphery and one in the centre) were each placed in a bucket trap to monitor male populations. Three MATDs of each lure were similarly used in each of the control orchards to monitor population trends of male *B. invadens* and *Ceratitis* spp. In addition, Torula yeast was used in McPhail traps in both treated and untreated areas because this lure was not in competition with the MAT lures. MATDs and traps were placed on 15 March 2008 and fruit flies were collected at 15 day-intervals through 19 July.

In addition to adult fruit fly population monitoring, 100 fruit per variety (10 from each of 10 trees) were collected on each sampling date during two consecutive weeks of the peak maturity period for that variety. The trees used for sampling were distributed as follows: four trees in the periphery, another four at intermediate distance to the centre, and two trees in the centre of the orchard. All fruit were inspected for signs of infestation by fruit flies, weighed and incubated in plastic bins (with a 2 cm layer of moist sand in the bottom) in groups of 10 fruit to allow fruit fly larvae to leave the fruit and pupate. Pupae were removed at 3-4 day intervals and placed in Petri dishes with moist cotton and a small quantity of sugar and protein hydrolysate for emerging adults, which were later sexed and identified to species.

In addition to mango sampling, we collected (at two-week intervals) at least 20 fruit from each of several potential *B. invadens* and *Ceratitis* spp. hosts within 500 m from the borders of each of the experimental orchards. These included *S. latifolius*, *A. senegalensis*, *V. paradoxa* and *A. occidentale*. The identity and frequency of host species were recorded to assess the relative effect of fruit fly sources surrounding orchards on the efficacy of MAT to suppress fruit flies in the mango orchards.

Development of the Invader-B-lok

Research in Benin was conducted with blank M3s because at short notice an alternative absorbent material could not be found. While this trial work was being established we evaluated different materials in order to find a material similar to the cane fibres used by Steiner et al. (1965) that would be inexpensive. Stonehouse (2002) had used plywood in Pakistan so we decided to evaluate pieces of soft board (as used for noticeboards), SA pine, chipboard and Masonite hardboard for absorption and durability.

The comparison was started on 17 April 2008 and four square blocks (6 x 6 cm) were cut out of each material. The softboard and pine were 13 mm thick, the chipboard 16 mm and Masonite 3 mm. Each block had a hole drilled through it so it could be later suspended. Each block was weighed before being placed in water for 15 hours. After removing the blocks from the water they were placed on a rack to drain for 5 min before being weighed once more. The mean mass of water absorbed per block type was then determined.

Tanzania 2009/10

Attempts to conduct research on a large, government-owned citrus farm near Muheza failed because permission was not granted to enter the country for this purpose. We provided 40 kg Mazoferm bait to Dr Zuberi Seguni (MARI) there to evaluate against *B. invadens* but never received any results.

Uganda with NARL collaboration with C. Nankinga and I. Rwomushana

CRI signed an MOU with National Agricultural Research Laboratories in order to conduct some trials with MAT and bait sprays against *B. invadens* on citrus farms in north-eastern Uganda in the district of Soroti.

Trials were laid out at four sites in the villages Abuket and Sapir in Kyere sub-county, Obulin in Olio sub-county and Orupe in Kateta, but these sites were over 300 km from the research centre near Kampala so supervision of monitoring and bait applications was poor. Fruit flies were monitored by using two Moroccan traps per treatment containing 3-component BioLure (Suterra, sold by Chempack, South Africa) and a dichlorvos block. Monitoring and treatments took place between 7 May and 20 June 2010. The orchards were also only 1-2 ha in size so treatment size was limited to about 0.5 ha. Only two of the sites had all four of the treatments below, the other two smaller sites did not include Treatment 2 with MAT blocks alone. Being on the equator, the citrus orchards are harvested twice a year so in October 2010 the design was changed to only one or two treatments per site with a control in citrus trees 1 to 5 km away. Flies were again monitored with the use of two BioLure traps per treatment and control, and fallen fruit were collected to determine what flies would emerge.

Treatments evaluated:

1. Untreated control
2. Softboard blocks (12 per ha) impregnated with 3 ml methyl eugenol and 1 ml Malathion 500 EC
3. Hym-Lure 1L plus Malathion 500 EC 175 ml per 100 L water at 100-200 ml per tree side down every second inter-row. Apply fresh bait once a week in the morning.
4. MAT treatment (2) plus bait treatment (3)

Namibia 2010/1

A trial was conducted in Valencias on a commercial farm (Namfo) just outside Tsumeb, Namibia in May-June 2010 using protein baits and M3s, both with and without MAT blocks. Two replicate blocks were used per treatment (randomized in each half of the orchard) and each block was 0.97 ha and contained 650 trees (25 rows x 26 trees). Two BioLure three-component traps were placed in each replicate (in the seventh row in from opposite edges) and two control traps were placed on opposite edges of the farm approximately 500 m from the trial orchard. The orchard traps were hung 1.5 m above the ground inside the northern side of the canopy. Traps were first hung on 13 May 2010 when the M3s (360 per replicate) and MAT blocks (20 per replicate) were also hung and the first bait sprays applied. Monitoring and bait applications continued for eight weeks.

Treatments evaluated:

1. HymLure 0.8 L plus mercaptothion 500 EC 175 ml per 100 L water at 155 ml per tree side down every second inter-row (100 L bait per ha)
2. M3 bait stations at 360/ha
3. Softboard blocks (20/ha) impregnated with methyl eugenol and mercaptothion 500 EC in a 3:1 ratio plus Treatment 1.
4. Softboard blocks (20/ha) impregnated with methyl eugenol and mercaptothion 500 EC in a 3:1 ratio plus Treatment 2.

The trial was repeated in February-March 2011 in navel oranges using two replicates each of the same treatments but with treatment blocks containing ME separated from those without this attractant by 275 m of mango trees (Fig. 3.3.5.1). Two BioLure 3-component traps were hung per replicate as before and four control traps were hung 50 m apart along the marula tree windbreak on the northern edge of the farm 275 m from the trial citrus. MAT blocks and M3s were evenly distributed throughout the blocks where required. All treatments and monitoring started on 9 February 2011. Dead flies collected from the traps were collected by farm management and sent to CRI-Nelspruit for counting.

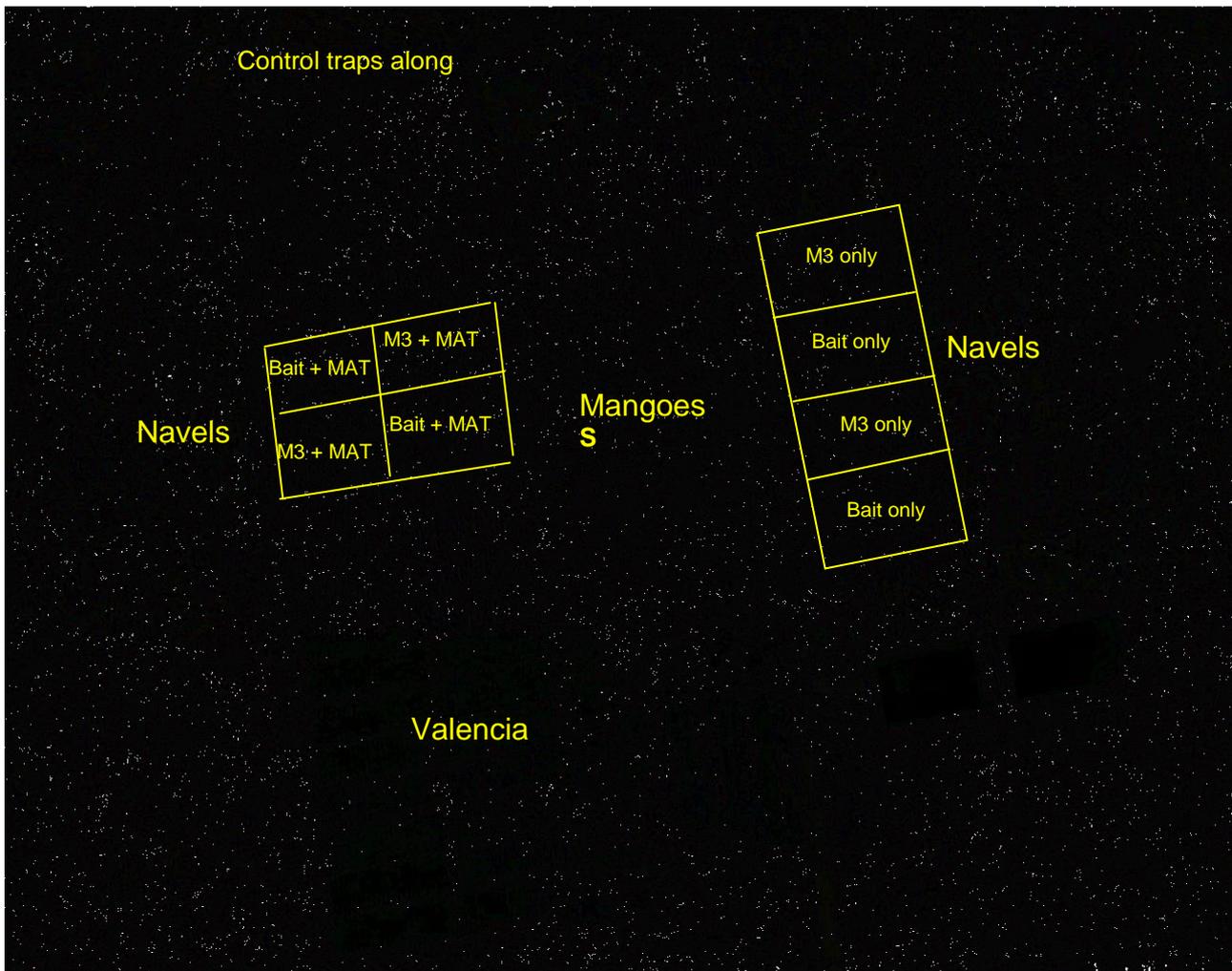


Figure 3.3.5.1. Layout of second trial near Tsumeb, Namibia started February 2011.

Kenya 2010/1-2012/3

Plans were made to conduct a trial similar to the one in Namibia on orange trees at Deka Plantations Ltd, on the Thika road, north of Nairobi, late in 2010. However, numbers of *B. invadens* remained low until March 2011 when MAT blocks were hung in all treatments and Morocco bucket traps (four per treatment) containing BioLure 3-component attractant and a dichlorvos block were also hung in each treatment. Fruit fly numbers remained low throughout March so bait and bait-station treatments only started in April 2011. Control blocks were not used but control traps were placed on the perimeter of the farm more than 0.5 km from the treated orchards. The treatments were each applied to a single 2 ha block of navel oranges as follows:

1. Softboard blocks (12/ha) impregnated with methyl eugenol and mercaptothion 500 EC in a 3:1 ratio (MAT blocks)
2. MAT blocks (1) plus Mazoferm 7 L plus mercaptothion 500 EC 175 ml per 100 L water at 100-200 ml per tree side down every second inter-row. Apply fresh bait once a week in the morning.
3. MAT blocks (1) plus Prolure 2 L plus mercaptothion 500 EC 175 ml per 100 L water applied as in (2)
4. MAT blocks (1) plus M3 bait stations at 400/ha

Flies were collected weekly from each trap and placed in labelled plastic bags for four weeks during April. The dead flies were sent to Nelspruit for identification and counting. The farm manager did do a rough count of the insects found in some traps and included his count with the flies but we ignored this.

The trial was repeated early in 2012 with MAT blocks (10 per ha) and BioLure 3-component traps (two per 2 ha block) being hung on 31 January 2012 before colour break on the oranges. This time a citrus variety block was used as a control that was distant from the treated orchards. The commercial combination of bait and insecticide: GF120, was also included in the trial. Bait applications and the hanging of M3s only started on 21 February at the first sign of fruit colour. The treatments were as follows:

1. Softboard blocks (10/ha) impregnated with methyl eugenol and mercaptothion 500 EC in a 3:1 ratio (MAT blocks)

2. MAT blocks (1) plus Mazoferm 7 L plus mercaptothion 500 EC 175 ml per 100 L water at 100 ml per tree side (5 s per tree when spraying with a knapsack) down every second inter-row. Fresh bait was applied once a week in the morning.
3. MAT blocks (1) plus Prolure 2 L plus mercaptothion 500 EC 175 ml per 100 L water applied as in (2) at 100 ml mixture per tree.
4. MAT blocks (1) plus M3 bait stations at 400/ha
5. MAT blocks (1) plus GF120 (containing spinosad) 1 L plus 24 L water applied per ha, or approximately 50 ml per tree (2.5 s per tree when spraying with a knapsack)

Traps were emptied once a week and the dead flies placed in plastic bags and refrigerated until they were sent to South Africa for identification. The last time the traps were emptied was on 27 March 2012 so there were five weeks of trap counts after all treatments started.

Results and discussion

Benin with IITA collaboration from R. Hanna and D. Gnanvossou 2008-9

Ceratitis spp. populations in TA traps were 5-fold higher in MAT orchards compared with control orchards on the first sampling date (Fig. 3.3.5.2). During subsequent dates, *Ceratitis* spp. declined to substantially lower levels (with about 65% reduction at peak population density) in MAT compared with control orchards. To our knowledge, this is the first demonstration in sub-Saharan Africa of the utility of terpinyl acetate in the suppression of *Ceratitis* spp. populations.

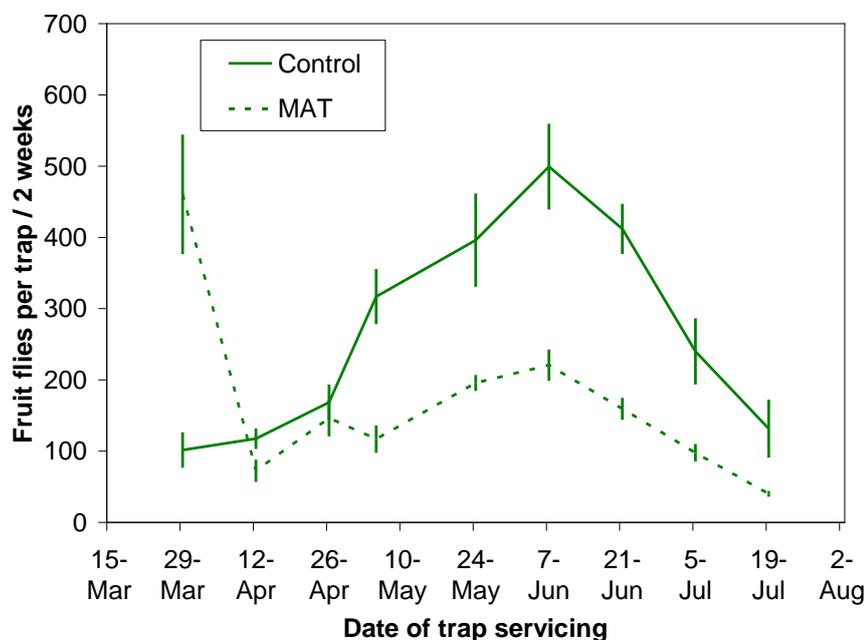


Figure 3.3.5.2. Population trends of male *Ceratitis* spp. in terpinyl acetate traps in control (solid line) and MAT orchards (dashed line).

Bactrocera invadens displayed considerably different dynamics than *Ceratitis* spp. Unlike *Ceratitis* spp., *B. invadens* populations were nearly absent until early May, which is typical for northern Benin, where *B. invadens* disappears during the dry season from January to April, and reinvades after the beginning of the rainy season. As in the case of *Ceratitis* spp. in TA traps, *B. invadens* populations were relatively higher in the MAT orchards compared with control orchards on the first sampling date, but remained very low in all orchards during the month of April (Fig. 3.3.5.3). The trend reversed by early May when *B. invadens* populations increased steeply in the control orchards reaching an average of $5\,024.8 \pm 644.4$ (mean \pm SE) individuals per trap per two week period by the last sampling date on 19 July, while *B. invadens* populations in the MAT orchards remained relatively flat and at levels of less than 10% of those in the MAT orchards, reaching a peak of 431.5 ± 153.1 individuals per trap per two weeks on 19 July. To our knowledge, this is also the first study to demonstrate the utility of MAT to suppress populations of *B. invadens* anywhere where this species has been found. Results from Torula yeast traps were not provided because the sorting of the many species of flies from these traps was very time consuming.

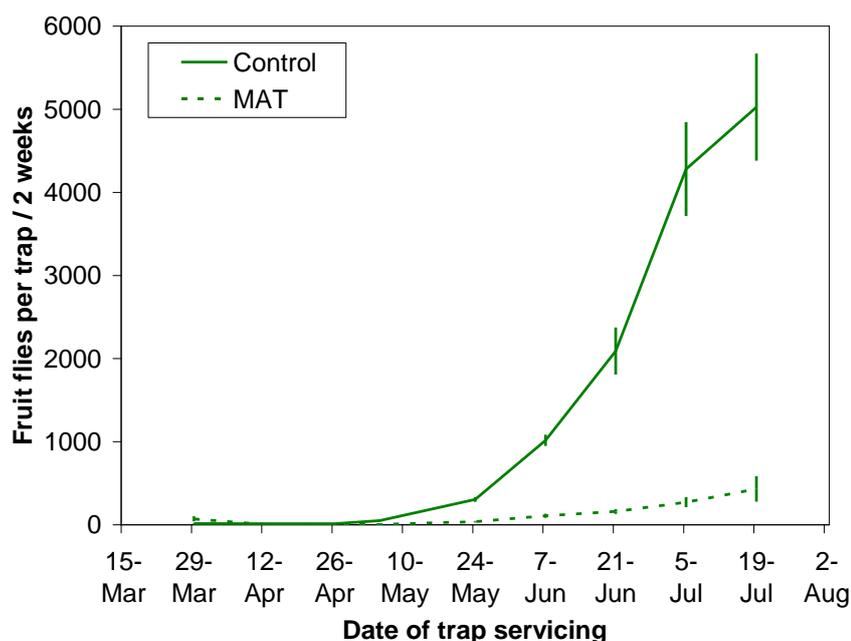


Figure 3.3.5.3. Population trends of male *Bactrocera invadens* in methyl eugenol traps in control (solid line) and MAT orchards (dashed line) during 2008.

Fruit fly infestations of two mid-season varieties (Eldon and Kent) are presented in Table 3.3.5.1. These infestations mirrored the reductions in fruit fly adult populations, particularly those of *Ceratitidis* spp. The percentage of fruits infested by fruit flies (all species) were similar for the two varieties (49.8 ± 2.67 for Eldon and 46.2 ± 2.98 for Kent) in the control orchards, but in the MAT orchards infestations of both varieties were significantly less than in the control orchards, with infestation of Kent being less than those of Eldon. MAT reduced fruit fly infestations by 39.8% for Eldon and 46.8% for Kent. Unfortunately, this infestation refers to both *B. invadens* and *Ceratitidis* spp. (mostly *C. cosyra*) because at the time of writing this report the identification of the emerging flies was not complete. As the TA MAT device was less efficient at controlling *Ceratitidis* due to its volatility it is likely that many of the flies were *Ceratitidis* spp.

Table 3.3.5.1. Fruit fly infestation levels in MAT and control orchards, 2008 mango season in northern Benin.

Mango variety	Fruit infestations (mean \pm SE)		Percent infestation reduction with MAT
	Control	MAT	
	Percent infested fruits		
Eldon	49.8 ± 2.67	30 ± 2.1	39.8
Kent	46.2 ± 2.98	24.6 ± 2.42	46.8
	Pupae per kg/fruit		
Eldon	26.3 ± 3.10	12.9 ± 2.38	56.1
Kent	19.7 ± 2.57	5.61 ± 1.11	68.8

The results of this one year study were encouraging. Not only did MAT reduce adult male populations of both *Ceratitidis* spp. and *B. invadens* adult male populations, but more importantly it led to substantial reductions in fruit fly infestations and in turn greater harvest of marketable mango. This was the first demonstration of the utility of MAT in fruit fly suppression in mango orchards in sub-Saharan Africa. However, it was clear that for commercial control of *B. invadens*, bait sprays would also be required to reduce the numbers of females. MAT is likely to be more effective in citrus than mango because *B. invadens* is the dominant species in Benin and citrus does not appear to be as susceptible to *B. invadens* as mango. The M3 as a MATD was not practical because it could only hold a few millilitres of lure and toxicant and therefore required recharging. A cheaper product that could hold a larger volume of liquid would be more effective.

Plans were made to repeat this work in citrus the next year but the IITA research focus changed to the use of parasitoids so no further research was conducted there and a final report was never received.

Acknowledgements

We thank IITA core donors for their financial support, and the five farmers – Caboura Sounon, Chala Dama Julien, Gounou Gbéba, Gbéléjähé Louis (2 orchards) and Chabi Barka Claude, for allowing us to conduct this study in their orchards. Innocent Agnontcheme, Lazare Dossoumon, Yakpa Simon, Marcellin Amande and Marius Sessou provided the needed technical and logistical assistance in carrying out this research.

Development of the Invader-B-lok

Chipboard held the most water relative to the original dry mass of the block (Table 3.3.5.2) but after hanging in the air for a few days these blocks disintegrated. The softboard held the next most water relative to its original mass and these blocks remained durable after soaking. Softboard is also considerably cheaper than the next most absorbent material SA pine. We therefore opted to use softboard in creating MAT blocks for evaluation in Uganda but the size of the blocks was reduced to 50x50x10 mm. A mixture by volume of 3 methyl eugenol (ME) to 1 mercaptothion 500 EC was made up and the blocks initially soaked in this for 12 hours. Then it was found that the ME and mercaptothion EC is absorbed much more rapidly than water so later blocks were made by only soaking for 30 min. These blocks contained approximately 18 g of ME and mercaptothion mixture. This technique was then provided to River Bioscience for the manufacture of Invader-B-lok and this was the MAT block used in further research (Fig. 3.3.5.4).

Table 3.3.5.2. Amount of water held as a percentage of the dry mass of 6 x 6 cm squares of wood product

Block	Softboard	SA Pine	Chipboard	Masonite
1	80.3	54.1	156.4	22.5
2	78.6	59.8	154.8	22.3
3	81.1	58.2	160.3	23.1
4	85.8	63.1	159.4	23.0
Mean	81.5	58.8	157.7	22.7



Figure 3.3.5.4. MAT blocks (Invader-B-lok) killing *B. invadens* in the back of a pick-up truck before being hung in the orchard in Kenya

Uganda 2009/10-2010/1

In April/May 2010 there were good numbers of *B. invadens* in the orchards and these were seen when changing ME traps (Fig. 3.3.5.5). However, neither of the seasons in 2010 produced results that showed significant differences between treatments in catches with BioLure or even showed any trends between

treatments at any of the four sites. This is attributed to the plot size being too small to have a control and treatments that contained MAT blocks adjacent to one another because the ME attracted flies out of the control blocks. The monitoring data were also unreliable with traps in some treatments sometimes being emptied on different days to other treatments. Fly numbers in the controls in the second season of October and November were very low so any benefit of having treatments further apart was not quantified. Very few flies were recovered from fallen fruit in the second season so these data also did not show treatment effects. However, farmers said that they were able to pick a crop of mangoes for the first time in three years from trees that were mixed in with, or adjacent to the citrus, so the treatments clearly had an impact in reducing infestation levels. The only quantifiable result was that over all treatments and sites in the second season, BioLure traps caught 318 *B. invadens* compared with 73 *Ceratitis fasciventris*, 59 *C. cosyra* and 53 *C. capitata* (Fig. 3.3.5.6).

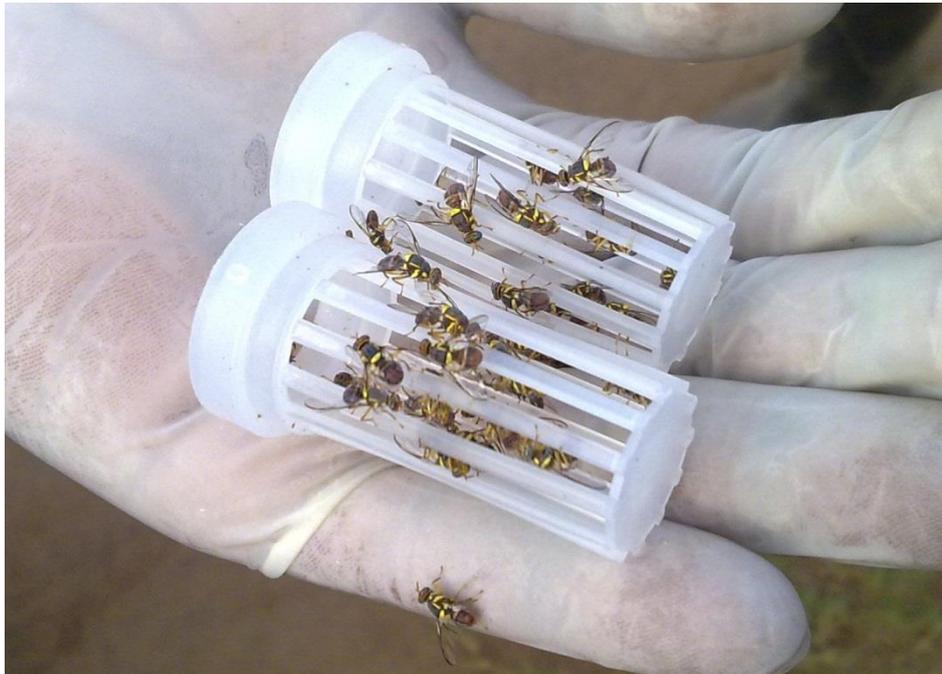


Figure 3.3.5.5. *B. invadens* males coming to baskets that had previously held ME lures in bucket traps in Uganda.

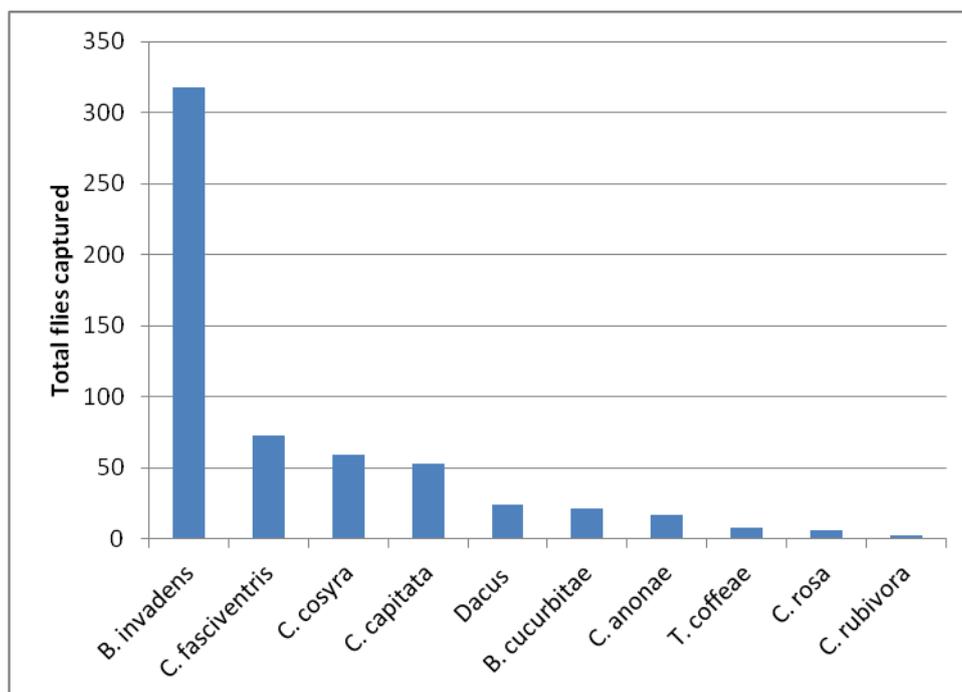


Figure 3.3.5.6. Fruit fly species composition as determined by total fruit fly catches in BioLure-baited traps in 4 citrus orchards in Uganda from September to October 2010.

Namibia 2010/1

In the first trial in Valencias the numbers of *B. invadens* crashed with the onset of cold, dry weather in mid to late May 2010 and no flies were recovered from any of the treatments or boundary trees. We were therefore not able to record any treatment differences. In late summer of 2011, the numbers of trapped *B. invadens* on the farm remained low, even though unusually high rainfall had been received that year and conditions were considered suitable. The reason may be due to the farmer using M3 bait stations and MAT blocks on other parts of the farm and suppressing fruit fly numbers in the whole area. The traps were therefore only emptied on two occasions. No Natal flies, *C. rosa* Karsch, were recovered in the BioLure traps and the numbers of *B. invadens* and Medfly were very low (Table 3.3.5.3). Neither of these species was caught in the marula windbreak but only a total of 27 *C. cosyra* in the four traps over the eight week period. Due to the low numbers of flies, statistical analysis was not possible but the fact that only two *B. invadens* flies were caught in the two treatments containing MAT blocks compared with a total of 10 in the two treatments without MAT blocks suggests that ME was not pulling *B. invadens* from other treatments as had happened with smaller plot sizes. Perhaps the only definite conclusion that can be drawn is that under these circumstances, all treatments provided commercial control including those with M3s alone or HymLure bait sprays alone.

Table 3.3.5.3. Numbers of *B. invadens* and Medfly caught in two BioLure 3-component traps per replicate in treated citrus near Tsumeb, Namibia

Treatments	First evaluation period 12 d		Second evaluation period 44 d		Total pest flies* caught in 8 weeks	Total per trap per week
	Replicate A	Replicate B	Replicate A	Replicate B		
M3s only	1 Bi	0	2 Bi 1 Med	1 Bi 1 Med	6	0.19
Bait only	3 Bi	1 Bi	1 Bi	1 Bi	6	0.19
M3s + MAT	0	0	2 Med	2 Bi 1 Med	5	0.16
Bait + MAT	1 Med	0	0	0	1	0.03

*A combination of *B. invadens* and *C. capitata*.

Kenya 2010/1-2012/3

In the first trial in 2011, the numbers of *B. invadens* were significantly ($P < 0.05$) lower in all treatments compared to the untreated border (Table 3.3.5.4) and all treatments were considered by the grower to provide commercial control, although an intervention threshold has not been developed for BioLure in bucket traps. A few specimens of Medfly and Natal fly were caught in the traps and these were added to the numbers of *B. invadens* and expressed as citrus flies in Table 3.3.5.4. The best treatment was the combination of MAT blocks and M3s. Marula flies were more abundant on the farm than other *Ceratitis* spp., due to the proximity of mangoes. The untreated border was close to a mango block so that explains the high numbers of Marula flies caught there compared to the block with MAT blocks only which should not have affected Marula fly numbers. Prolure is similar to the attractant used in M3 bait stations and this is known to be effective against Marula fly so this explains why the numbers are lower in those treatments than in the Mazoferm treatment (Table 3.3.5.4). Flies were kept at ambient temperature in plastic bags for several months before sending to South Africa for identification so many were mouldy and difficult to identify (Fig. 3.3.5.7).

Table 3.3.5.4. Mean numbers of fruit flies caught per BioLure 3-component trap per week during the month of April 2011 after MAT blocks had been present throughout March in orange orchards in Kenya.

Treatments	Mean no. <i>B. invadens</i> per week per trap	Mean no. citrus flies* per week per trap	Mean no. <i>C. cosyra</i> per week per trap
Untreated border	8.4 a	8.6 a	74.1 a
MAT only	2.3 b	3.1 ab	18.2 ab
MAT plus Mazoferm 7% plus mercaptothion	4.4 b	4.8 ab	39.2 ab
MAT plus Prolure 2% plus mercaptothion	1.4 b	1.7 b	10.8 b
MAT plus M3 bait stations 400/ha	0.9 b	1.2 b	12.8 b

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

*A combination of *B. invadens* and *C. capitata*.

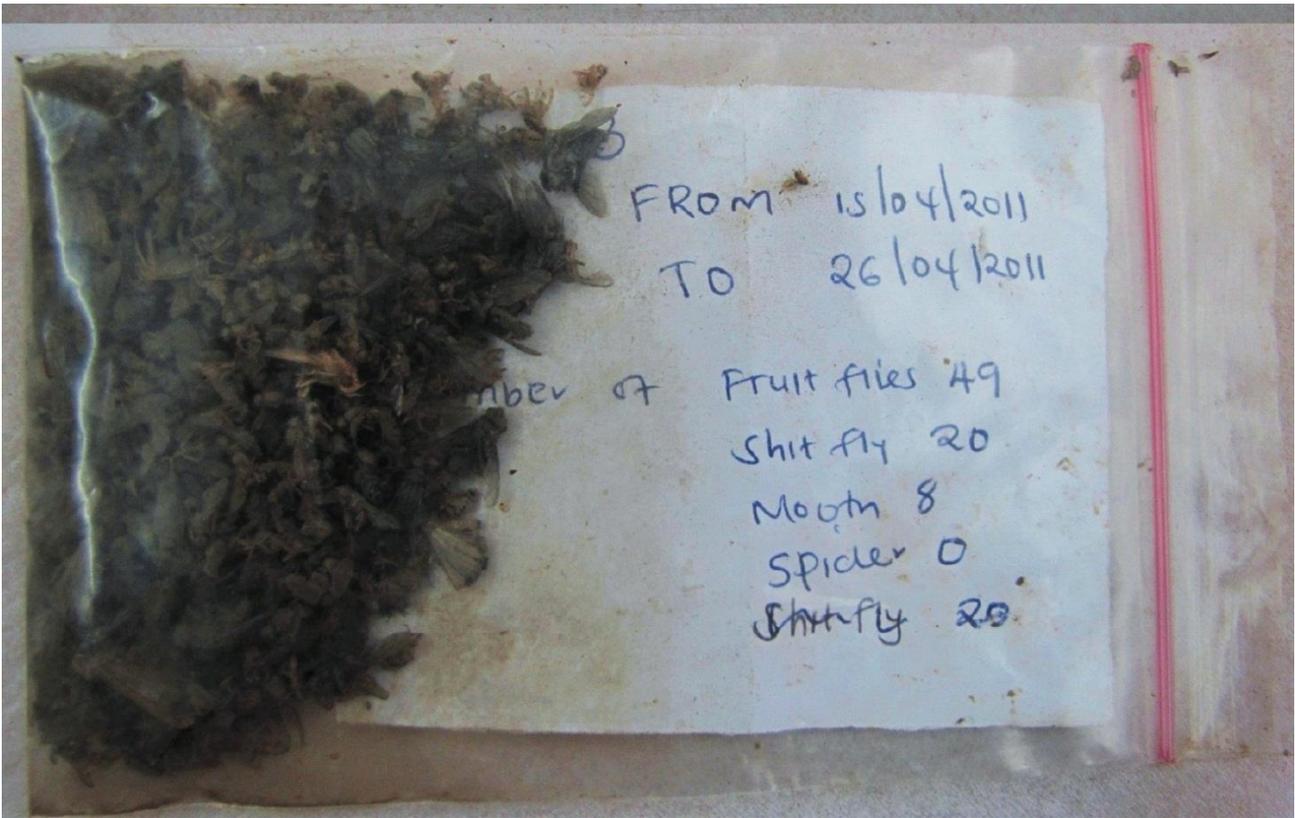


Figure 3.3.5.7. Mouldy insects (with farm manager notes) that had to be identified several months after collection.

In the second trial in 2012, traps in the untreated control were seldom emptied and had to be excluded. Variation in the numbers of flies trapped within treatments was extreme and statistical analysis was not possible so numbers in both traps per treatment were pooled in order to provide reasonable data for inter-treatment comparisons. Numbers of *B. invadens* females per trap per week in the MAT-only treatment remained twice as high as in all the other treatments that also contained a form of bait (Fig. 3.3.5.8). This therefore confirmed the need to have a bait treatment in addition to male annihilation in order to obtain commercial control of females. The combination of MAT blocks and M3s resulted in low numbers of trapped *B. invadens* females throughout the trial, whereas numbers increased in the MAT plus Mazoferm treatment.

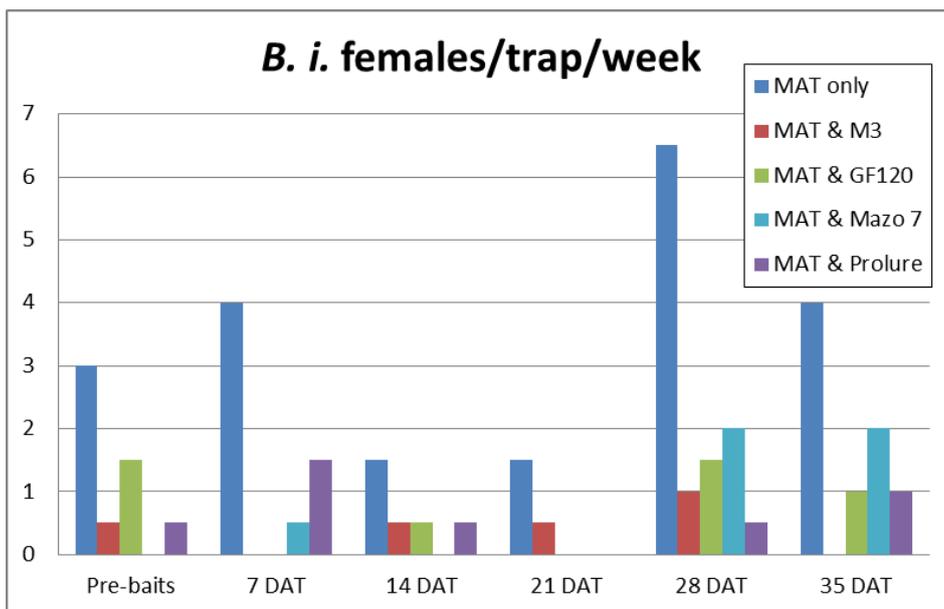


Figure 3.3.5.8. Mean catches of female *B. invadens* per BioLure trap per week in differently treated navel orange orchards in Kenya in February-March 2012.

Medfly numbers were extremely high at the beginning of the trial due to a neighbouring coffee farm. When their numbers were combined with *B. invadens* in Fig. 3.3.5.9 it was clear that it took two weeks to get the combined fruit fly numbers under control. After five weeks, the MAT blocks plus M3 bait stations were once again giving the lowest number of flies and MAT plus GF120 was also effective.

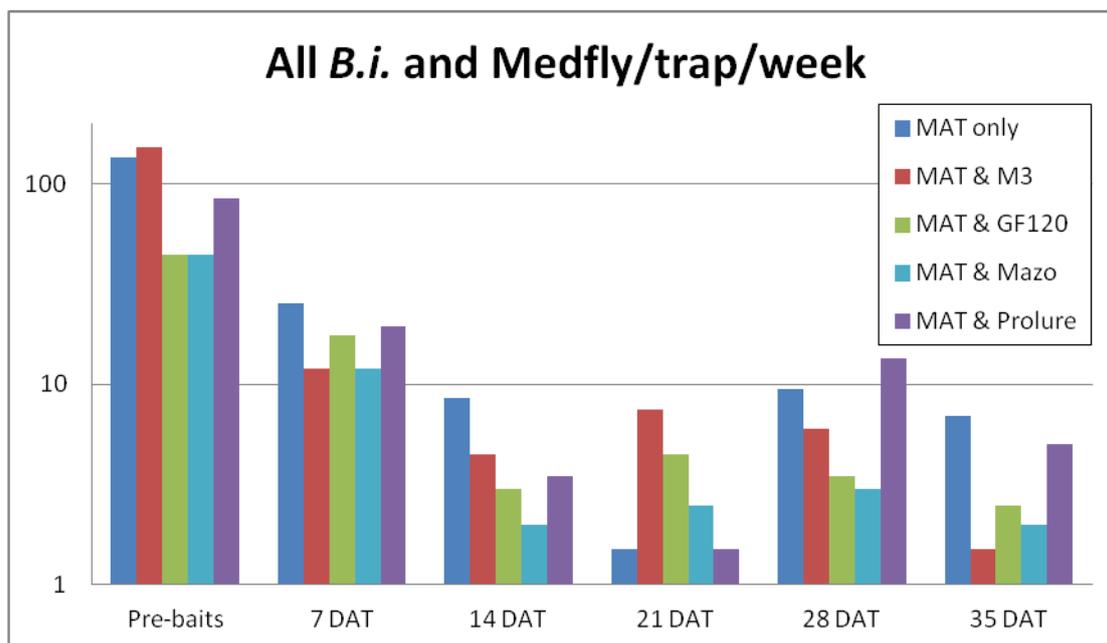


Figure 3.3.5.9. Mean catches of both sexes of *B. invadens* and Medfly per BioLure trap per week in differently treated navel orange orchards in Kenya in February-March 2012.

Conclusion

After trials in four countries, often without good supervision and less than ideal trial sites, we can conclude that commercial control of *B. invadens* is possible in citrus when both MAT blocks and a form of baiting for females are used. The combination of M3 bait stations and MAT blocks resulted in the lowest numbers of trapped flies but the use of M3s alone was only evaluated in Namibia where the *B. invadens* population was low.

Future research

Once *B. invadens* is established in South Africa and research can be conducted first-hand we will need to establish how many MAT blocks are required per hectare and whether control can be achieved with bait sprays or bait stations alone.

Technology transfer

Talks have been presented on this research at the 2010 and 2012 Citrus Research Symposia held in the Drakensberg, South Africa. Results of this project were also presented at the meeting of the Tephritid workers of Europe, Africa and Middle East in Crete, Greece in July 2012. An SA Fruit Journal article will be published on parts of this research.

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3.3.6 FINAL REPORT: Determine the potential global distribution for *Bactrocera invadens* using CLIMEX

Project: SU De Villiers (April 2010 – June 2013) by M de Villiers (CRI at SU), Vaughan Hattingh (CRI), Marc de Meyer (Royal Museum of Central Africa, Belgium), Jean-François Vayssières (CIRAD, Benin), Antonio Sinzogan (University of Abomey-Calavi, Benin), Sunday Ekesi (ICIPE, Kenya), Samira Faris (ICIPE, Kenya), Maulid Mwatawala (Sokoine University of Agriculture, Tanzania), Faiza Salah (University of Gezira, Sudan), Hayder Abdelgader (The Agricultural Research Corporation, Sudan), Chiluba Mwape (Zambia Agriculture Research Institute, Zambia)

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 phenology in Africa. Twenty three 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, were used to monitor the flies. Results showed that *B. invadens* is adapted to a wide range of climates, including the hot extremes bordering the Sahara desert. Two key factors appear to influence its phenology, namely moisture, either in the form of rain or irrigation, and host availability. A preliminary CLIMEX model has been constructed based on its distribution, relative abundance and seasonal phenology in Africa, as well as information on its biology. This will be fine-tuned to incorporate several irrigation scenarios to achieve optimal fit between trapping data and climatic suitability as predicted by the model.

Opsomming

Sedert die eerste verskyning van die indringer vrugtevlieg, *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 potensieële globale verspreiding van *B. invadens* met CLIMEX te bepaal, gebaseer op die vlieg se verspreiding, relatiewe volopheid en seisoenale voorkoms in Afrika. Drie-en-twintig 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 perseel, is gebruik om die vlieë te monitor. Resultate het gewys dat *B. invadens* 'n wye reeks van klimate kan oorleef, insluitend die warm woestynklimatiese aangrensend tot die Sahara. Twee faktore blyk om die vlieg se fenologie te beïnvloed, naamlik vog, óf in die vorm van reën óf reënval, en gasheer beskikbaarheid. 'n Voorlopige CLIMEX model is opgestel gebaseer op die vlieg se verspreiding, relatiewe volopheid en seisoenale fenologie in Afrika, sowel as inligting oor die biologie van die vlieg. Hierdie model sal verfyn word om verskeie besproeiing scenarios in te sluit om optimale passing tussen moniteringsdata en klimatologiese geskiktheid, soos deur die model voorspel, te verkry.

Introduction

The African Invader fly, *Bactrocera invadens* (Diptera: Tephritidae), is a fruit fly pest species of Asian origin that was detected on the African mainland in 2003 (Lux et al., 2003; Drew et al., 2005) and now found in approximately 35 countries in Africa, including the islands of Madagascar, Cape Verde, Comoros and Mayotte. The pest had spread as far south as northern Namibia, northern Botswana, northern Zimbabwe and Mozambique (Fruit fly database held at the Royal Museum for Central Africa, accessed via Marc de Meyer, 2013; Mguni, 2013). Recently, the pest was declared present in specified areas in the the northern parts of South Africa (Manrakhan & Hattingh, 2013:). *Bactrocera invadens* is a pest of economic importance to the fruit industry in Africa (Mwatawala et al., 2004) and is of phytosanitary concern, being listed as a quarantine pest in the European and North American Plant Protection Organisations (CPC, 2011; EPPO, 2013).

It is likely that this pest species will continue to expand its range in Africa given the fragmented agricultural landscape of the continent and the polyphagous and aggressive nature of the pest. *Bactrocera invadens* is already recorded on almost 80 plant species (De Meyer et al., 2012) and seems also to be capable of displacing native *Ceratitis* pest species such as *C. cosyra* (Hala et al., 2006; Ndiaye et al., 2008; Ekesi et al., 2009; Rwhomushana te al., 2009).

The potential geographical distribution of *B. invadens* has been recently modelled using the correlative ecological niche modelling techniques, GARP and MaxEnt, with known occurrence records of the pest in Africa and in Asia (De Meyer et al., 2010). Results from the models suggest that *B. invadens* prefers hot and humid environments and the pest seems well suited for an equatorial climate. Although most of the known occurrences in Africa fell within the predicted ranges of the GARP model, the predicted distribution does not include the northern boundaries of the species' actual distribution in countries such as Sudan, Chad, Niger and Mali. The MaxEnt model showed a much narrower range of suitability, excluding many of the occurrence records from the Royal Museum for Central Africa fruit fly database. For instance, large parts of Kenya and Tanzania, where the pest is present and quite extensively, were found to be climatically unsuitable for *B. invadens*. The short falls of the climatic models for *B. invadens* were attributed to lack of known distributional records of the pest in its native region and lack of continuous trapping data to indicate fly abundance in areas in Africa where the pest does occur.

Stated objectives

The objectives of this study were to gather data on the distribution and year round abundance and phenology of *B. invadens* in Africa, and to use the data acquired to build up a more accurate climatic model for prediction of the range of expansion of *B. invadens*, using CLIMEX software.

Materials and methods

To determine the relative abundance and seasonal phenology of *B. invadens* in Africa, sampling sites were chosen in Senegal, Ghana, Benin, Niger, Sudan, Kenya, Tanzania and Zambia (Fig. 3.3.6.1). These sites are representative of different climatic regions, according to the Köppen-Geiger classification system (Kriticos et al., 2012) in which the pest is known to occur. Yellow Chempac bucket traps baited with methyl eugenol were used to trap the flies. Dichlorvos was used to kill the attracted flies inside the traps. At each site, three traps were set up and traps were active for one week during every month. Trapping was continued for a period of two years. Traps were placed mainly in mango trees, either in orchards or home gardens. Information was also gained on the periods of irrigation in each area and the fruit ripening periods of the main hosts surrounding the traps. In addition, a literature search was undertaken to determine the current distribution of this pest.

To determine the relative abundance at each sampling site, the fly counts of the three traps were averaged for each week. At each site, the average value for each of the sampling weeks was then added together and divided by the total number of weeks (24 for a full two-year cycle) during which sampling was conducted to get one value expressed as the average number of flies caught per trap per week. In areas where all data have not yet been obtained or where sampling was not done every month, averages were taken for the period during which monitoring was performed, even though it was not for a full two-year cycle. The following classes of abundance were assigned: very low: <10 flies per trap per week; low: 10-50 flies per trap per week; moderate: 51-250 flies per trap per week; high: 251-500 flies per trap per week; very high: >500 flies per trap per week.

The data that were collected over the two-year trapping period, together with presence data from across the known distribution range of the species in Africa and Asia, and information on the biology of the species are used to estimate the species' climatic niche using CLIMEX (Hearne Scientific Software Pty Ltd., Australia) (Sutherst & Maywald, 1985; Sutherst et al., 2007).

Results and discussion

African distribution

The current distribution of the species in Africa as listed by the Fruit fly database held at the Royal Museum for Central Africa (accessed via Marc de Meyer, 2013) is shown in Fig. 3.3.6.2. This does not include all the sites shown in Fig. 3.3.6.1, where the species was found during the present study, so those will be added to the list in future. As can be seen, *B. invadens* is adapted to a wide range of climates, including tropical,

temperate and even arid climates. However, in the arid climates, the locations where it occurred were mostly alongside rivers (dotted blue lines shown in Fig. 3.3.6.2).

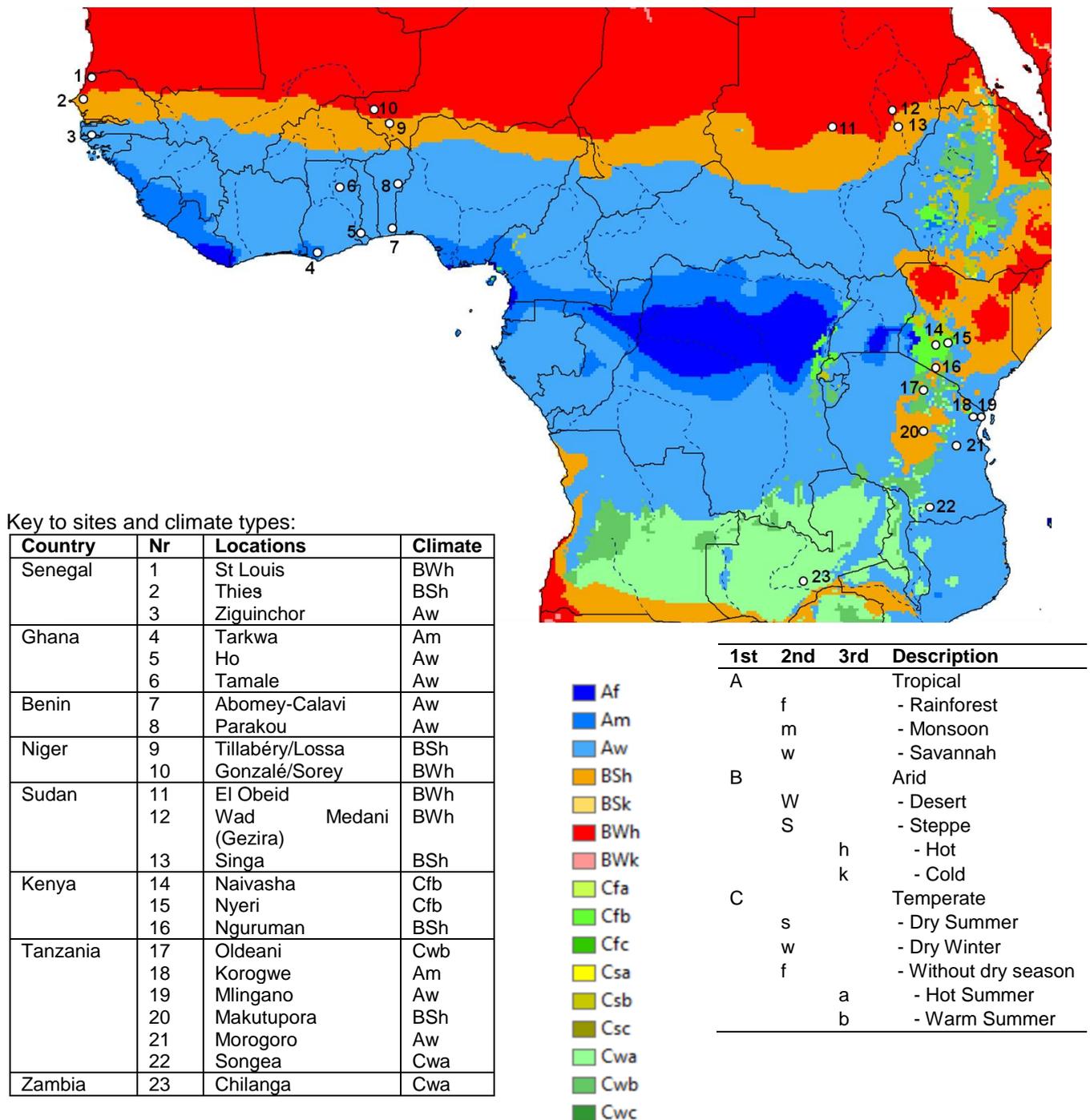
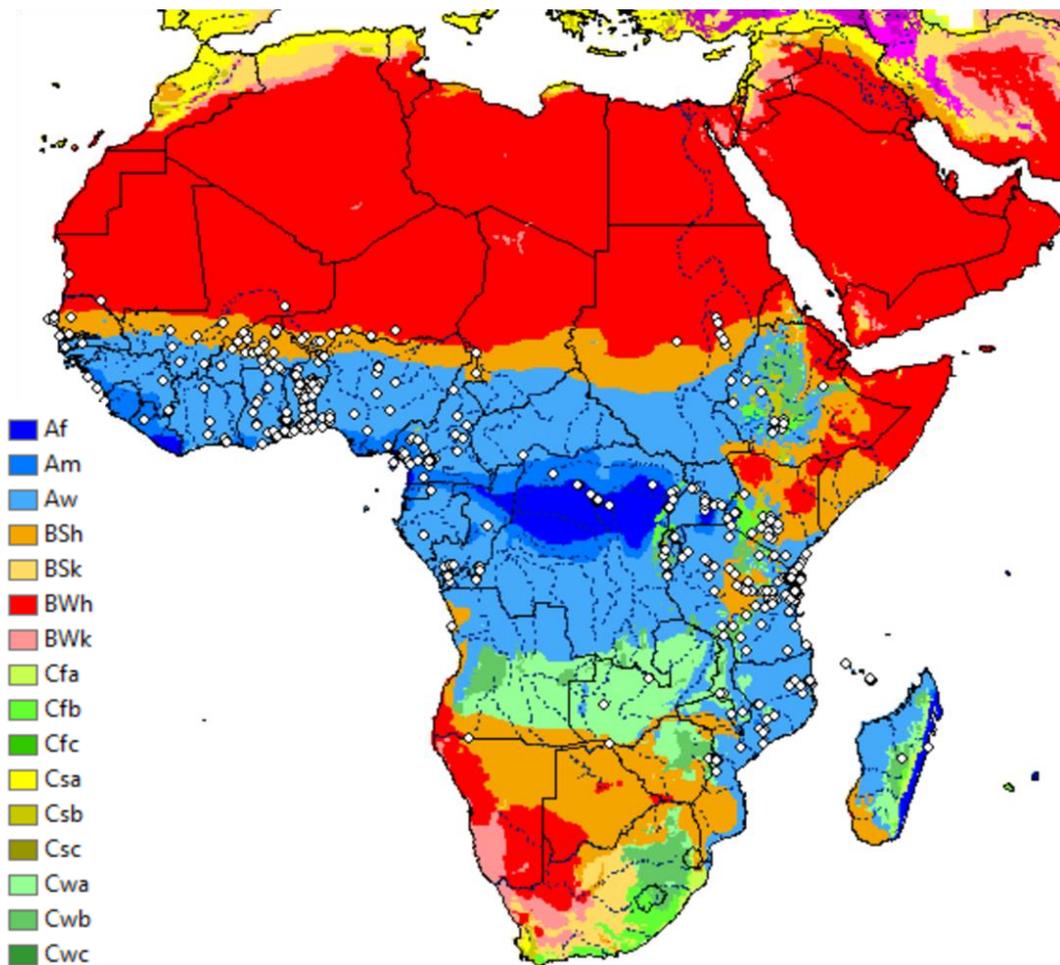


Figure 3.3.6.1. Sampling sites for monitoring *Bactrocera invadens* across different climatic regions of Africa according to the Köppen-Geiger climate classification system (Kriticos *et al.*, 2012).



1st	2nd	3rd	Description
A			Tropical
	f		- Rainforest
	m		- Monsoon
	w		- Savannah
B			Arid
	W		- Desert
	S		- Steppe
		h	- Hot
		k	- Cold
C			Temperate
	s		- Dry Summer
	w		- Dry Winter
	f		- Without dry season
		a	- Hot Summer
		b	- Warm Summer

Figure 3.3.6.2. The current distribution of *Bactrocera invadens* in Africa (Fruit fly database held at the Royal Museum for Central Africa, accessed by Marc de Meyer, 2013) shown with the Köppen-Geiger climatic zones (Kriticos et al., 2012).

Relative abundance and seasonal phenology

Relative abundance and seasonal phenology data is shown in Table 3.3.6.1. With the exception of Tarkwa and Tamale (Ghana), all sites with a tropical climate (Am and Aw) had high to very high counts. At Tarkwa and Tamale counts were low and moderate respectively. This may be due to trap placement. At these two sites, traps were placed in mango trees in home gardens with few to no surrounding host plants, which may have caused a lack of food source for the flies to persist in larger numbers. The high numbers found in these tropical areas corresponds with the model predictions of De Meyer et al. (2010).

In Makutupora (Tanzania) with an arid climate (BSh) and where no irrigation was applied, counts were moderate. However, in Nguruman (Kenya), St Louis, Thies (Senegal) and the Niger and Sudan sites, which all fell into arid climatic regions (BSh or BWh), counts were mostly high to very high. This can be attributed to the fact that all these sites were under irrigation, highlighting the influence of moisture on the persistence of the species. Exceptions to this were El Obeid (Sudan), where counts were low and Gonzalé/Sorey (Niger) where counts were moderate. Perhaps the levels or frequency of irrigation were lower at these sites.

In the moderate Cwa climates of Songea (Tanzania) and Chilanga (Zambia), counts were high and moderate respectively, showing that these climates are also suitable for the species, although, in some areas, maybe less so than the tropical climates. In the moderate Cfb climates of Naivasha and Nyeri (Kenya), counts were very low and in Oldeani (Tanzania) with a moderate Cwb climate, counts were low. One would expect higher suitability in these areas. However, this is probably due to high altitude, with altitudes higher than 2200 m, 1700 m and 1400 m at Naivasha, Nyeri and Oldeani respectively. *Bactrocera invadens* is mainly a lowland pest, decreasing in numbers with increasing altitude (Ekesi et al., 2006; Mwatawala et al., 2006a, b; Geurts et al., 2012). Also, the classes for relative abundance were chosen arbitrarily and counts between 10 to 50 flies per trap per week (classified as low counts here), which was found in Oldeani, will probably not be considered low in the field.

In Benin and Ghana, numbers started to increase with an increase in rainfall during March or April, coinciding with the mango ripening season. Generally, when the ripening periods of the mangoes ended, the numbers started to decrease even though rainfall was still high. During the drier season from November to February (December to February at Tarkwa), numbers were lower, probably both due to the lower moisture levels and lack of available hosts. In St Louis (Senegal), the mango ripening period was from April to September. However, numbers only started to increase during June, probably because it was too dry prior to this (irrigation was initiated during March, but was not done continuously) and the rain started to increase from July onwards. Numbers decreased at the end of the rainy season which also coincided with the end of the mango ripening period. In both Thies and Ziguinchor (Senegal), the phenology closely followed the irrigation period (March to July in Thies and March to May in Ziguinchor), rainfall (rainy season is from June to October at both locations) and fruit ripening patterns (ripe fruit available from June to October in Thies and from May to July in Ziguinchor). In El Obeid (Sudan), highest fly activity occurred during the rainy season from May to October. However, this was not the case in the other sampling sites in Sudan (Wad Medani and Singa). In Wad Medani, large numbers occurred outside the rainy season, which was also from May to October, probably as a result of irrigation. In Singa, fly numbers started to decrease from May to July (during the second year of monitoring), when the rainfall started to increase. This can be attributed to a lack of hosts during this period. In Chilanga (Zambia), the phenology pattern followed the rainfall pattern (which started to increase from November, peaking in January and again decreasing towards May) and fruit ripening patterns (December to April). In Niger, counts were low during the period of irrigation (March to June) and the period of mango ripening (April to June). Counts only started to increase during July after an increase in rain towards June. It is possible that there were other host plants, besides mango, supporting populations during this climatically favourable period and that the levels and frequency of irrigation supplied prior to the rainy season was not enough to support persistence of the fly. In Nguruman (Kenya), irrigation is applied all year round and the phenology of the flies generally followed the fruit ripening periods, with ripe fruit available from October to December and May to June. In Naivasha and Nyeri (Kenya), counts were too low to see what the phenology patterns are linking to. In Tanzania, the phenology generally appeared to be linked to a combination of rainfall and the ripening patterns of mango and or citrus. The importance of rainfall and fruit ripening periods in the seasonal phenology of *B. invadens* has also been demonstrated by Kwasi (2008), Mwatawala et al. (2006b), Ndiaye et al. (2008), Vayssières et al. (2005, 2009a, b, c, 2010).

CLIMEX modelling and potential global distribution

A preliminary model was constructed based on the trapping results and known biology of the species. However, the current model does not take irrigation into account. It therefore only shows a restricted distribution of the species and does not include areas such as Sudan and the northern parts of Senegal where the species occurs in high abundance. Their occurrence in these dry areas is due to the irrigation applied as this species is heavily influenced by irrigation. Since different irrigation methods were used at the different sampling sites, we will explore several irrigation scenarios to obtain optimal fit between relative

abundance and seasonal phenology data and suitability as predicted by CLIMEX. The model will be refined when visiting Prof Darren Kriticos in Australia during May 2013. A manuscript is being prepared where the final model will be presented.

Conclusion

Results showed that *B. invadens* is well adapted to survive a wide range of climates, from the hot climates bordering the Sahara desert to the moderate temperate climates on the African continent. There seems to be a link between rainfall and the seasonal occurrence of the species, generally increasing along with an increase in rainfall. Irrigation in dry regions also seemed to result in higher fly numbers than with an absence of irrigation. Another important factor in seasonal phenology was the availability of fruit. Therefore, it seems that the key factors in the persistence of the species in an area are humidity and host availability.

Future research

None.

Technology transfer

None.

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Table 3.3.6.1. Abundance and phenology data of *Bactrocera invadens* across Africa.

Climatic zone	Country	Location	Average number of flies per trap per week (Standard errors in brackets)														
			Jan 2011	Feb 2011	Mar 2011	Apr 2011	May 2011	Jun 2011	Jul 2011	Aug 2011	Sep 2011	Oct 2011	Nov 2011	Dec 2011	Jan 2012	Feb 2012	Mar 2012
Tropical Monsoon (Am)	Ghana	Tarkwa	-	103.3 (12.8)	103.3 (29.6)	90.7 (65.5)	49.3 (18.3)	28.0 (19.1)	45.3 (27.4)	30.0 (14.0)	42.0 (9.0)	26.7 (8.7)	8.0 (3.5)	9.0 (2.9)	12.3 (5.4)	30.7 (10.7)	47.7 (9.5)
	Tanzania	Korogwe	-	-	271.3 (84.7)	618.3 (150.9)	841.3 (88.9)	828.3 (264.0)	1331.7 (479.5)	1031.7 (319.3)	675.3 (121.7)	397.7 (124.2)	274.0 (41.6)	980.7 (140.4)	866.3 (459.6)	86.3 (40.7)	73.0 (35.5)
Tropical Savannah (Aw)	Benin	Abomey-Calavi	216.3 (39.3)	191.0 (35.8)	196.0 (26.7)	584.0 (35.7)	852.0 (181.2)	481.7 (68.5)	407.7 (90.1)	210.7 (43.0)	139.0 (28.9)	103.0 (31.5)	125.0 (23.6)	75.7 (21.2)	121.7 (18.0)	122.3 (11.7)	574.3 (103.3)
	Benin	Parakou	0.7 (0.3)	6.3 (3.4)	35.3 (13.3)	167.0 (45.5)	374.3 (59.0)	767.7 (50.0)	842.0 (67.8)	603.3 (26.7)	175.7 (24.7)	55.7 (8.4)	64.7 (20.4)	23.3 (6.7)	27.7 (7.0)	8.7 (4.6)	91.7 (28.5)
	Ghana	Ho	-	143.0 (49.2)	318.7 (103.0)	1186.7 (437.6)	1864.3 (176.8)	1127.3 (178.3)	640.0 (82.4)	391.7 (51.0)	357.0 (142.5)	447.0 (69.5)	173.3 (27.7)	334.0 (37.4)	307.3 (43.1)	252.3 (15.4)	394.3 (175.3)
	Ghana	Tamalé	24.7 (18.7)	17.3 (14.8)	6.7 (4.4)	11.7 (2.7)	18.3 (4.8)	56.7 (16.0)	205.7 (65.7)	181.3 (14.8)	208.0 (94.1)	84.3 (80.3)	79.0 (76.0)	12.0 (12.0)	7.7 (7.2)	11.3 (11.3)	6.7 (5.7)
	Senegal	Ziguinchor	30.0 (11.9)	57.7 (34.3)	88.0 (30.4)	929.0 (436.2)	1615.0 (442.2)	2983.7 (1009.6)	1819.0 (213.9)	1485.3 (259.4)	355.0 (186.0)	150.0 (40.6)	88.3 (18.5)	28.7 (12.8)	58.3 (19.5)	78.0 (20.4)	73.3 (34.7)
	Tanzania	Mlingano	-	-	85.7 (46.2)	242.3 (107.7)	205.7 (89.2)	725.0 (295.6)	549.3 (230.3)	267.7 (47.4)	141.0 (15.5)	535.7 (176.7)	867.7 (334.6)	1053.3 (433.3)	637.3 (196.5)	271.3 (18.3)	211.7 (51.7)
	Tanzania	Morogoro	-	-	-	-	-	930.3 (310.9)	816.3 (235.4)	366.7 (89.5)	291.3 (91.6)	421.0 (282.5)	1421.7 (489.1)	1421.7 (489.1)	2402.0 (586.1)	1254.0 (169.1)	702.0 (57.6)
Hot Arid Desert (BWh)	Niger	Gonzalé/Sorey	-	-	-	-	4.3 (1.5)	10.0 (5.0)	205.0 (128.9)	99.7 (33.0)	142.0 (66.6)	324.3 (96.9)	30.0 (22.8)	3.7 (2.3)	0 (0)	0 (0)	0.3 (0.3)
	Senegal	St Louis	0.3 (0.3)	1.0 (0.6)	0.3 (0.3)	1.7 (1.7)	0 (0)	76.0 (14.7)	1170.0 (165.0)	1013.3 (100.2)	670.0 (79.2)	25.0 (9.9)	9.3 (1.8)	4.7 (1.5)	0 (0)	0 (0)	0 (0)
	Sudan	El Obeid	-	-	0.7 (0.7)	0.7 (0.7)	0.3 (0.3)	1.0 (1.0)	**	**	**	10.7 (0.9)	1.0 (0.6)	0 (0)	0 (0)	0.3 (0.3)	0.3 (0.3)
	Sudan	Wad Medani (Gezira)	Counts cannot be used as <i>B. zonata</i> infested the area and was not separated from <i>B. invadens</i>													146.0 (45.7)	212.0 (73.8)

Table 3.3.6.1. Continued. Rel Abun = Relative abundance (see text for calculation).

Climatic zone	Country	Location	Average number of flies per trap per week (Standard errors in brackets)														Rel Abun
			Apr 2012	May 2012	Jun 2012	Jul 2012	Aug 2012	Sep 2012	Oct 2012	Nov 2012	Dec 2012	Jan 2013	Feb 2013	Mar 2013	Apr 2013	May 2013	
Tropical Monsoon (Am)	Ghana	Tarkwa	45.7 (20.3)	28.7 (6.7)	22.0 (7.8)	23.7 (13.8)	21.3 (10.9)	13.7 (4.8)	9.3 (0.9)	5.7 (0.7)	16.7 (2.7)	--	--	--	--	--	35.4
	Tanzania	Korogwe	127.0 (44.9)	299.0 (154.0)	1223.3 (548.3)	1409.3 (340.5)	283.0 (89.6)	137.0 (22.5)	372.0 (121.3)	694.3 (198.6)	410.3 (160.1)	819.7 (291.3)	33.7 (14.1)	--	--	--	586.9
Tropical Savannah (Aw)	Benin	Abomey-Calavi	978.3 (85.5)	881.7 (31.9)	817.7 (86.4)	429.7 (23.0)	142.7 (23.0)	89.3 (24.2)	122.3 (52.5)	62.3 (13.7)	123.7 (39.3)	--	--	--	--	--	335.3
	Benin	Parakou	1353.3 (238.4)	3767.0 (476.7)	2112.7 (226.3)	1049.3 (197.8)	557.0 (103.0)	210.3 (75.1)	151.0 (66.0)	45.0 (21.0)	138.3 (120.9)	--	--	--	--	--	526.2
	Ghana	Ho	946.3 (432.3)	1121.0 (300.0)	662.7 (87.6)	471.0 (31.8)	322.7 (69.3)	212.3 (86.5)	235.0 (95.3)	86.7 (19.1)	139.7 (39.7)	--	--	--	--	--	527.6
	Ghana	Tamalé	9.7 (7.2)	14.3 (10.3)	19.3 (9.4)	38.7 (3.8)	67.7 (7.7)	83.0 (20.4)	37.0 (17.1)	24.7 (16.8)	23.3 (9.1)	--	--	--	--	--	52.0
	Senegal	Ziguinchor	199.0 (58.2)	513.0 (241.5)	2215.3 (162.4)	3695.3 (1310.7)	405.7 (120.8)	305.0 (61.4)	103.0 (6.2)	87.7 (32.7)	46.3 (3.7)	--	--	--	--	--	725.4
	Tanzania	Mlingano	300.0 (56.7)	210.3 (91.9)	628.3 (161.3)	877.3 (282.2)	675.7 (342.7)	135.7 (43.4)	830.0 (73.8)	763.0 (44.0)	1193.3 (316.8)	261.0 (25.5)	166.7 (17.9)	--	--	--	493.1
	Tanzania	Morogoro	297.7 (96.1)	538.3 (195.2)	286.0 (119.5)	351.7 (117.6)	159.0 (45.2)	128.7 (62.5)	103.3 (55.7)	362.0 (238.5)	1123.7 (122.5)	995.3 (179.1)	756.3 (284.4)	1160.3 (329.6)	936.5 (570.5)	Still to do last collection	749.0
Hot Arid Desert (BWh)	Niger	Gonzalé/Sorey	19.7 (6.4)	7.0 (3.5)	24.3 (4.7)	16.0 (7.8)	11.3 (8.1)	10.7 (7.4)	65.7 (36.0)	73.3 (40.6)	42.7 (27.7)	*	*	*	*	--	54.5
	Senegal	St Louis	0 (0)	0 (0)	717.7 (106.6)	3964.7 (74.4)	3009.0 (637.2)	3668.0 (2737.9)	788.3 (342.7)	173.3 (67.6)	180.0 (48.9)	--	--	--	--	--	644.7
	Sudan	El Obeid	6.3 (1.7)	5.0 (2.0)	22.0 (1.2)	38.3 (1.9)	59.3 (7.6)	46.0 (5.5)	42.0 (1.7)	8.0 (2.1)	0 (0)	4.3 (1.5)	11.3 (1.5)	--	--	--	12.3
	Sudan	Wad Medani (Gezira)	776.7 (443.8)	185.3 (43.7)	405.0 (297.5)	2123.0 (365.0)	1023.0 (407.0)	754.0 (98.0)	1076.7 (315.6)	971.3 (269.7)	1228.3 (178.4)	--	--	--	--	--	777.4

Table 3.3.6.1. Continued.

Climatic Zone	Country	Location	Average number of flies per trap per week (Standard errors in brackets)														
			Jan 2011	Feb 2011	Mar 2011	Apr 2011	May 2011	Jun 2011	Jul 2011	Aug 2011	Sep 2011	Oct 2011	Nov 2011	Dec 2011	Jan 2012	Feb 2012	Mar 2012
Hot Arid Steppe (BSh)	Kenya	Nguruman	-	226.7 (153.7)	219.3 (34.2)	273.3 (82.7)	677.3 (352.1)	895.3 (317.1)	1431.0 (572.8)	731.0 (168.6)	452.7 (88.0)	300.7 (6.9)	577.0 (133.5)	998.3 (133.2)	872.3 (293.0)	**	278.7 (68.8)
	Niger	Tillabéry/ Lossa	-	-	-	-	7.7 (7.7)	34.0 (17.1)	417.7 (167.0)	667.3 (123.8)	1083.3 (217.8)	1343.3 (334.9)	64.3 (52.9)	53.0 (49.0)	1.7 (1.2)	9.7 (7.7)	5.7 (3.5)
	Senegal	Thies	7.7 (2.7)	3.3 (1.5)	7.7 (3.9)	9.7 (3.9)	10.7 (4.3)	426.7 (205.8)	1139.3 (279.7)	1796.7 (163.6)	763.0 (210.7)	318.7 (75.6)	103.7 (9.8)	50.7 (10.8)	7.0 (3.2)	1.0 (0.6)	16.7 (6.1)
	Sudan	Singa	32.7 (19.2)	14.3 (1.2)	**	0.3 (0.3)	3.7 (1.7)	51.0 (24.6)	**	**	**	**	**	179.0 (0)	317.0 (96.0)	306.3 (62.9)	473.3 (88.5)
	Tanzania	Makutupora	-	-	59.3 (45.3)	39.0 (7.8)	12.3 (4.8)	12.0 (5.6)	14.7 (6.6)	5.7 (3.2)	8.0 (6.1)	5.7 (3.8)	25.3 (20.5)	36.0 (16.2)	36.0 (16.2)	141.7 (100.0)	90.0 (35.9)
Temperate with dry winter & hot summer (Cwa)	Tanzania	Songea	-	-	92.3 (13.9)	68.0 (20.8)	27.0 (19.6)	7.3 (1.9)	15.7 (2.9)	0.7 (0.3)	63.0 (38.4)	26.0 (9.8)	454.3 (131.4)	306.0 (59.3)	306.0 (59.3)	565.7 (313.2)	504.0 (245.3)
	Zambia	Chilanga	-	-	-	-	-	-	-	-	-	-	-	63.0 (30.7)	164.7 (81.1)	175.3 (41.0)	179.0 (52.2)
Temperate with dry winter & warm summer (Cwb)	Tanzania	Oldeani	-	-	61.7 (17.0)	159.7 (83.3)	80.3 (41.4)	11.7 (2.9)	14.0 (3.0)	9.0 (4.2)	2.3 (0.3)	0.3 (0.3)	2.7 (0.7)	14.3 (9.0)	85.3 (38.0)	36.7 (8.4)	49.0 (9.3)
Temperate with warm summer & no dry season (Cfb)	Kenya	Nyeri	-	0.7 (0.3)	0.3 (0.3)	3.0 (1.0)	3.3 (0.9)	3.0 (1.0)	2.7 (1.2)	0.7 (0.3)	2.0 (1.5)	1.0 (0.6)	2.7 (0.9)	1.7 (1.7)	2.0 (1.5)	**	1.0 (0.6)
	Kenya	Naivasha	-	0 (0)	0 (0)	0 (0)	1.0 (0.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**	0 (0)

Table 3.3.6.1. Continued. Rel Abun = Relative abundance (see text for calculation).

Climatic Zone	Country	Location	Average number of flies per trap per week (Standard errors in brackets)														
			Apr 2012	May 2012	Jun 2012	Jul 2012	Aug 2012	Sep 2012	Oct 2012	Nov 2012	Dec 2012	Jan 2013	Feb 2013	Mar 2013	Apr 2013	May 2013	Rel Abun
Hot Arid Steppe (BSh)	Kenya	Nguruman	187.0 (12.7)	303.7 (90.1)	364.0 (53.5)	531.7 (83.5)	360.0 (80.4)	989.7 (467.2)	388.0 (65.9)	511.7 (99.7)	235.0 (22.1)	563.7 (5.3)	416.3 (155.4)	--	--	--	532.7
	Niger	Tillabéry/ Lossa	69.7 (30.9)	35.7 (16.8)	105.3 (49.4)	191.3 (52.6)	31.7 (3.7)	35.0 (14.0)	269.3 (168.8)	582.0 (470.0)	216.3 (128.0)	*	*	*	*	--	261.2
	Senegal	Thies	36.0 (19.7)	128.7 (73.2)	269.7 (235.2)	440.7 (81.2)	2389.3 (93.1)	1080.7 (139.6)	752.7 (52.6)	439.7 (66.4)	146.3 (70.1)	--	--	--	--	--	431.1
	Sudan	Singa	983.3 (84.6)	442.0 (83.6)	108.7 (11.5)	51.0 (9.9)	**	**	591.0 (150.3)	558.0 (147.8)	515.7 (135.6)	476.3 (149.1)	--	--	--	--	289.2
	Tanzania	Makutupora	**	52.7 (17.1)	20.7 (5.8)	32.7 (8.4)	44.7 (21.6)	30.3 (19.2)	11.5 (8.5)	34.7 (30.2)	73.0 (66.0)	277.7 (253.4)	108.3 (70.9)	--	--	--	51.0
Temperate with dry winter & hot summer (Cwa)	Tanzania	Songea	301.7 (72.8)	193.0 (95.1)	525.0 (201.6)	68.3 (37.1)	83.7 (7.3)	35.7 (11.5)	30.7 (3.8)	328.0 (70.1)	1265.7 (76.4)	952.0 (141.2)	445.3 (74.8)	--	--	--	277.7
	Zambia	Chilanga	90.3 (20.4)	66.7 (25.2)	22.7 (2.7)	19.0 (8.5)	11.0 (4.4)	Have gotten no further feedback from Zambia									88.0
Temperate with dry winter & warm summer (Cwb)	Tanzania	Oldeani	**	13.7 (6.6)	2.3 (0.9)	26.0 (9.0)	0 (0)	6.7 (2.6)	1.0 (1.0)	0.7 (0.7)	102.0 (23.3)	270.7 (47.4)	67.7 (20.6)	--	--	--	44.3
Temperate with warm summer & no dry season (Cfb)	Kenya	Nyeri	0.7 (0.3)	2.3 (0.9)	0 (0)	0 (0)	0.3 (0.3)	0 (0)	0.7 (0.7)	0.7 (0.3)	1.7 (1.2)	2.0 (1.5)	4.7 (2.3)	--	--	--	1.5
	Kenya	Naivasha	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (0.3)	--	--	--	0.1

- Monitoring not yet started; -- Monitoring finished; * Monitoring performed, but data not yet received; ** Monitoring not performed, either due to inaccessibility of traps due to rain, or for reasons not known.

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

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

Summary

Further evaluation of the fumigant GRASFUM has shown that grain chinch bug is very susceptible to the fumigant and 100% mortality of 3 623 insects was obtained after 6 h at 0.75x full dosage. The main research focus during the report period was to determine the maximum mortality that could be obtained of internal pests such as fruit fly and false codling moth (FCM) without being detrimental to the fruit. At full dosage for 24 h, fruit fly mortality in oranges reached 100% and the mortality of FCM larvae was approximately 70%. However, an off-taste was obtained in Shamoutis after this treatment. Two further tests with full dosage for 24 h on Valencia oranges gave no off-taste so cultivars may vary in their susceptibility. For this reason and the high cost of the fumigant, further research will be conducted at 0.75x full dosage and this treatment followed by short cold treatments to guarantee 100% mortality.

Opsomming

Verdere evaluering van die berokingsmiddel GRASFUM het getoon dat die graanstinkluis baie vatbaar vir die berokingsmiddel is, en 100% mortaliteit van 3 623 insekte is ná 6 ure by 'n 0.75x volle dosis verkry. Die hoofnavorsing gedurende die verslagperiode het daarop gefokus om die maksimum mortaliteit van interne plaë soos vrugtevlug en valskodlingmot (VKM) te bepaal wat verkry kan word sonder om skadelik vir die vrugte te wees. Teen die volle dosis vir 24 uur, het vrugtevlug-mortaliteit in lemoene 100% bereik, en die mortaliteit van VKM-larwes was ongeveer 70%. 'n Afsmaak is egter ná hierdie behandeling in Shamoutis verkry. Twee verdere toetse, met die volle dosis vir 24 uur op Valencia lemoene, het geen afsmaak gegee nie, dus kan kultivars in hul vatbaarheid varieer. Om hierdie rede en die hoë koste van die berokingsmiddel, sal verdere navorsing by 'n 0.75x volle dosis uitgevoer word. Hierdie behandeling sal met kort koue behandelings opgevolg word om 100% mortaliteit te waarborg.

3.3.8 **PROGRESS REPORT: Determine the least susceptible immature life stage of Medfly to cold disinfestation at 1°C**

Project 1054 (2012/3 – 2013/4) by Tim Grout, Peter Stephen and Kim Stoltz (CRI)

Summary

In our attempts to get the temperature for cold disinfestation of Medfly in fruit going to Japan increased to 1°C, the Japanese have requested a repeat of some of the earlier work but using their protocol. They insisted that we use older eggs which are reportedly more tolerant to the cold, but after holding the eggs in water for at least 24 h their development was delayed and an additional day had to be added to the protocol to give time for the larvae to develop to the right size. The first replicates using Clementines and grapefruit that were conducted without the additional day will have to be repeated due to poor survival in the controls. The adjusted protocols for the other replicates should hopefully be acceptable and the complete dataset will then be submitted to the Japanese for consideration.

Opsomming

In ons pogings om die temperatuur vir koue disinfestasië van Medvlieg in vrugte wat vir Japan bestem is, na 1°C te verhoog, het die Japanese versoek dat vroeër werk herhaal moet word, maar dat hul protokol gebruik moet word. Hulle het daarop aangedring dat ouer eiers gebruik word wat na bewering meer bestand teen die koue is, maar nadat die eiers in water vir ten minste 24 uur gehou is, was hul ontwikkeling vertraag. 'n Bykomende dag moes by die protokol gevoeg word om aan die larwes tyd te gee om tot die regte grootte te ontwikkel. Die eerste herhalings waarin Clementines en pomelo's gebruik is, maar sonder die bykomende dag, sal herhaal moet word weens die swak oorlewing in die kontroles. Die aangepaste protokolle vir die ander herhalings sal hopelik aanvaarbaar wees en die volledige dataset sal dan aan die Japanese vir oorweging voorgelê word.

3.3.9 FINAL REPORT: Use of molecular techniques to distinguish between *Bactrocera invadens* and local fruit fly pest species

Project 1047 (April 2012 – March 2013) by A. Manrakhan, M. De Villiers & G. Cook (CRI)

Summary

Diagnostic keys to differentiate between the invasive fruit fly - *Bactrocera invadens* (Drew Tsuruta and White), and other fruit fly pests rely mainly on adult characters. As such, fruit fly larvae in infested fruit have to be either reared to adulthood for species identification or identified using molecular techniques. We tested the polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) method to distinguish *B. invadens* from local fruit fly pest species in South Africa using published primers and restriction enzymes. PCR-RFLP analyses using the primer pair: LR-J-12883 and LR-N-13398 from the 16S ribosomal RNA gene and restriction enzyme DraI enabled the distinction of *B. invadens* from *Ceratitidis capitata* (Wiedemann), *C. rosa* Karsch and *C. cosyra* (Walker). Using the same primer pair and restriction enzyme, local fruit fly pest species and *B. invadens* could also be distinguished from two other exotic fruit fly species *B. zonata* (Saunders) and *B. cucurbitae* (Coquillett). The PCR-RFLP analysis can be completed using the facilities available at Citrus Research International. Results from PCR-RFLP analyses were available within less than 48 h. The results from this method in the form of gel patterns were easy to interpret. This method could therefore be used to distinguish *B. invadens* and two other high risk exotic fruit fly pests from local fruit fly pests in South Africa. However the consistency of the method would have to be first tested by analysing different populations of the target fruit fly species, in particular the local fruit fly pest species, before fully recommending this technique as a diagnostic tool.

Opsomming

Die diagnostiese sleutels wat gebruik word om onderskeid te tref tussen die indringer vrugtevlug - *Bactrocera invadens* (Drew, Tsuruta en White), en ander vrugtevlug plae is hoofsaaklik op volwasse karaktereieenskappe gebaseer. Dit beteken dat vrugtevlug larwes in besmette vrugte toegelaat moet word om volwassenheid te bereik om sodoende die identifisering van die spesie te vergemaklik of anders dan geïdentifiseer word met behulp van molekuleêre tegnieke. Ons toets die polimerase kettingreaksie-beperkings lengte polimorfisme (PCR-RFLP) metode om *B. invadens* te onderskei van plaaslike vrugtevlug plaagspesies in Suid-Afrika met behulp van gepubliseerde DNA voorlopers. Onderskeid is getref tussen *B. invadens*, *Ceratitidis capitata* (Wiedemann), *C. rosa* Karsch en *C. cosyra* (Walker) met behulp van PCR-RFLP ontledings deur gebruik te maak van die DNA voorlopers: LR-J-12.883 en LR-N-13398 van die 16S-ribosomale RNA-geen en die Dra I beperkings-ensiem. Deur ook gebruik te maak van dieselfde voorlopers en beperkings-ensiem, is plaaslike vrugtevlug plaagspesies en *B. invadens* ook onderskeibaar van twee ander eksotiese vrugtevlug spesies, *B. zonata* (Saunders) en *B. cucurbitae* (Coquillett). Die fasiliteite beskikbaar by Citrus Research International is voldoende om PCR-RFLP analises uit te voer en resultate kan binne 48 uur verkry word. Die patrone kan maklik onderskei word en die metode kan dus gebruik word om *B. invadens* en twee ander hoë risiko eksotiese vrugtevlug plae te onderskei van plaaslike vrugtevlug plae in Suid-Afrika. Voor die tegniek ten volle aanbeveel kan word as 'n diagnostiese toets, moet die herhaalbaarheid van die metode getoets word deur die ontleding van verskillende bevolkings van die teiken vrugtevlug spesies te toets, veral in die geval van die plaaslike vrugtevlug spesies.

Introduction

In South Africa, there are three main fruit fly pest species that are problematic to commercial fruit production because of phytosanitary concerns and yield losses. The main fruit fly pests belong to the genus *Ceratitidis* and are the Mediterranean fruit fly, *C. capitata* (Wiedemann), the Natal fly, *C. rosa* Karsch, and the marula fly, *C. cosyra* (Walker) (Annecke and Moran, 1982). Female fruit flies cause damage to fruit by inserting their eggs just underneath the fruit skin (White and Elson-Harris, 1994). These eggs hatch into larvae which feed inside the fruit causing decay. In May 2010 however, the fruit fly problem in South Africa was further compounded with interceptions of the invasive fruit fly, *Bactrocera invadens*, in two separate areas in Limpopo Province (Anon 2010b; 2010c). *Bactrocera invadens* was successfully eradicated from these areas using a combination of protein baiting and male annihilation treatments (Anon 2010a; 2011). However in subsequent years, further interceptions of this invasive pest occurred in the northern parts of South Africa. In 2011, *B. invadens* was successfully eradicated in all areas where it was detected. In 2012 although eradication was declared successful in many areas where *B. invadens* was detected, some areas still remained problematic. In 2013, *B. invadens* was declared present in specified areas in the northern parts of South Africa and under official control (Anon 2013). Given that *B. invadens* is still a regulated pest in South Africa; areas affected by the pest should be quarantined.

When an area is quarantined for *B. invadens*, movement of fruit outside of the area is usually regulated (Manrakhan et al., 2012). Fruit intended for movement from production areas are usually inspected for

damage symptoms. Symptoms of fruit fly damage include oviposition punctures on the fruit skin and decay. Only fruit consignments that are free of *B. invadens* are allowed outside of the quarantine area. If a consignment is found to be infested with fruit fly larvae, it is currently very difficult to identify the pest to species level. There are very few published keys for fruit fly larvae and these keys are based mainly on morphological features of third instar or mature larvae (White and Elson-Harris, 1994). Moreover, some fruit fly pest species of major economic importance such as *C. rosa* have been omitted from these keys (White and Elson-Harris, 1994). In order to accurately determine the fruit fly species, infested fruit would have to be incubated within the quarantine area and reared through to adult flies where they can be identified. This is however time consuming (taking about 2-3 weeks at temperatures between 25°C and 27°C) and risky since the fly larvae might not survive. Molecular techniques such as DNA barcoding and polymerase chain reaction (PCR) based methods are available for identification of fruit fly larvae although there are some limitations with these techniques (Jenkins et al., 2012). Among the currently available molecular techniques, DNA barcoding was identified as a more accurate, standard, robust and reproducible method for identification of invasive fruit fly species (Armstrong and Ball, 2005). However for institutes which do not have in-house sequencing facilities, identification using DNA barcodes would not be as quick and could cost more than other PCR-based methods.

We proposed to test the ability of one of the PCR-based methods: PCR-restriction fragment length polymorphism (PCR-RFLP) to distinguish between *B. invadens* and local fruit fly pests in South Africa. PCR-RFLP was previously successfully used for identification of several fruit fly species including the local fruit fly pests in South Africa - *C. capitata*, *C. rosa* and *C. cosyra* (Armstrong et al., 1997; Barr et al., 2006; Muraji and Nakahara, 2002). This technique has however never been tested on *B. invadens*.

Stated objectives

To diagnose *B. invadens*, *C. capitata*, *C. rosa*, *C. cosyra*, *Dacus ciliatus*, *D. frontalis* and *D. bivittatus* using PCR-RFLP analyses.

The three *Dacus* species which will also be included in the study are pests of cucurbits which often occur in areas where citrus is grown. Some cucurbit crops are also hosts of the invasive fly, *B. invadens*.

Materials and methods

Insect materials

Second instar larvae of *B. invadens*, *B. dorsalis*, *C. capitata*, *C. rosa* and *C. cosyra* obtained from established laboratory colonies at the International Centre of Insect Physiology and Ecology (ICIPE), Kenya (*B. invadens*), International Atomic Energy Agency Seibersdorff (IAEA) facilities (*B. dorsalis*) and CRI, Nelspruit, South Africa (*Ceratitis* species), as well as *Dacus bivittatus* (Bigot) adults from Limpopo province, South Africa, were used in this study. *Dacus frontalis* Becker specimens could not be obtained and this species was therefore not included. *Dacus ciliatus* Loew was also not included due to the difficulty in acquisition and identification of specimens. Larvae (instars unknown) of two exotic fruit fly species, present in other African countries, *B. cucurbitae* (Coquillett) and *B. zonata* (Saunders), obtained from Reunion and a secondary insect pest found in infested fruit, *Drosophila* spp, obtained from Nelspruit, were also included in the study. All samples were preserved in 90% ethanol.

DNA extraction

A CTAB-based (Murray and Thompson, 1980; Rogers and Bendich, 1985) manual DNA extraction protocol was used. Larvae and adults were removed from ethanol and each sample was placed into a sterile 1.5 µl Eppendorf tube. For adults, the heads were used for the extractions. Depending on the size of the larvae and heads, 100-150 µl CTAB buffer, preheated at 60°C, was added. The samples were macerated with a sterile micropestle, and macerates were incubated in a water bath at 65°C for 30 minutes. Thereafter, 100-150 µl chloroform was added and the extract was mixed. The mixture was then centrifuged for 5 minutes at 13 000 rpm. The supernatant was then transferred to a sterile 1.5 µl Eppendorf tube. 250-300 µl pre-cooled 100% ethanol was added, where after the mixture was placed in a -80°C freezer for 45 minutes to precipitate the DNA. This was followed by centrifugation for 15 minutes at 13 000 rpm. The supernatant was discarded and the pellet was washed with 500 µl 70% ethanol. The mixture was then centrifuged for 10 minutes at 13 000 rpm. The supernatant was discarded and the pellet was dried, where after it was dissolved in 25-50 µl (depending on the size of the initial insect sample) of sterile distilled water.

PCR-RFLP analysis

PCR amplifications were performed using an Applied Biosystems 2720 thermal cycler. Three mitochondrial DNA (mtDNA) fragments were amplified by PCR using three primer pairs, LR-J-12883 and LR-N-13398 (targeting the 16S rRNA gene) (Barr et al., 2006), AFP2 and ARP1, and DFP1 and DRP2 (targeting portions of the 16S and 12S rRNA genes) (Muraji and Nakahara, 2002). GoTaq® Hot Starter Green Master Mix (Promega, Madison, USA) was used for PCR reactions. Theroocycling parameters were: PCR products were

digested in separate reactions using two restriction enzymes: DraI and MseI (Fermentas) as per manufacturers' instructions. PCR-RFLP products were separated on 4% MS-8 agarose gels (Conda, Madrid, Spain) and O'GeneRuler™ 100bp and Ultra Low Range DNA ladders (Fermentas) were used as size markers. Gels were stained with ethidium bromide and visualized under UV light.

Results and discussion

Initially only three *Ceratitis* species, *B. invadens* and *B. dorsalis* were tested to establish the most appropriate primer set to differentiate between species. Different banding patterns were obtained for the *Ceratitis* and the *Bactrocera* species tested using the LR-J-12883 & LR-N-13398 primers and digestion with Dra I restriction enzyme (Fig. 3.3.9.1). The three *Ceratitis* species yielded different restriction profiles while the same profile was obtained for *B. invadens* and *B. dorsalis*.

In PCR-RFLP analyses using primers AFP2/ ARP1, and DFP1/ DRP2 and Dra I restriction enzyme, *C. rosa* and *C. capitata* were found to have the same banding patterns (Fig. 3.3.9.2). *Ceratitis cosyra* was however distinguishable from the two other *Ceratitis* species (Fig. 3.3.9.2). *Bactrocera* species banded differently to all *Ceratitis* species but the two *Bactrocera* species could not be differentiated (Fig. 3.3.9.2).

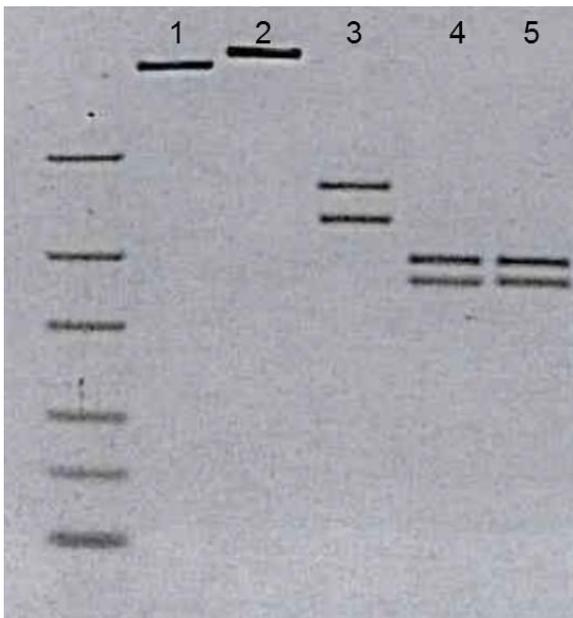


Figure 3.3.9.1. Representative gel (4%) for RFLP profiles obtained using the LR-J-12883 and LR-N-13398 primers and Dra I restriction enzyme: This extreme left lane is O'GeneRuler™ Ultra Low Range DNA Marker followed by RFLP products; lane 1. *C. rosa*; 2: *C. capitata*, 3: *C. cosyra*, 4: *B. invadens* and 5: *B. dorsalis*

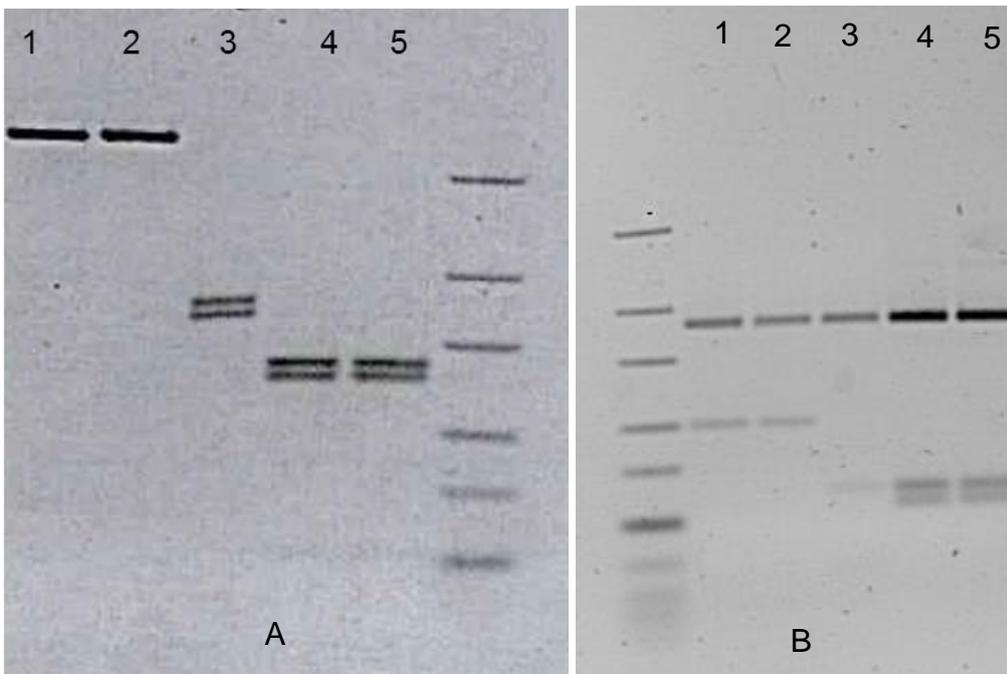


Figure 3.3.9.2. Representative gels (4%) for RFLP profiles obtained using primer sets (A) AFP2/ ARP1 and (B) DFP1/DRP2, both digested with Dra I. O'GeneRuler™ Ultra Low Range DNA Marker is loaded at the extreme right (A) and extreme left (B). RFLP products are loaded sequentially in each gel; 1. *C. rosa*; 2: *C. capitata*, 3: *C. cosyra*, 4: *B. invadens* and 5: *B. dorsalis*

PCR products using the three tested primer pairs and digestion with Mse I restriction enzyme yielded different restriction profiles for *B. invadens* and the *Ceratitidis* species. However some or all of the three *Ceratitidis* species tested were not differentiated using Mse I.

The best differentiation between species was obtained using the LR-J-12883 / LR-N-13398 primers and Dra I restriction enzyme and further tests were standardised with the latter system. Additional species were included in the tests namely: *B. cucurbitae*, *B. zonata*, *D. bivittatus* and *Drosophila* spp. Results showed that, *B. zonata*, *B. cucurbitae*, *D. bivittatus*, as well as *Drosophila* species could also be distinguished from the local *Ceratitidis* species (Fig. 3.3.9.3) The test was repeated and the same profiles were obtained as in the first analysis.

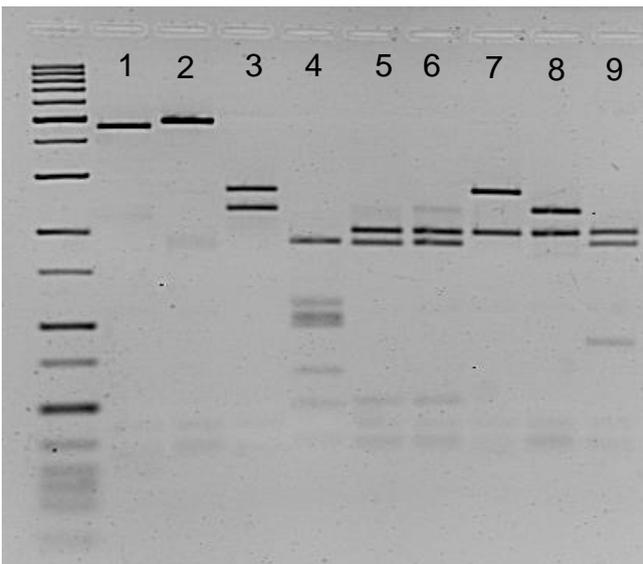


Figure 3.3.9.3. Representative gel (4%) for RFLP profiles obtained using primer set (LR-J-12883/ LR-N-13398) and Dra I restriction enzyme: O'GeneRuler™ Ultra Low Range and 100bp DNA Marker is loaded at the extreme left. RFLP products are loaded sequentially; 1. *C. rosa*; 2: *C. capitata*, 3: *C. cosyra*, 4: *Drosophila*, 5: *B. invadens*, 6: *B. dorsalis*, 7: *B. cucurbitae*, 8: *B. zonata*, 9: *D. bivittatus*.

The digestion profiles of *C. capitata* and *C. cosyra* obtained in this study were similar to the profiles obtained by Barr et al. (2006) using the same primer set and restriction enzyme.

The method tested here relied mainly on laboratory reared samples and needs to be validated by testing samples from different populations of the targeted species. The validation process was initiated in October 2012 with samples of *Ceratitidis* flies which were collected in traps from different regions of South Africa during a 2006-2009 CRI funded project. However, problems were encountered due to poor storage of samples and resulting poor quality DNA being obtained. The validation process will be completed in 2013 using DNA extracts of other *Ceratitidis* samples.

Conclusion

RFLP analysis using primers targeting the 16S rRNA gene in combination with the Dra I restriction enzyme distinguished exotic *Bactrocera* species, including *B. invadens*, from locally occurring *Ceratitidis* pest species. However, *B. invadens* could not be differentiated from *B. dorsalis*.

Future research

PCR-RFLP analyses as described above would have to be tested on samples from different populations of *Ceratitidis* species in South Africa in order to validate the use of this technique to differentiate *B. invadens* from the locally occurring *Ceratitidis* pest species.

Technology transfer

Not applicable

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3.3.10 FINAL REPORT: Determining the sensitivity of *Bactrocera invadens* to methyl eugenol

Project 1032 (April 2012 – March 2013) by A. Manrakhan and J-H. Daneel (CRI) & M. Mwatawala (Sokoine University of Agriculture, Tanzania)

Summary

Sensitivity of *Bactrocera invadens* (Drew, Tsuruta and White) males to methyl eugenol was quantified using mark-release recapture studies in two sites containing citrus and mango trees in Mikese, Morogoro, Tanzania. Recapture rates and frequency of capture of >0 flies increased with an increased density of methyl-eugenol baited traps. Results indicated that a density of 4 methyl eugenol baited traps per km² in production areas would be able to detect a new incursion of *B. invadens* containing as few as 48 young males.

Opsomming

Bactrocera invadens (Drew, Tsuruta and White) mannetjies se sensiwiteit ten opsigte van metiel-eugenol was gekwantifiseer deur gebruik te maak van die merk-vrylaat en hervangsmetode in twee studie areas bestaande uit sitrus en mango bome, in Mikese, Morogoro, Tanzanië. Hervangskoerse en gereeldheid van vangste van >0 vlieë het vermeerder as die digtheid van metiel-eugenol gelaaide lokvalle verhoog is. Die resultate toon aan dat 'n digtheid van 4 metiel-eugenol gelaaide lokvalle per km² in produksie areas, genoeg is om 'n nuwe inkursie van *B. invadens*, met so min as 48 jong mannetjies, op te spoor.

Introduction

Bactrocera invadens is an invasive exotic fruit fly species of Asian origin that was detected for the first time in Africa in 2003 and now found present in 30 countries on the continent (Ekesi and Muchugu 2006). In May 2010, *B. invadens* was intercepted in two areas in northern Limpopo, South Africa. The pest was successfully eradicated thereafter in both areas using protein baiting and male annihilation treatments (Anon 2010; 2011). However in subsequent years, further interceptions of this invasive pest occurred in the northern parts of South Africa. In 2011, *B. invadens* was successfully eradicated in all areas where it was detected. In 2012 although eradication was declared successful in many areas where *B. invadens* was detected, some areas still remained problematic. In 2013, *B. invadens* was declared present in specified areas in the northern parts of South Africa and under official control (Anon 2013). Given that *B. invadens* is still a regulated pest in South Africa, areas affected by the pest should be quarantined.

Bactrocera invadens responds to the parapheromone - methyl eugenol (ME) (Lux et al., 2003) which is used for monitoring and control of the fly. Currently the recommendation for surveillance of *B. invadens* in fruit production areas is 1 ME baited trap per km² (Manrakhan et al., 2010) and complies with the international trapping guide (IAEA, 2003). When *B. invadens* is detected, a delimiting survey is carried out whereby the density of ME traps is increased by 10 fold in the core area around the point of interception. Moreover, the current recommendation for eradication of *B. invadens* is at 400 ME baited blocks per square km based on recommendation for eradication of other methyl-eugenol responding *Bactrocera* flies such as *B. papayae* and *B. dorsalis* (Hancock et al., 2000; Seewooruthun et al., 1999).

Although methyl eugenol is a very powerful lure (Cunningham, 1989) and has been reported to attract *Bactrocera dorsalis*, a species closely related to *B. invadens*, from a distance of over 2 km (Steiner, 1952) the sensitivity of *B. invadens* to methyl eugenol has actually not been tested.

Stated objectives

The aim of this study was therefore to test the sensitivity of *B. invadens* to methyl eugenol in order to determine whether the current trapping and control strategies based on this parapheromone are appropriate to the pest. This study was carried out using a mark-release-recapture method.

Materials and methods

Fruit fly materials. *Bactrocera invadens* used in the studies originated from laboratory colonies maintained at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya (EKESI et al., 2007). Newly emerged pupae were shipped via road to the Sokoine University of Agriculture (SUA), Morogoro, Tanzania. Upon arrival at SUA, pupae were marked with selected fluorescent dyes. Four fluorescent pigments were used: Stellar Green 8, Magenta 10 and Lunar Yellow 27 from Swada, Cheshire, United Kingdom (FTX series) and White from Bastion Paint, KwaZulu-Natal, South Africa. The amount of dye used per pupa was 0.02 g per 100 pupae for the first 6 releases. Thereafter the amount of dye per pupa was

reduced to 0.01 g per 100 pupae in order to improve adult emergence. Pupae of each colour were placed separately inside a 9 L aerated plastic container. In each container, water and a mixture of sugar and yeast hydrolysate (3 parts sugar to 1 part yeast hydrolysate) were added. Three days after adult emergence, males of each colour were transferred to 200 ml aerated plastic containers for releases at specific points. In the smaller containers, water and food were provided as described above. Prior to release, pupae and adult males were held at 28°C and 75% RH. *Bactrocera invadens* males were released at approximately 4 days after adult emergence.

Field sites. Studies were carried out at two sites at Mikese, Morogoro District, Tanzania between 23 August and 16 November 2012. A distance of \approx 3.3 km separated the two sites. One site contained mainly mango (*Mangifera indica* L.) and a few other fruit trees (see (Mwatawala et al.2006) (Block 050: S06° 46' 36.4" E37° 54' 47.2") and the other site contained mango and citrus trees (Block 051: S06° 48' 23.6" E37° 54' 47.2"). The coverage of fruit trees at both sites was less than 50% with natural bush vegetation and cultivated maize or cassava between trees. During the study period, mean maximum and mean minimum temperatures were 31.83° C \pm 0.21°C and 18.85°C \pm 0.20°C respectively. Mean relative humidity was 65.83% \pm 0.51%. Total rainfall recorded during the study period was very low (25.8 mm).

Trapping. We used yellow Lynfield traps (River Bioscience, Port Elizabeth, South Africa) baited with Invader-Lure (River Bioscience), a fibre board block impregnated with 15 g of methyl eugenol. The Invader-Lure was fitted to the lid of the trap using a metal hook. A 3 g tablet with 19.5% dichlorvos was placed at the bottom of the trap to kill attracted flies. The methyl eugenol dispenser and insecticide in each trap were replaced with fresh ones before each test.

Distance-specific releases and central trapping. At each site, a trap was placed in the centre and was suspended on a host tree (mango in block 050 and citrus in block 051) at approximately 1.5 m above the ground. *Bactrocera invadens* males were released at four distances from the central trap: 25 m, 50 m, 100m and 250 m. Release points were marked along the four cardinal directions. Flies released at each distance were marked with a different coloured dye. Males were released at three densities: 48, 128 and 160, separately per site and in separate weeks. Equal numbers of males were released at each of 16 release points per site such that total fly densities were maintained. There were 6 replicates for the lowest release density (one replicate per week across 3 consecutive weeks at each site) and 4 replicates for each of the higher release densities (one replicate day per week across 2 consecutive weeks at each site). The trap in each site was checked and emptied after 7 days.

Central release and high density trapping. At each site, four traps were placed at 100 m from a central point along the four cardinal directions. Forty eight males were released from the centre of each block. Males released in the two blocks were marked with different coloured dyes. Releases were replicated 4 times (2 sites over 2 consecutive weeks). Traps in each site were checked and emptied after 7 days.

Fly identification. Flies captured in traps were identified to species, sex and colour. For colour determination, adult *B. invadens* males were checked under UV light. In cases where the colour was not visible on the body surface, the head of the adult fly was crushed gently on a glass slide under a binocular microscope using pointed forceps in order to extrude the ptilinum. The slide was then placed under UV light for identification.

Data analysis. Data were summarized as percentage of flies recovered (out of total released). Differences between percentage of flies recovered between distances and release densities were determined using a non-parametric Kruskal-Wallis test (XLSTAT Version 2012.4.02, Addinssoft) since values were not normally distributed (Shapiro-Wilk test: $P < 0.05$).

Results and discussion

With a density of 1 trap per study site, a total of 48 *B. invadens* males were recaptured out of 1440 released (3.33%) (Table 3.3.10.1). Recapture rates did not differ significantly between the three *B. invadens* release densities tested ($P > 0.05$). A higher recapture rate - 6.77% (192 released) was however obtained when a higher trapping density was used - 4 traps per study site (Table 3.3.10.2). There was no statistical difference in recapture rates between release distances ($P > 0.05$), although numerically higher numbers of *B. invadens* males were captured when released at 50 m and 100 m from the central trap. This was in contrast to previous findings on other methyl-eugenol respondent flies such as *B. dorsalis* where decreases in captures were observed at increased release distances from a methyl eugenol trap (from 25 m to 300 m) (Shelly et al., 2010). Given that the fluorescent pigments used in our study were not rotated among the four release distances tested, we cannot rule out the possibility that some pigments were less distinctive than others and could have therefore been misidentified.

Table 3.3.10.1. Mean recapture (\pm SE) of marked *B. invadens* males when released at different fly densities and at different distances from one centrally placed methyl eugenol baited Lynfield trap in each of two sites in Mikese, Morogoro, Tanzania.

Distance from trap/m	Mean (\pm SE) number of <i>B. invadens</i> males captured at different fly release densities (males per release point)			Mean % (\pm SE) of flies captured
	12	32	40	
25	0.50 \pm 0.34	0.00 \pm 0.00	0.00 \pm 0.00	1.79 \pm 1.29
50	1.17 \pm 0.75	2.25 \pm 0.25	0.25 \pm 0.25	10.12 \pm 3.17
100	2.33 \pm 1.80	2.00 \pm 0.82	0.50 \pm 0.29	14.29 \pm 6.62
250	0.17 \pm 0.17	0.75 \pm 0.25	0.00 \pm 0.00	2.38 \pm 1.04

Table 3.3.10.2. Total number of marked *B. invadens* males recaptured in 4 methyl eugenol baited traps placed along 4 cardinal directions at 100 m from a central release point.

Release date	Site	Total number of <i>B. invadens</i> males released	Total number of <i>B. invadens</i> males recaptured
08/11/2012	Block 050	48	2
	Block 051	48	1
16/11/2012	Block 050	48	5
	Block 051	48	5

Recapture rates were lower than expected, especially when considering very high captures of *B. invadens* observed within small time intervals in previous surveys using ME baited traps (Lux et al.2003). Recapture rates of *B. invadens* found in this study were also generally lower than general recapture rates of *B. dorsalis* reported in previous studies which varied from 15% to 22% in grids or trapping layout with less than 500 m distance interval between releases and traps (Shelly et al., 2010; Shelly and Edu, 2010). The low recapture rates observed in our study could be as a result of the age of *B. invadens* males used. Shelly et al. (2010) and Shelly and Edu (2010) used 2 weeks old males in their mark-release-recapture studies. Shelly et al. (2008) found that the attraction response of mature 2 weeks old *B. dorsalis* males to methyl eugenol was 8 fold higher than the attraction response of 3 day old *B. dorsalis* males. Fruit fly incursions in an area are often a result of either anthropogenic movement of infested fruit from which new flies would emerge or extensive dispersive movement of post-teneral adults, a movement characteristic of dacine fruit flies (Fletcher, 1987). Often, therefore new fruit fly incursions could contain a higher number of younger than older males.

When 48 young males were released per site, frequency of capture of > 0 flies with one trap was 33.33%. With more than 100 young males released, frequency of capture of > 0 flies increased to 75.00%. With four traps per site, frequency of capture of > 0 flies for a population containing 48 males was 100%.

Conclusion

A density of 4 ME baited traps per km² would be able to detect a *B. invadens* incursion containing as few as 48 young males, based on the above data and assuming traps placed in each site covered an area of 1 km².

Future research

Since mature *B. invadens* males could be more attracted to methyl eugenol, this study should be repeated with older flies before any change in current recommendation is warranted. Moreover, more replicates would be required to strengthen findings. Finally, trials should be conducted in areas with uniform host patches which are more reflective of the production areas in South Africa.

Technology transfer

A manuscript for publication as a short communication has been prepared and is under review within CRI. If deemed publishable, findings will also be presented as a poster for the next CRI symposium.

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3.4 PROGRAMME: MEALYBUG AND OTHER MARKET ACCESS PESTS

Programme coordinator: Sean D Moore (CRI)

3.4.1 Programme summary

Three of the seven mealybug species known to occur on citrus in South Africa are considered as phytosanitary threats for certain export markets. Three of the four projects reported within this programme address different aspects of mealybug management. The final one looks at carob moth, which is often closely associated with mealybug. Carob moth is not a phytosanitary pest for most markets. However, there are exceptions, such as China. In the first of the mealybug projects, which was funded by the International Atomic Energy Agency (IAEA), gamma irradiation at 150 Gy was evaluated on a probit-8.7 scale to confirm this dose's value as a potential phytosanitary treatment (3.4.2). All reproductive females were fully sterilized by the treatment. In the second project on mealybug, the parasitoid *Anagyrus* sp. nr. *pseudococci* was investigated as a biological control candidate for inundative releases (3.4.3). Mealybug infestation in Navel oranges was reduced by up to 80%. It was demonstrated that as few as 5000 parasitoids could be released per hectare, or possibly even 2500 parastoids in a second season. In the third project, the potential of entomopathogenic fungi for biological control of mealybug was demonstrated in the laboratory (3.4.4). An initial screening dose gave between 64 and 67.5% mortality of mealybug crawlers with the three most effective isolates. Screening against thrips was also attempted but was not yet successful at the time of this report. The final project in this programme looks at the morphology and ecology of carob moth on citrus, with the ultimate aim being to assess the threat status of carob moth on citrus and other hosts associated with

citrus (3.4.5). Valid morphological descriptions of each life stage are being prepared in order to avoid confusion with false codling moth.

Programopsomming

Drie van die sewe witluis spesies wat op sitrus in Suid-Afrika bekend is word as 'n fitosanitêre bedreiging vir sekere uitvoer markte beskou. Drie van die vier projekte in hierdie program wat hier gerapporteer word, spreek verskillende aspekte van witluis bestuur toe. Die finale een kyk na karobmot wat gereeld met witluis geassosieer word. Vir meeste markte is karobmot nie 'n fitosanitêre plaag nie, maar daar bestaan sekere uitsonderings soos China. In die eerste van die witluis projekte, wat deur die Internasionale Kernenergie Agentskap (IAEA) bevonds is, is gammabestraling van 150 Gy op 'n probit 8.7 skaal geëvalueer om die dosis se waarde as 'n moontlike fitosanitêre behandeling te bepaal (3.4.2). Alle reprodutiewe wyfies is ten volle deur die behandeling gesteriliseer. In die tweede projek op witluis is die parasiet *Anagyrus* sp. nr. *pseudococci* as 'n biologiese beheer kandidaat vir aanvullende loslatings ondersoek (3.4.3). Witluis besmetting in Nawellemoene is met tot 80% verminder. Dit is bepaal dat so min as 5000 parasiete per hektaar losgelaat kon word, of selfs miskien 2500 parasiete in 'n tweede seisoen. In die derde projek is die vermoë van entomopatogeniese swamme vir biologiese beheer van witluis in die laboratorium bewys (3.4.4). 'n Inisiële loodsproef dosis het tussen 64 en 67.5% mortaliteit van witluis kruipers gegee met die drie mees doeltreffende swam isolate. Biotoetse teen blaaspoetjie is ook probeer maar was nog nie suksesol teen die tyd van verslaskrywing. Die finale projek in hierdie program kyk na die morfologie en ekologie van karobmot op sitrus met die uiteindelijke doel om die dreigings status van karobmot op sitrus en ander verwante gashere te evalueer (3.4.5). Geldige morfologiese beskrywings van elke lewensstadium word voorberei om verwarring met valskoldlingmot te vermy.

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

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

Summary

An experiment was conducted on a small scale to assess the radio-tolerance of reproductive mealybugs to 150 Gy of ionizing radiation. All females were fully sterilized and similarly to non-ovipositing females, no progeny developed.

The efficacy of 150 Gy was consequently evaluated on a probit-8.7 scale to confirm this dose's value as a potential phytosanitary treatment. Two experiments were conducted with 70 440 and 3 150 reproductive females respectively. In the control treatments of both experiments females oviposited normally on butternuts. The F1 progeny from these females developed into non-ovipositing females before the control treatments were terminated. In the ionizing radiation treatments a small number of 1st instar F1 nymphs were produced before treatment. Subsequent to treatment, these nymphs either died *in situ*, or migrated to fresh butternuts where they also died without further development. All reproductive females were fully sterilized by the treatment and continued production of a new generation was prevented. The results are regarded as adequate to meet the requirements of Research Contract 15634/RO and the study is concluded.

Opsomming

'n Proef is op klein skaal uitgevoer om die radiovatbaarheid van reprodutiewe witluiswyfies vir 150 Gy ioniserende straling vas te stel. Alle wyfies is ten volle gesteriliseer en soortgelyk aan volwasse nie-reprodutiewe wyfies, het geen nageslag ontwikkel nie.

Die doeltreffendheid van 150 Gy ioniserende straling is vervolgens op probit-8.7 vlak geëvalueer om dié dosis se waarde as 'n potensieële fitosanitêre behandeling te bevestig. Twee proewe is met onderskeidelik 70 440 en 3 150 reprodutiewe wyfies uitgevoer. In die kontrolebehandelings van beide proewe het die onbehandelde wyfies normaal eiers op botterskorsies gelê. Die F1-nageslag van dié wyfies het tot volwasse wyfies ontwikkel voordat die kontrole-behandelings tot niet gemaak is. In die ioniserende stralingbehandeling is 'n klein aantal 1^{ste} instar F1-nimfe alreeds voor behandeling geproduseer. Ná behandeling is hulle almal óf *in situ* dood óf het na vars botterskorsies migreer waar hulle sonder verdere ontwikkeling gevrek het. Alle reprodutiewe wyfies is ten volle deur die behandeling gesteriliseer en voortgesette voortplanting is voorkom. Die resultate is as voldoende vir die vereistes van navorsingskontrak 15634/RO beskou en die studie is afgesluit.

3.4.3 FINAL REPORT: Assessment of the potential of *Anagyrus* sp. as a biocontrol agent for mealybug

Project 1017 (April 2011 – March 2014) by Sean Moore, Wayne Kirkman (CRI), Rob Stotter (Xsit), Moshe Cohen, Rami Friedman and Shimon Steinberg (BioBee, Israel)

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 3rd 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. From the Eastern Cape, 34% of all parasitized mealybug collected was parasitized by *Anagyrus*, whereas 84% was *Anagyrus* in the Western Cape. Laboratory trials to investigate the biology and host preference of *Anagyrus* have been delayed and will be reported separately in next year's annual report.

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^{de} 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 verskille tussen behandelings getoon nie, waarskynlik omdat die behandelde blokke nie voldoende van mekaar geskei was nie. Van die Oos-Kaap is 34% van alle geparasiteerde witluis wat versamel is deur *Anagyrus* geparasiteer, waar 84% in die Wes-Kaap *Anagyrus* was. Laboratorium proewe om die biologie en gasheer voorkeur van *Anagyrus* te ondersoek is vertraag en sal apart in volgende jaar se verslag gerapporteer word.

Introduction

Seven different species of mealybug are known to infest citrus in South Africa (Hattingh et al., 1998). At least three of these are regarded as phytosanitary pests for important export markets (Hattingh & Moore, 2003). It is therefore imperative that mealybug be well controlled on citrus. Concern has been expressed for several years now that there is an over-dependence on organophosphate insecticides for achieving this. There is not only a risk of the development of resistance but also the imminent threat of organophosphate tolerance being withdrawn by important western export markets. It is therefore urgent that alternatives to these organophosphate insecticides be sought.

On the other hand, chemical control on its own cannot effectively keep mealybug under control. Once residual efficacy has expired, only natural enemies can maintain mealybug at acceptably low levels. Apart from naturally occurring parasitoids and predators, which can have an immense impact on mealybug, it has been shown that inoculative augmentation of parasitoids can significantly enhance control. This has been shown with *Coccidoxenoides perminutus* against citrus mealybug, *Planococcus citri* (Hattingh et al., 1997; Moore et al., 1997), which has subsequently been commercially employed in the citrus industry and adopted by many citrus growers. One shortcoming with the use of *C. perminutus* is its specificity for *Planococcus* spp – therefore *P. citri* on citrus (Hattingh & Tate, 1997). Consequently, the other important mealybug species are left inadequately controlled or uncontrolled by these releases.

Hattingh et al. (1993) showed that along with *C. perminutus*, *Anagyrus* sp. is the most important parasitoid species against *P. citri* on citrus in South Africa. Moore & Kirkman (2006) and Johnson & Gilliomee (2010) showed that *Anagyrus* sp. also attacks the oleander mealybug, *Paracoccus burnerae* – almost certainly the most prolific mealybug species on citrus, after *P. citri*. However, it is not known how effective *Anagyrus* sp. is against *P. burnerae* and therefore if it is effective enough to be considered as a biocontrol candidate for this species – or for that matter, any of the other mealybug species occurring on citrus. However, *Anagyrus* sp. has been recorded as a parasitoid of the Karoo thorn mealybug, *Nipaecoccus viridis* (= *vastator*) (Prinsloo, 1984) and is regarded as having a relatively broad host range (Daane et al., 2004).

Nevertheless, due to successes with *Anagyrus* spp. as biocontrol agents for mealybug control, including as an inoculative augmentation candidate (Daane et al., 2004; Abd-Rabou, 2005), there is strong justification for testing the same in the context of mealybug on citrus in South Africa.

Du Roi IPM has already begun to rear *Anagyrus* sp. near *pseudococci* with a fair degree of success, therefore giving the proposed research some early impetus. However, the *Anagyrus* sp. parasitoids used in the field trials conducted were obtained from BioBee in Israel. It is exactly the same species as that which occurs in South Africa and is being reared by Du Roi IPM. The proposed biological studies are currently underway at Rhodes University and will be reported in next year's report.

Objectives

The objectives of this study are therefore to:

- Establish a rearing technique for *Anagyrus* sp., suitable for large scale production. (Funded outside of this proposal).
- Comparatively test at least *P. citri* and *P. burnerae* as hosts for *Anagyrus* sp.
- Conduct augmentation trials with *Anagyrus* sp.

Only the results of the augmentation trials are reported (incompletely) here.

Materials and methods

Sites.

Eastern Cape

Inoculative augmentation trials were conducted at two sites in the Eastern Cape, at Sun Orange and Good Hope farms in the Sundays River Valley. The treatment blocks at Sun Orange farm (Fukumoto Navel oranges) were each 1.4 hectares in size, with at least one buffer block between treatments (Fig 3.4.3.1). The treatment blocks at Good Hope Farm (Delta Valencia oranges) were approximately 1 ha each (Fig 3.4.3.2). Scouting was conducted in each treatment block on 9 November 2011, to determine the levels of mealybug infestation and site suitability.

Western Cape

The trial site used last season (not reported in the CRI research report), where a repeat treatment was done in the current season, was an orchard of Lina Navel oranges on the farm Robyn. The orchard was selected as it had a history of citrus mealybug infestation, and as a prior trial was conducted there starting January 2011. The orchard was 4.16 hectares in area, consisting of 13-year old trees. The entire orchard was used for the trial, with blocks being set out so that each of the 3 treatment blocks was between 1 and 1.2 ha in area, with a 5-row buffer between each treatment (Figure 3.4.3.3).

The second trial site, on the farm Hexrivier, was an orchard of Palmer Navel oranges. The orchard had a history of citrus mealybug infestation. The orchard was 3.5 ha in area, and consisted of 40-year old trees. The orchard was surveyed prior to the trial being initiated. The entire orchard was used for the trial. Treatment blocks were set out so that each treatment block was approximately 0.8-0.9 ha in area, with a 5-6 row buffer between each treatment (Figure 3.4.3.4).



Figure 3.4.3.1. Trial layout at Sun Orange farm, showing rates of *Anagyrus* sp released per hectare.



Figure 3.4.3.2. Trial layout at Good Hope farm, showing rates of *Anagyrus* sp released per hectare.



Figure 3.4.3.3. Layout of trial site (orchard 1014) on the farm Robyn, showing the two treatment blocks (T2 and T3) and control block (T1) and release points of *Anagyrus* sp (black dots; second release points in T2 shown by red dots). Trees sampled randomly are shown by white shaded area.

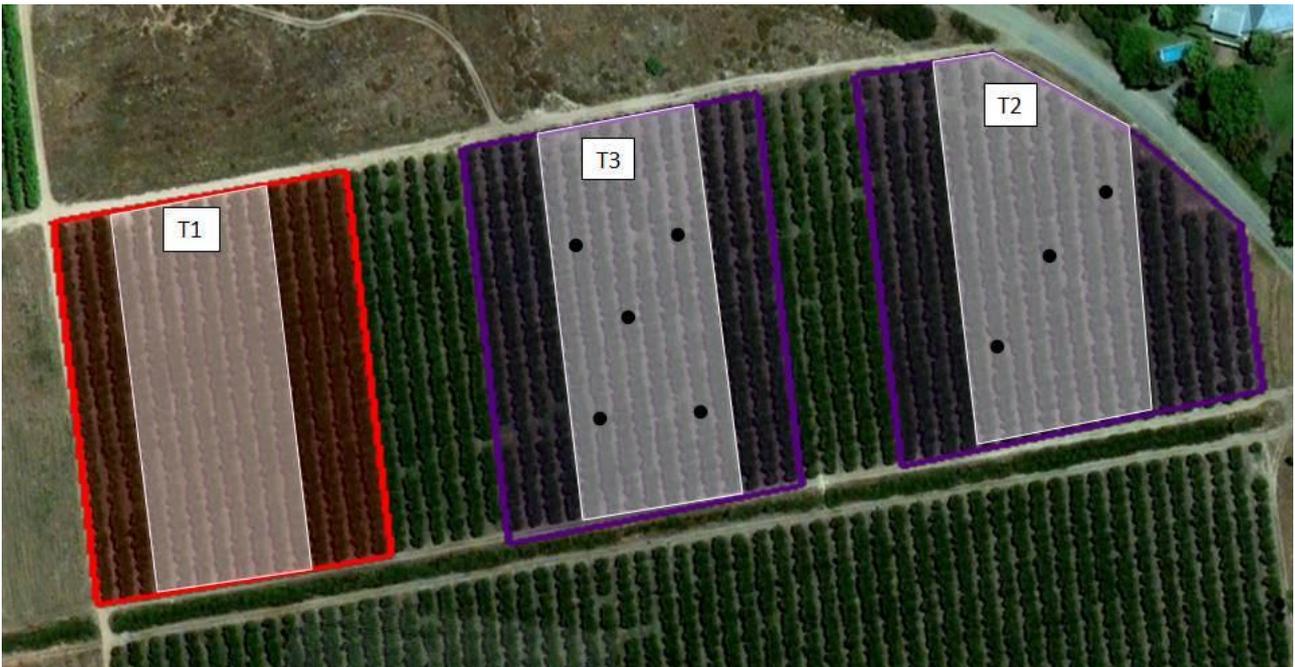


Figure 3.4.3.4. Layout of trial site (orchard Vlei 1 Navels) on the farm Hexrivier, showing the two treatment blocks (T2 and T3) and control block (T1) and release points of *Anagyrus* sp (black dots; second release points in T2 shown by red dots). Trees sampled randomly are shown by white shaded area.

Releases

Eastern Cape

Three rates of *Anagyrus* were released: 2500, 5000 and 30000 per hectare per season. Parasitoids were released in two instalments – on 15 November and 20 December 2011. The parasitoids were released in the form of parasitized mealybug mummies, contained in plastic bottles, each containing approximately 500 individuals (Fig 3.4.3.5). These plastic bottles were hung in trees regularly distributed throughout the blocks.

Western Cape

The first batch of *Anagyrus* sp. parasitoids were received in Citrusdal from BioBeel on 16 November 2011. The parasitoids were cold-stored at 10°C overnight and were released the following morning into both trial orchards. The second batch was received on 21 December 2011 and released on the same day. One bottle arrived empty on this occasion, and therefore only 500 *Anagyrus* sp were released in orchard Vlei 1 at the farm Hexrivier, meaning that this particular treatment only received a total of 2000 *Anagyrus* sp/ha, and not the intended 2500/ha.

Quality control

Each consignment of *Anagyrus* sp. contained one extra bottle of pupae. These were kept in the laboratory until all adults had emerged, and the adults were counted. One bottle from each treatment was collected at various intervals after releases, to determine the percentage eclosion at each date. In the Eastern Cape, mealybug infested sprouting potatoes were placed in release blocks, and at distances of 50 and 100 m from the blocks, in order to trap parasitoids and to determine the distance they could migrate.

Monitoring of mealybug infestation and parasitism

Six fruit from each of 20 randomly selected trees per treatment were collected every second week. The fruit were then evaluated in the laboratory for mealybug infestation and percentage parasitism. Parasitised mealybug and mummies were collected, parasitoids were allowed to eclose, and were kept in alcohol for identification.



Figure 3.4.3.5. *Anagyrus* sp dispenser taped onto a citrus tree.

Results and discussion

Task table:

Objective / Milestone	Achievement
A. Rear <i>Anagyrus</i>	
A.1. Sustainable culture through multiple generations	Succeeded for a few generations but culture subsequently collapsed. Culture was transferred to a climate controlled room and has been revived very well.
A.2. Adequate <i>Anagyrus</i> for lab studies	Achieved
A.3. Adequate <i>Anagyrus</i> for field trials	Imported from BioBee
B. Lab studies	Initiated in August 2012
B.1. Parasitoid biology	As above
B.2. Host preference	As above
C. Field trials – augmentative release	Successfully completed in 2011/12 season. Will not be repeated

	in 2012/13 as originally planned.
C.1. Eastern Cape	Completed
C.2. Limpopo	Not conducted, however, Western Cape used instead.

Sites.

Eastern Cape

The mealybug infestation in the treatment blocks at Sun Orange farm on 9 November ranged from 8 to 14 percent. At Good Hope Farm infestation ranged from 14 to 18%. This showed that infestation at both sites was sufficiently high for that time of season, and treatment blocks were comparably infested.

Western Cape

On Robyn Farm, the orchard had an average of 3.33% of fruit infested by citrus mealybug in mid-November 2011, and a very even spread of the pest throughout the orchard.

At Hexrivier Farm the orchard had between 5.8 and 8.3% of fruit infested in mid-November, prior to releases of *Anagyrus* sp.

Quality control

Eastern Cape

Four hundred and ninety eight *Anagyrus* sp. adults emerged from a sample bottle retained from the November batch, and 539 from the December batch – both acceptably close to the “approximately 500” stated on the label.

For the November application, the average percentage emergence of parasitoids from bottles retrieved from release orchards was 61% nine days after application, 77% after 18 days and 80% after 27 days. For the December application, an average of 87 % of parasitoids had emerged from the mummies 15 days after application. The higher percentage eclosion in December/January could be as a result of higher temperatures at that time.

Unfortunately the mealybug-infested potatoes used for measuring dispersal of the released *Anagyrus* sp. were either stolen or removed by monkeys, so no data could be collected.

Western Cape

Eclosion of *Anagyrus* sp from mummies from the first batch released was 82% at both release sites on average. Eclosion of the second batch of *Anagyrus* sp. was lower at 72% and 67% at Hexrivier and Robyn Farms respectively. The lower percentage eclosion may have been due to higher temperatures and lower humidity experienced at this stage of the season. A similar occurrence happened during the trial at Robyn the previous season where an average of 65% eclosion was recorded in January 2011.

Monitoring of mealybug infestation and parasitism

Eastern Cape

Infestation

Mealybug infestation of Fukumoto Navel oranges at Sun Orange farm was reduced by 6%, 73% and 80%, relative to the control block, in the 2500, 5000 and 30000 parasitoid per ha release blocks, respectively (Fig 3.4.3.6). At Good Hope Farm, the trial did not show any difference between treatments, probably because there was inadequate spatial separation between treatment blocks (Fig 3.4.3.7).

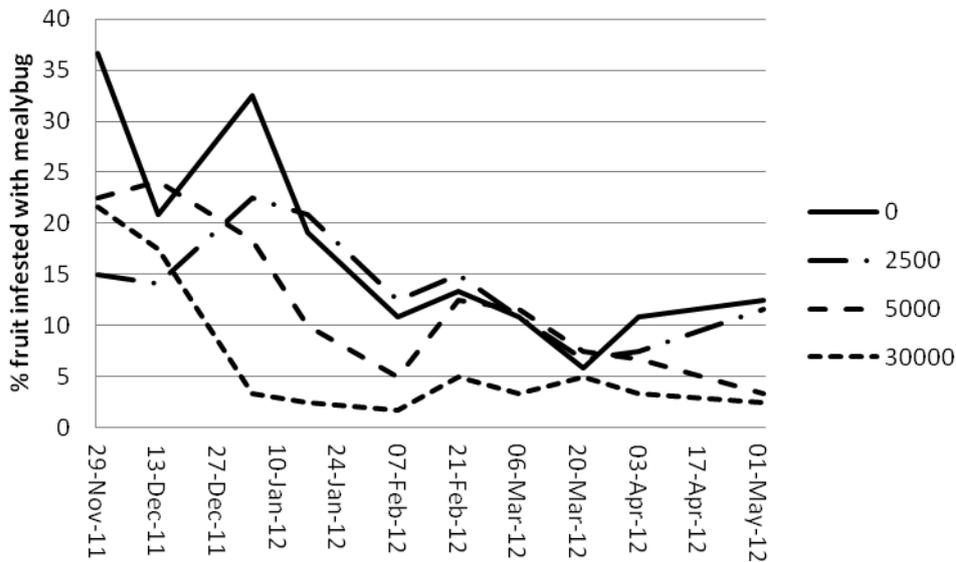


Figure 3.4.3.6. Percentage Fukumoto Navel oranges infested with live mealybug for different release rates of *Anagyrus* sp. at Sun Orange farm, evaluated from 29 November 2011 to 01 May 2012.

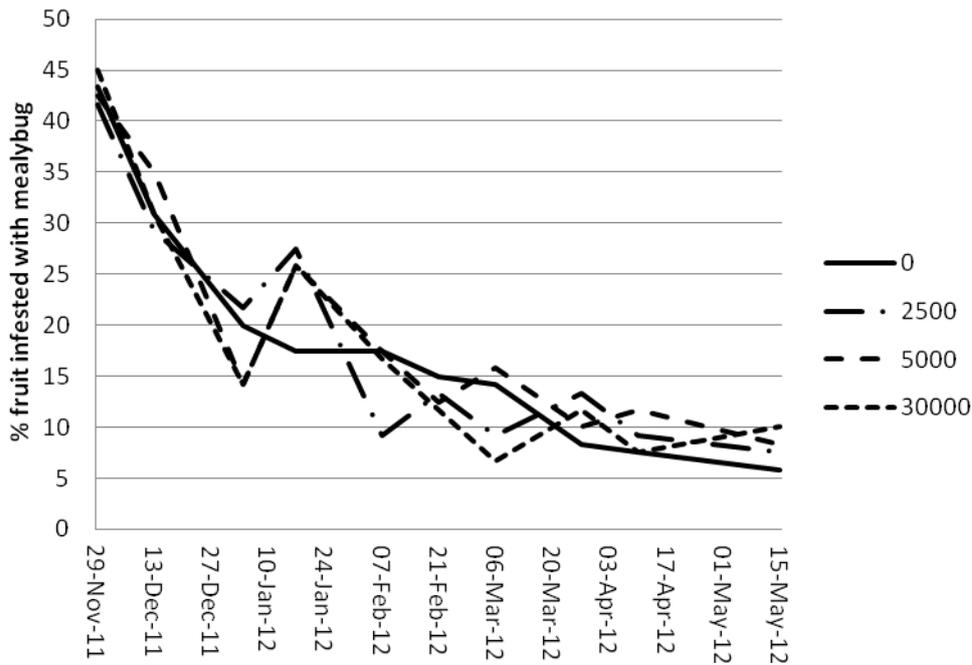


Figure 3.4.3.7. Percentage Midnight Valencia oranges infested with live mealybug for different release rates of *Anagyrus* sp. at Good Hope Farm, evaluated from 29 November 2011 to 15 May 2012.

Parasitism

Anagyrus sp. made up 34% of the total parasitoids which were reared from parasitized mealybug (mummies) recovered from fruit during the evaluation (Table 3.4.3.1). This was 35.4% in release blocks and 30.8% in control blocks. As one might have expected this difference to be greater, it might have been obscured by the small sample size from the control. *Coccidoxenoides perminutus* and *Leptomastix* sp. were also prevalent. This highlights the potential of biocontrol of mealybug under favourable conditions in the Eastern Cape.

Table 3.4.3.1. Parasitoids emerged from parasitized mealybug (mummies) recovered from infested fruit in the Eastern Cape, between 29 November 2011 and 15 May 2012 (percentage of total in brackets).

Treatment	Parasitised mealybug collected	Parasitoid species			
		<i>Anagyrus sp. nr psdeudococci</i>	<i>Coccidoxenoides perminutus</i>	<i>Leptomastix sp.</i>	Other
Release	65	23 (35.4)	27 (41.5)	11 (16.9)	4* (6.2)
Control	13	4 (30.8)	7 (53.8)	2 (15.4)	0 (0)

*Two specimens were unidentifiable due to damage; the other two specimens have been sent for identification.

Western Cape

Infestation

Fruit infestation on the farm Robyn was very uniformly spread through the orchard pre-release in mid-November 2011. An average of 3.3% infestation was recorded for all three blocks in the trial site. By the end of December 2011, infestation had noticeably increased, particularly in the control block, where infestation was 36% compared to 27% in Treatment 2 (2500 *Anagyrus* sp/ha) and 11% in Treatment 3 (5000 *Anagyrus* sp/ha) (Fig 3.4.3.8). This positive trend continued for the rest of the season until harvest. Just prior to harvest (21 April 2012), infestation was 24% in the control block, compared to 10% in treatment 2 and 7% in treatment 3 respectively. The differences between treatments and control were significant. During the previous season, infestation in treatment blocks ranged from 8 – 14% at harvest compared to 3% and 15% in chemically-treated blocks.

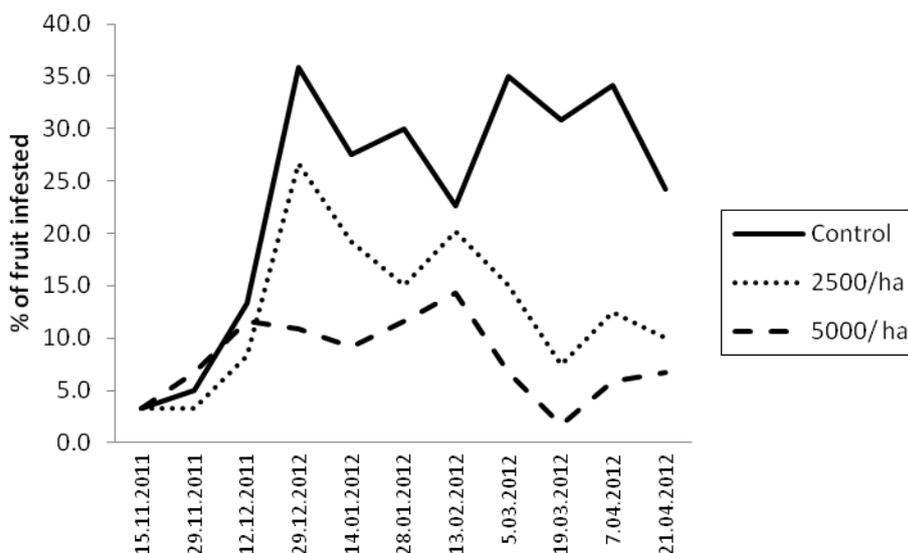


Figure 3.4.3.8. Percentage of Lina Navel oranges infested with live mealybug for different release rates of *Anagyrus* sp at Robyn Farm, evaluated from 15 November 2011 to 21 April 2012.

At Hexrivier Farm, a very different trend was observed (Fig. 3.4.3.9). Pre-release in mid-November, infestation of fruit ranged from 6-9% throughout the orchard, more than double the infestation that was observed at Robyn Farm. Infestation remained at a similar level for the first month after application of the first release of *Anagyrus* sp, after which infestation increased rapidly. By late January, infestation of fruit ranged from 30-40% in all three blocks, with no difference between the control block and two treatment blocks. On 2 February 2012, Buprofezin was applied. Four weeks after application, infestation in the orchard reached a peak, ranging from 31% in the 5000/ha block, to 66% in the 2500/ha block. Two weeks later, in mid-March, infestation in all three blocks had dropped dramatically to around 10%. This was due to the Buprofezin application, which took approximately 5 weeks to show its full effects. Interestingly, within 2 weeks, the infestation in the control block rose very quickly, whilst the infestation in the two treatment blocks was maintained at a lower level. This could be attributed to the higher numbers of *Anagyrus* sp in these blocks. It can therefore be argued that another chemical spray would have been required in the control block, whereas the *Anagyrus* sp releases in the other blocks, would have saved a chemical spray.

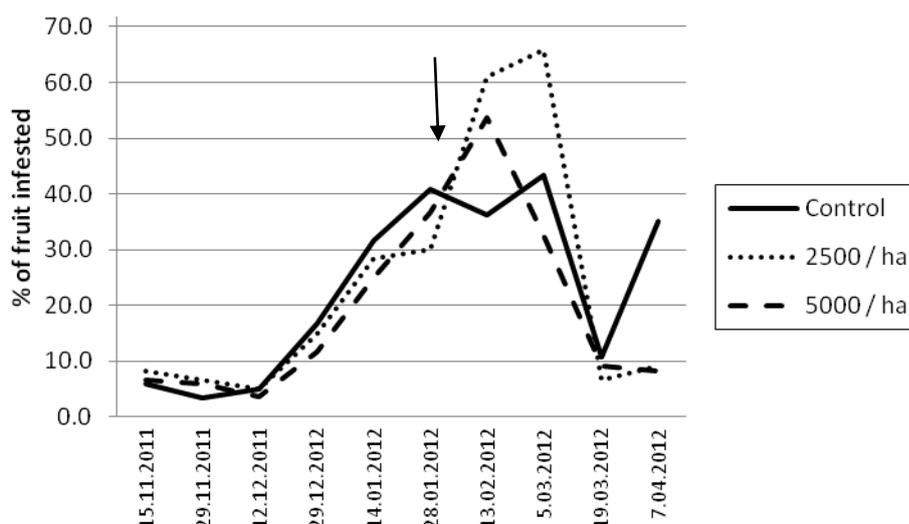


Figure 3.4.3.9. Percentage Palmer Navel oranges infested with live mealybug for different release rates of *Anagyrus* sp at Hexrivier Farm, evaluated from 15 November 2011 to 7 April 2012. The black arrow denotes a Buprofezin application.

Parasitism

Anagyrus sp. made up 84% of the total parasitoids which were reared from parasitized mealybug (mummies) recovered from fruit during the evaluation (Table 3.4.3.2) The bulk of the remaining 16% was *Leptomastix* sp. It was interesting to note that no *C. perminutus* parasitoids were recovered during the evaluation. No differentiation was made between collections from release and control orchards.

Table 3.4.3.2. Parasitoids emerged from parasitized mealybug (mummies) recovered from infested fruit in the Western Cape, between 29 November 2011 and 21 April 2012.

Parasitised mealybug collected	Parasitoid species			
	<i>Anagyrus</i> sp. nr <i>psdeudococci</i>	<i>Coccidoxenoides perminutus</i>	<i>Leptomastix</i> sp.	Other
85	70	0	12	1*

*No details available.

Conclusion

Augmentation with *Anagyrus* sp. nr *pseudococci* appeared to successfully reduce mealybug infestation. The trials will be monitored until harvest before final conclusions are drawn, but it seems that releases should be conducted at a rate of at least 5000 parasitoids per ha in the first year, and possibly a lesser quantity from the second year onwards. Parasitoids recovered from the trial sites will still be identified and will be reported in the final report.

Technology Transfer

A poster was presented at the Citrus Research Symposium in August 2012, and an oral paper will be presented at the Entomological Society Congress in July 2013.

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3.4.4 PROGRESS REPORT: Screening of entomopathogenic fungi against citrus mealybug and citrus thrips

Project 1048 (2012/3 – 2013/4) by J.F. Dames (RU), M. Hill (RU) and S.D. Moore (CRI)

Summary

A mealybug starter culture was obtained from Du Roi and is being reared by the Entomology Department of Rhodes University per Du Roi's protocols on chemical free butternut. Fungal isolates of *Metarhizium anisopliae* and *Beauveria bassiana* from the Rhodes University fungal collection were subcultured and are being maintained on Sabouraud Dextrose 4% Agar (SDA). Isolates are regularly checked for viability and contamination. To determine infectivity of the 17 fungal isolates, they were assayed using 5th instar false codling moth (FCM) larvae. Larvae were placed onto SDA after death to allow the fungus to sporulate and grow externally. Of the 17 fungal isolates, 14 were successfully assayed through the FCM larvae and pure cultures were obtained. Stocks of the cultures are maintained and were examined microscopically to confirm fungal identity.

Mealybug crawlers and adults were used for pilot trials of the fungal bioassays. These were carried out in 24 well plates. The method was modified to include 2 rows of wells filled with sterile water to increase the humidity within the plate. Into each of the remaining 16 replicate wells, sterile filter paper and washed citrus leaf sections were placed. Fungal conidial suspension (1×10^7 spores per ml) was used to inoculate crawlers and adults. Crawlers were inoculated with 10 μ l of the test fungus, while adults received 14 μ l. In the initial tests one of the major problems was the escape of the crawlers from the wells thus preventing accurate data from being collected. To prevent the crawlers from escaping sterilised folded up tissue paper was placed between the tops of the wells and lid and then secured with elastic bands.

After optimisation of the method and an initial screening, a final screening was conducted. This was performed on 10 fungal isolates from the Rhodes collection (8 *B. bassiana*, 2 *M. anisopliae*) and negative and positive (commercially available *B. bassiana* product, Becker Underwood) controls were included. A commercial *M. anisopliae* product obtained from Real IPM (ICIPE 69) could not be included due to lack of fungal growth. This final screening was performed with 5 replicates per life stage, per fungal isolate and percentage mortality was calculated based on the average mortality from each of the isolates. Statistical analysis is being conducted. From the results obtained, indications are that 3 isolates have shown biological control potential, particularly of crawlers. The isolates GAR 17 B3 (*B. bassiana*) and FCM AR 23 B3 (*M. anisopliae*), both with 67.5% crawler mortality, and GB AR 23 13 3 (*B. bassiana*) with 64% crawler mortality, are being used for dose dependent assays and these isolates will be molecularly identified. The mortality rates among adults were notably lower, the highest being 53.75% with most having 40% mortality. This is believed to be a result of the increased waxy outer layer which covers adult mealybugs and prevents the fungal spores from reaching the cuticle.

An initial screening with thrips, collected from the field, was also attempted but low concentrations due to rainfall and low survival rates resulted in the postponement of the screening. Collections of thrips will be done as soon as possible, weather permitting, and an alternative method is being investigated.

Opsomming

'n Witluis kultuur is van Du Roi gekry en word deur die Entomologie Departement van Rhodes Universiteit volgens Du Roi se protokolle op chemiese vrye botterskorsies geteel. *Metarhizium anisopliae* en *Beauveria bassiana* swam isolate is van die Rhodes Universiteit se versameling gekry en op Sabouraud Dekstrose 4% Agar (SDA) onderhou. Isolate is gereeld getoets vir lewensvatbaarheid en kontaminasie. Infektiwiteit van die 17 swam isolate is teen 5de instar valskodlingmot (VKM) larwes getoets. Na afsterwing is larwes op SDA geplaas om die swam te laat sporuleer en ekstern te groei. Uit die 17 isolate is 14 suksesvol deur VKM hergesirkuleer en suiwer kulture is gekry. Voorrade van die kulture is onderhou en mikroskopies ondersoek om swam identiteit te bevestig.

Witluis kruipers en volwassenes is vir swam biotoets loodsproewe gebruik. Hierdie is in 24-put plate uitgevoer. Die metode is gemodifiseer om twee rye te gebruik vir steriele water om humiditeit in die plaat te verhoog. Steriele filter papier en gewasde stukkie sitrusblare is in die oorblywende 16 putte gesit. Kruipers en volwassenes is met 'n konidiële suspensie (1×10^{-7} spore per ml) geïnokuleer. Kruipers is met 10 μ l en volwassenes met 14 μ l geïnokuleer. In die eerste proewe was daar 'n probleem met die ontsnapping van kruipers wat akuraatheid van data gekompromie het. Om die ontsnapping van kruipers te verhoed is opgevoerde gesteriliseerde papier tussen die putte en deksel geplaas en met rekbande vasgemaak.

Na verfyning van die metode en loodsproewe voltooi is, is finale ondersoek proewe gedoen. Hierdie is met 10 swam isolate van die Rhodes versameling gedoen (8 *B. bassiana*, 2 *M. anisopliae*). Negatiewe en positiewe kontroles (kommersieel beskikbare *B. bassiana* produk, Becker Underwood) is ingesluit. 'n Kommersiele *M. anisopliae* produk van Real IPM (ICIPE 69) kon nie ingesluit word nie as gevolg van 'n tekort aan swam groei. Hierdie proewe is met 5 replikate per lewensstadium per swam isolaat uitgevoer. Persentasie mortaliteit is gebaseer op die gemiddelde mortaliteit vir elke isolaat. Die data word tans statisties ontleed. Drie isolate het potensiaal vir biologiese beheer, veral van kruipers, gewys. Die isolate GAR 17 B3 (*B. bassiana*) en FCM AR 23 B3 (*M. anisopliae*) het albei 67.5% mortaliteit van kruipers veroorsaak en GB AR 23 13 3 (*B. bassiana*) het 64% mortaliteit gegee. Hierdie drie isolate word tans vir dosis-respons biotoetse gebruik en word molekuler geïdentifiseer. Mortaliteit van volwassenes was duidelik laer, met 'n maksimum van 53.75% en gewoonlik 40%. Hierdie is heel waarskynlik as gevolg van die beskermende waks laag wat volwassenes dek en hulle dus teen swamspore beskerm.

'n Eerste proef met blaaspootjie, versamel van die veld, is ook uitgevoer, maar lae getalle, as gevolg van reen, en lae oorlewing in proewe het gelei tot opskorting van die proef. 'n Alternatiewe metode word ondersoek en blaaspootjie sal weer versamel word sodra die weer dit toelaat.

3.4.5 **PROGRESS REPORT: The morphology and ecology of the Carob moth in citrus orchards** Project US/ENT-11-A3 (2012/7-2014/12) by P. Addison, G. Morland and H.G. Geertsema (SU)

Summary

The Carob moth *Ectomyelois ceratoniae* is a field pest that occurs on growing fruits and carob tree pods. It has caused up to 40% damage in both the date and pomegranate industries. It is also a stored product pest. It is a species that originated from the Mediterranean region. It is difficult to tell how much damage the Carob moth has caused in the South African citrus industry, because in its larval stage can be easily confused with the false codling moth *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae). The aims of this study are to produce a valid morphological description of each life stage of the carob moth, as well as to determine its seasonal cycle within citrus orchards of the Western Cape. Other objectives include a life table study and a wing morphometrics study. To achieve these aims a series of field tests and laboratory tests will be done. The field tests include the hanging of yellow delta traps baited with chemically synthesized pheromone within orchards, as well as a damage assessment. The laboratory tests will be done by rearing a colony, which will then be tested at temperature gradients to determine intrinsic rates of increase and other relevant developmental parameters. The predicted outcome of the study is to assess the threat status of Carob moth on citrus and other hosts associated with citrus in order to limit crop losses.

Opsomming

Die Carob mot *Ectomyelois ceratoniae* is 'n plaag wat op vrugte en karob peule voorkom. Die mot het 'n Mediterreense oorsprong. Tot 40% skade is in die dadel en granaat bedrywe aangetoon. Karob mot is ook 'n

plaag van gestoorde produkte. Dit is moeilik om Karob mot skade op sitrus in Suid Afrika te skat, omrede dit dikwels met die valskodlingmot, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), verwar kan word. Die doel van hierdie studie is om 'n geldige morfologiese beskrywing van elke aspek van die lewenssiklus te skep, asook om die seisoenale siklus binne sitrusboorde in die Wes-Kaap te bepaal. 'n Lewenstabel en vlerk-morfologie studie sal ook uitgevoer word. Om hierdie doele te bereik, sal 'n reeks veld en laboratorium studies uitgevoer word. Die veld toetse behels die uitplaasing van geel delta lokvalle asook n' skatting van skade. Laboratorium toetse sal uitgevoer word deur 'n kolonie aan te teel en diet aan temperatuur gradiënte bloot te stel. Daardeur sal intrinsieke vermeerderings koerse en ander relevant ontwikkelings parameters bepaal kan word. Die voorspelde uitkoms van die studie is om 'n bedreigingstatus op te stel vir karobmot op sitrus en ander gashere wat met sitrus geassosieer is. Uiteindelik sal oesverliese voorkom kan word.

3.5 PROGRAMME: MINOR PESTS AND MITES

Programme Coordinator: Tim G Grout (CRI)

3.5.1 Programme summary

Pests may be considered minor because they only occur sporadically, only affect a low percentage of citrus production areas or cause little economic damage. However, when they do occur they are capable of causing serious damage or indirectly increasing numbers of other key pests. Woolly whitefly is now present in many citrus production regions in South Africa but is mainly problematic where few pesticides are used for the control of red scale and mealybug. Research has shown that sprays of buprofezin, pyriproxyfen and Movento as registered for other citrus pests are effective in controlling woolly whitefly. Indigenous natural enemies such as *Encarsia* parasitoids and coniopterygids do suppress it, but cannot control it (3.5.2). The project on woolly whitefly has now been terminated but the pest will still receive attention under a new project on the control of pests shortly before harvest. This new project (1061) will also address the control of leafhoppers which have been very rare in the last few seasons and it has not been possible to conduct any trials on them (3.5.3). In the southern production regions snails can be problematic and are difficult to control once they get into the trees. Contract research was conducted on a new snail bait from Villa Crop Protection but although it lasted longer than the standard bait it did not offer much better control. Sprays of Nordox to the foliage were ineffective in reducing snail numbers in the tree (3.5.4).

Programopsomming

Plae kan as minder belangrik beskou word wanneer hul slegs sporadies voorkom, slegs 'n lae persentasie van sitrus produksie-areas affekteer of min ekonomiese skade veroorsaak. Wanneer hulle egter voorkom kan hulle ernstige skade veroorsaak, of indirek, getalle van ander sleutelplae laat toeneem. Wollerige witvlieg is nou in baie sitrus produksie-areas in Suid-Afrika teenwoordig, maar is hoofsaaklik problematies waar 'n paar insekdoders vir die beheer van rooi dopluis en witluis gebruik word. Navorsing het getoon dat bespuitings met buprofezin, pyriproxyfen en Movento, soos geregistreer vir ander sitrusplae, effektief in die beheer van wollerige witvlieg is. Inheemse natuurlike vyande soos *Encarsia* parasitoïede en Coniopterygidae onderdruk dit, maar kan dit nie beheer nie (3.6.2). Die projek oor wollerige witvlieg is gestaak, maar die plaag sal steeds aandag, as deel van 'n nuwe projek oor die beheer van plae kort voor oes, geniet. Hierdie nuwe projek (1061) sal ook die beheer van blaarspringers aanspreek, wat baie skaars in die laaste paar seisoene was, en daarom was dit nie moontlik om enige proewe met hulle te doen nie (3.6.3). In die suidelike produksie-areas kan slakke problematies wees en dit is moeilik om hulle te beheer as hulle eers in die bome beland het. Kontraknavorsing is op 'n nuwe slak-lokmiddel van Villa Crop Protection gedoen, maar alhoewel dit langer as die standaard lokmiddels gehou het, het dit nie veel beter beheer verskaf nie. Bespuitings van Nordox op die blare was oneffektief in die vermindering van slakgetalle in die boom (3.6.4).

3.5.2 FINAL REPORT: Managing woolly whitefly

Project 975 (2010/1 – 2012/3) by T. Grout, S.D. Moore, P.R. Stephen, W. Kirkman (CRI), K. Kruger (UP), T. Fullard, M. Hill (RU) and J. Giliomee (SU)

Summary

As woolly whitefly spreads across the country it is slowly becoming more problematic in commercial citrus orchards where few scalicides are applied. It is typically found in home gardens for a year or two before being found in commercial citrus farms nearby. Woolly whitefly has now spread north to Polokwane 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 spray trials in the Eastern Cape showed buprofezin 30 g/hl water plus BreakThru 5 ml/hl to be 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 in project 1061.

Opsomming

Soos wat die wollerige witvlieg oor die land versprei, is dit stadig besig om meer problematies in kommersiële sitrusboorde te raak waar min doppluisdoders toegedien word. Dit word gewoonlik vir 'n jaar of twee in huistuine gevind voordat dit in kommersiële sitrusplase daar naby gevind word. Wollerige witvlieg het nou noord tot Polokwane 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 bespuitingsproewe in die Oos-Kaap het gewys 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 in projek 1061 voortgaan.

Introduction

Although woolly whitefly, *Aleurothrixus floccosus* (Maskell), is not yet considered a serious pest in most commercial citrus orchards in South Africa, it is becoming well known as a pest of lemon trees in domestic gardens in most cities (Giliomee and Millar 2009). Woolly whitefly is thought to have originated in South America where it is very widespread, but it also occurs in Central America, parts of North America, the Mediterranean countries, Middle East, India, Japan and most of Africa, but not Australia. Woolly whitefly (WWF) is the woolly white pest that produces copious amounts of sticky honeydew from the lower surface of leaves of citrus and certain other plants in home gardens in the Western and Eastern Cape, Gauteng, the North-West as far as Zeerust, Nelspruit in Mpumalanga, Stanger in KwaZulu-Natal and as far north as Polokwane in Limpopo Province. Many reports of this pest have been received from home owners which have shown that it has been present from around 2006 near George. Woolly whitefly is very widespread in other parts of the world and South Africa seems to be one of the last countries to get it. In the southern part of Africa it occurs in Angola, Zambia, DRC, Malawi, Uganda, Kenya and Tanzania but has not yet been officially recorded from Zimbabwe, Namibia, Botswana or Mozambique. In other parts of the world it became problematic in commercial citrus orchards and the parasitoid *Cales noacki* was introduced and it now largely controls the pest. In South Africa it is becoming a serious pest on farms where few insecticides are being used: particularly in the Western and Eastern Cape and near Brits in the North-West Province. Unfortunately, *C. noacki* cannot be released in South Africa without proving that it will not attack any of the indigenous whitefly species. As *C. noacki* has previously been found to attack at least one species besides WWF and it has now been found to belong to a species complex, attempts to run non-target effect testing will probably be fruitless and very expensive. The research approach in South Africa therefore must be to determine what natural enemies are having an impact, whether they could be introduced to regions where they do not occur, and learning how to control the pest with chemical applications.

Objectives

1. Determine what natural enemies are attacking WWF in South Africa
2. Determine what plant protection products are effective against it and how they should be used.

Materials and methods

During 2010/1 and 2011/2, research was conducted in four different regions of the country in order to determine whether the natural enemy complex was the same in all regions and to be able to conduct trials on chemical treatments in areas where it was most abundant and widespread. The collaborators were based at Stellenbosch, Port Elizabeth, Grahamstown, Pretoria and Nelspruit. During 2010/1, researchers tried to find out as much as they could about its distribution, natural enemies and susceptibility to chemicals. In 2011/2 the IPM research committee cut the funding to only allow for identification of natural enemies in the four provinces and some efficacy trials in the Eastern Cape. In 2012/3 funding only went to the Eastern Cape

and Nelspruit with the emphasis on some more chemical control trials in the Eastern Cape and identification of natural enemies in the northern parts of its distribution.

Western Cape

Research on WWF in the Western Cape was limited to the identification of WWF in new areas and collection of natural enemies for identification.

Eastern Cape

Chemical trials 2010/1

An orchard containing the required number of trees that were infested with WWF was sourced. Spurwing Farm just outside of Knysna, South Africa (33°56'56"S, 22°58'51"E) has an orchard containing ±600 Nules Clementine trees. Sixty trees, similarly infested with WWF were identified and marked as test trees. Treatments were allocated using the randomized block design. Six replicate blocks were identified with 10 trees in each; there were nine treatment trees and a control per block. Treatments were allocated using the randomised block method. Of the nine treatments, two were stem applications, one was a soil drench (9 ml in 1 L water per tree) and six of the treatment types were foliar applications (Table 3.5.2.1). Foliar applications were applied using a Janisch handgun spray machine at an average rate of 15 L/tree, using 1.75 mm nozzle orifices and a pressure of 1 500 kPa. The spray applications were conducted on 13 September, 2010.

The trial was evaluated 21 days after application on 4 October, 2010. Due to the low infestation levels of WWF the evaluation took the form of a quantified observation. Trees were analysed for two minutes, 60 seconds on either side of the tree, and the number of leaves with visible honeydew were recorded; honeydew is only produced by live insects so this was presumed to be a good measure of life as it is difficult to determine live WWF from dead. Between 4 and 17 of the most infested leaves were collected per tree and placed in emergence chambers. Emergence chambers were constructed using plastic lined, 1 litre cardboard milk cans with a transparent vial attached to a hole in the lid. The numbers of emergent WWF were counted and divided by the number of leaves in the chamber to calculate the average number of WWF per leaf.

Table 3.5.2.1. Ten treatments that were used for the chemical trial, their trade names when relevant and the concentrations or amounts applied to each tree. All spray treatments were applied with the Janisch handgun spray machine.

	Treatment Active ingredient	Trade name	Application concentration	Method of application
1	Untreated control			-
2	Imidacloprid	Imidor	9 ml/tree	Soil drench
3	Acetamiprid	Mospilan	6 ml/tree	Paintbrush
4	Methamidophos	Citrimet	12.5 ml/tree	Syringe
5	Medium range oil	Citrole 100	1 L/ 100L	Spray
6	Abamectin + oil	Biomectin (+oil)	(20 ml/100L) + (300 ml/100L)	Spray
7	Buprofezin + Agral 90	-	(30 g/100L) + (18 ml/100 L)	Spray
8	Methomyl	Lannate	20 g/100L	Spray
9	Chlorpyrifos	-	(100 ml/100L)	Spray
10	Wetcit	-	(200 ml/100L)	Spray

Chemical trials 2011/2

A trial was conducted in an orchard of two-year-old lemon trees (planted 2009) on CJ Potgieter Boerdery near Kirkwood in the Sundays River Valley, Eastern Cape province. Five treatments were applied and an untreated control was used (Table 3.5.2.2). One row of 50 trees was used for each treatment. Treatments were sprayed on 13 June 2011 using hand guns equipped with 1.5 mm nozzle orifices and using a pressure of 1 500 kPa. An average of 2.6 L was applied per tree, extrapolating to 1 443 L per ha. A second application of the two entomopathogenic fungi was made one week later on 20 June 2011. An average of 2.4 L per tree was applied, extrapolating to 1 332 L per ha. The trial was evaluated on 7 July 2011. Five infested leaves were picked from each of 10 randomly-selected trees per treatment. Leaves were taken back to the laboratory and placed into dark emergence bags (one per data tree). Eclosing adult WWF flew into a clear glass vial attached to the emergence bag. Numbers of WWF which had eclosed were counted on 2 August 2011.

Parasitoid survey 2010/1

Thirteen sample sites were used to determine the presence of any parasitoids on trees infested with WWF in the Eastern Cape, South Africa. Leaves infested with WWF were collected and placed in a plastic packet for transportation. After less than 24 hours, leaves (no more than 25 per chamber) were placed in emergence chambers (the same design as described above). Emergent adult WWF and any emergent parasitoids were attracted to the light admitted by the vial and could be collected (protocol modified from Miklasiewicz & Walker, 1990). Adult parasitoids were preserved and identified to family. Samples were sent to Dr. Gerhard Prinsloo (National Collection of Insects – Accession number RHAC843) for further identification. Percentage parasitism at Spurwing Farm, Knysna was calculated according to the following equation:

$$P = \frac{p}{w + p}$$

Where P is the percentage parasitism, p is the number of emergent parasitoids and w is the number of emergent woolly whitefly adults.

Parasitoid survey 2011/2

Since January 2012 WWF was collected from six sites in Grahamstown, two sites in Port Elizabeth, one site in King Williams Town and five sites in East London. The aim of the study was to assess the WWF populations on citrus in gardens around the Eastern Cape, ranging from Port Elizabeth to East London, and quantify any levels of parasitism. This was accomplished by picking WWF infested leaves off the particular lemon trees and placing them into emergence chambers as described above.

Gauteng

Since October 2009, leaves from three citrus trees were collected at time intervals ranging between one and four weeks in a home garden in Pretoria. Leaves were placed in ventilated glass vials or emergence boxes to allow for emergence of parasitoids. At the same time when leaves were collected, adult whiteflies were counted on 15 young leaves of each tree to obtain an indication of whitefly abundance.

In addition to the collection of leaves in the home garden, leaves of rough lemon trees infested with nymphs of WWF were collected at the Experimental Farm of the University of Pretoria. Leaves were placed in emergence boxes to allow for emergence of parasitoids.

Emerged parasitoids were collected and transferred to gelatine capsules and submitted to Drs G. Prinsloo/J. Kelly of the National Collection of Insects, ARC-Plant Protection Research Institute, for identification.

In 2011, T Grout identified WWF on a lemon tree in a home garden in Benoni.

Nelspruit and other northern regions

WWF probably only arrived in Nelspruit around 2009 and by 2011 only occurred on neglected citrus trees in home gardens and shopping centres. Natural enemies were collected for identification from Nelspruit, the Brits area and other parts where WWF was found.

Results and discussion

Western Cape

WWF primarily occurs in home gardens around the coast and is common in Cape Town, Stellenbosch, Hermanus and Worcester. By 2011 it had reached 125 km inland to De Doorns in the Hex River Valley but has not yet been found near Citrusdal or Clanwilliam. Only *Encarsia* sp. parasitoids have been recovered and identified and they are not very effective. Control of WWF in home gardens has been successful with frequent sprays of soapy water. Commercial farmers are trying to control it with treatments registered for the control of mealybug on citrus.

Eastern Cape

Chemical trials 2010/1

The buprofezin treatment had consistently low numbers of emergent WWF (Fig. 3.5.2.1). Abamectin had the next lowest number of emergent pests and a higher degree of variance while methomyl had similarly low levels of emergence and tighter variation. Chlorpyrifos and imidacloprid had similar levels of control. The medium range oil showed differing results between absolute numbers of emergent woolly whitefly and numbers relative to the number of leaves (Fig. 3.5.2.1). Acetamiprid, methamidophos and Wetcit® all had similar levels of control for both absolute and relative (Fig. 3.5.2.1) WWF. All treatments reduced the number of emergent WWF below the number that emerged from the untreated control.

The number of leaves with visible honeydew showed no significant differences between the different treatments. The results show that the two stem treatments (acetamiprid and methamidophos) and the soil drench (imidacloprid) were similar to or less effective than the untreated control. Only abamectin and methomyl showed clearly less live WWF than the untreated control according to the honeydew method of evaluation. Buprofezin had a high number of leaves with visible honeydew which contrasted with the low numbers of emergent WWF.

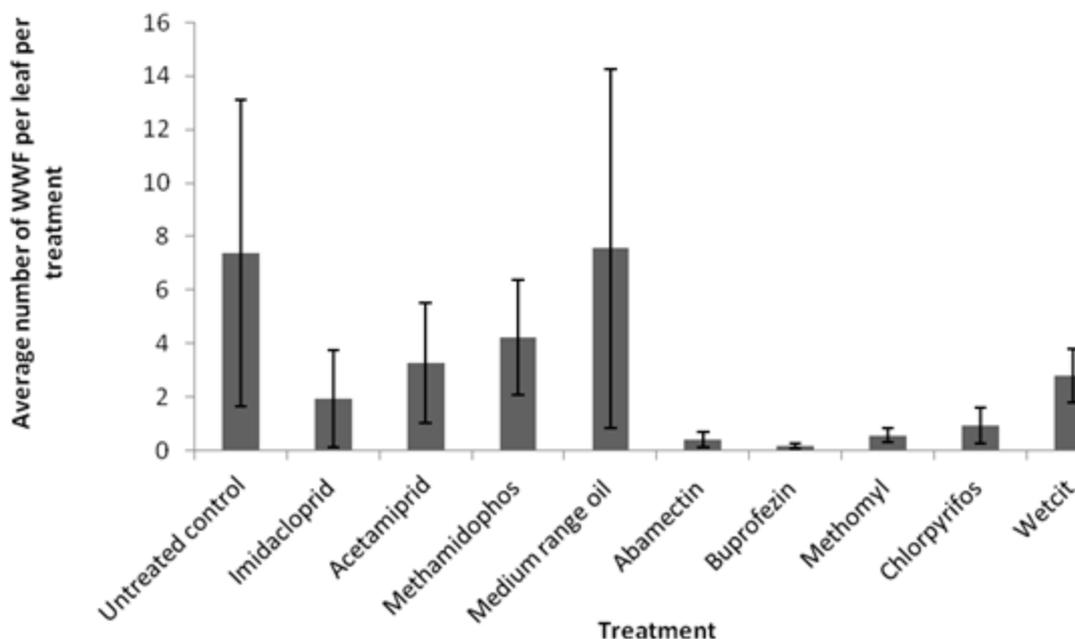


Figure 3.5.2.1. The number of woolly whitefly that emerged per chamber were divided by the number of leaves in that chamber to give the average number of woolly whitefly per leaf in each chamber. A Kruskal-Wallis analysis revealed no significant differences between treatments.

Chemical trials 2011/2

All three chemical treatments significantly and substantially reduced WWF infestation of trees (Table 3.5.2.2). *Beauveria bassiana* showed no efficacy at all. This was in contradiction to anecdotal reports received from some growers (always as multiple applications). However, as the trees in this trial were very small, conditions for good efficacy with a fungus were not at all favourable: the trees could not create a humid microclimate nor could they provide much protection against UV-irradiation (both important environmental conditions for good efficacy with a fungus). This considered, the experimental *Metarhizium anisopliae* performed relatively well, reducing infestation by almost 50% (even though this reduction was not statistically significant).

Table 3.5.2.2. Woolly whitefly (WWF) control on young lemon trees in the Sundays River Valley, Eastern Cape (June to August 2011)

Treatments (dosages per 100 L water) ¹	Mean adult WWF/tree (5 leaves/tree) ²	Infested trees (%)
Untreated control	9.4 a	80
Buprofezin (Applaud 500 WDG) 30 g	0.1 b	10
Pyriproxyfen (Scalex 100 EC) 30 ml	0.3 b	10
Spirotetramat (Movento 240 SC) 20 ml	0.5 b	20
Experimental <i>Metarhizium anisopliae</i> 13 ml	5.1 ab	50
<i>Beauveria bassiana</i> (4xe ⁹ spores/g) 67 ml	10.9 a	90

¹All spray treatments contained the wetter BreakThru 5 ml/100 L water

²Values followed by the same letter are not significantly different ($\alpha = 0.05$; Bonferonni LSD multiple range test)

Parasitoid survey 2010/1

Of the thirteen sample sites parasitoids were present at six of these locations. Knysna, Colchester and four of the five Grahamstown sites had parasitoids while at Port Elizabeth, Port Alfred, Bushmans, East London and Bathurst no parasitoids were found. These were all identified as *Encarsia* sp. (Hymenoptera: Aphelinidae) by PPRI Biosystematics.

Parasitoid survey 2011/2

To date, parasitism has been present in all Grahamstown samples, three East London samples and very little parasitism in the Port Elizabeth samples (Table 3.5.2.3). Parasitoid samples were sent off for identification and the wasps were again identified as *Encarsia* sp. This project ran until December 2012.

Table 3.5.2.3. Percentage parasitism of WWF by *Encarsia* sp. in the Eastern Cape in 2012. The “*” represents data not collected.

<u>Site</u>	<u>January</u>	<u>February</u>	<u>March</u>	<u>April</u>	<u>May</u>	<u>June</u>	<u>July</u>	<u>August</u>	<u>September</u>	<u>October</u>	<u>November</u>	<u>December</u>
23 Oatlands Road (GHT)	10.8	13.3	42.3	2.2	16.6	0	16.6	19	*	37.9	0	0
9 Beadle Street (GHT)	3.1	1.4	8.3	13	11.9	0	No infected leaves			14.3	6.7	15.4
Durban Street (GHT)	2.9	3.7	0	3.3	4.2	1	21.2	9	*	11.8	1.8	5.1
6 Charles Street (GHT)	10.5	5.8	19	10	14	5.5	Owner cut down tree					
1 Carslile Street (GHT)	7.8	2	14.3	16.6	40.8	34.2	5	11.9	*	16.7	0	0
New House (GHT, RU)	3	3.4	4	56	12.3	0	No infected leaves				No resident when sampling	
37 Two Rivers Drive (EL)	8.8	6.1	2.6	10.5	0	0	16.6	0	*	0	0	0
7 Raleigh Road (EL)	0	0	5.1	15.3	5	29	22.2	7.7	*	16.6	4.9	17.2
9 Da Gama Site House (KWT)	1.2	1.4	0	Branches cut off by owner								
58 Cassia Drive (PE)	0	4.8	0	0	4.5	21	0	3.3	*	No infected leaves		
3 Paddocks Complex (PE)	2.6	9	14.3	11.1	25	0	No longer participating in study					
4 Maynard Road (PE)	*	*	*	*	4.3	0	18.1	31.2	*	11.1	0	0
138 13th Ave (Gonubie)	6.3	12.5	12.3	8	0	22	0	14.8	*	8.8	0	12.2

GHT=Grahamstown; EL=East London; KWT=King Williams Town; PE=Port Elizabeth; RU=Rhodes University.

Gauteng

WWF and parasitoids were monitored for more than 18 months in a home garden in Pretoria (Fig. 3.5.2.2). Adult WWF occurring on the three trees were observed throughout the monitoring period with the exception of a period from end of September until end of November 2010 when no adult whiteflies were recorded. This is in accordance with findings at the Research Farm of the University of Pretoria where no whiteflies were found during this period. The absence of whiteflies in October/November in 2010 is in contrast to 2009 where whitefly numbers were relatively high during the same period in the home garden. WWF abundance has increased again since December 2010.

All parasitoids collected in 2010 (gardens: 87 individuals; research farm: 182 individuals) were sent to the National Collection of Insects, ARC-Plant Protection Research Institute, for identification. All specimens were examined and identified as *Encarsia* sp. (Hymenoptera: Aphelinidae) by Dr Janine Kelly. They were sent to Dr Andrew Polaszek (The Natural History Museum, London) in January 2011 for further identification. In general, parasitism has been low throughout. The mean percentage parasitism in home gardens from January 2010 until July 2010 when WWF was abundant ranged between 0.3 and 10%; percentage parasitism per leaf ranged between 0 and 29%. Collection of leaves infested with WWF nymphs to determine which parasitoid species occur in Pretoria, as well as their abundance, continued until March 2012.

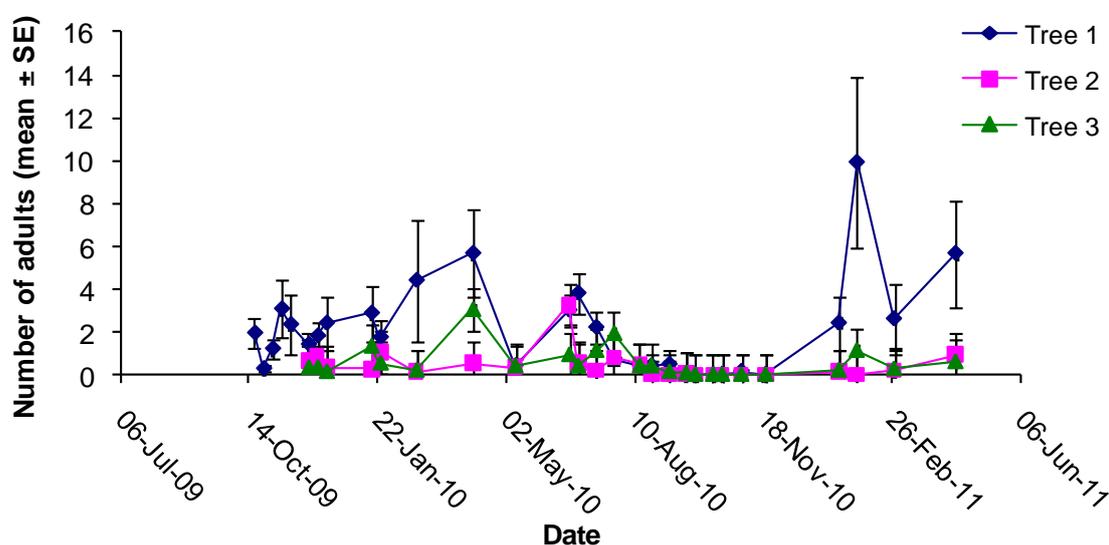


Figure 3.5.2.2. Abundance of *Aleurothrixus floccosus* on three citrus trees in a home garden in Pretoria (rough lemon: Tree 1 (height ca. 3.50 m), Tree 2 (height ca. 1.80 m); orange / rough lemon combined: Tree 3 (height ca. 1.50 m) (n = 15 leaves per tree).

Nelspruit and other northern regions

Of the parasitoids that have been collected in the northern areas, there were two different species of *Encarsia* and it is not yet known whether one of these is the same as the *Encarsia* sp. that is being found in other parts of the country. An *Eretmocerus* sp. was found in a Nelspruit home garden but this was in the presence of another unidentified whitefly so may not have been attacking WWF. However, parasitism does not provide control of the pest. Photographs from citrus orchards near Brits show high numbers of pupal cases of coniopterygids on foliage and these predators are apparently suppressing WWF. An investigation of home properties in Tzaneen in 2011 and a visit to a nursery there showed no signs of WWF but it was present in Mookgophong and Mokopane and arrived in Polokwane late in 2012 or early 2013. It was found as far west as Zeerust in early 2013 by JH Daneel. Nurseries in Johannesburg report of many people enquiring how to control it in their gardens in Johannesburg. MC Pretorius saw it in Stanger, KwaZulu-Natal in 2012 but no reports have been received of it being problematic in other parts of this province.

Conclusion

Parasitoids found in all regions except Nelspruit are *Encarsia* spp. but parasitism is low. Coniopterygids are beneficial in unsprayed orchards but these are not common in commercial, fresh fruit orchards. Spray trial results to date show that buprofezin and pyriproxyfen are the most efficacious.

Future research

Natural enemies will continue to be monitored and more treatments evaluated in project 1061. When *Cales noacki* arrives in the country, or the need to determine its effect on indigenous whiteflies before releases will be permitted is waived, a research project on the rearing and release of this parasitoid will be initiated.

Technology transfer

Advice for home-owners to spray 250 ml laundry soap powder (or 30 ml liquid) in 100 L water as a double application two weeks apart seems to have been successful. Several talks have been presented to growers on the results of the chemical trials.

An article was published in the SA Fruit J in the October/November 2012 issue: Grout, T.G., W. Kirkman and S.D. Moore. 2012. Woolly whitefly, *Aleurothrixus floccosus*, on citrus in South Africa. S.A. Fruit J. 11 (5): 77, 78, 81.

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3.5.3 FINAL REPORT: Treatments for the control of leafhoppers on citrus Project 942 (2008/9-2012/3) by Tim G. Grout and Peter R. Stephen (CRI)

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 seasons and no trial sites were found in either Mpumalanga or the Northern Cape. Further trials will be conducted under project 1061 when sites become available.

Opsomming

Alhoewel blaarspringers ernstige kosmetiese plaë kan wees, is hul uitbrake sporadies en geen chemiese bespuitings is vir hulle 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 seisoene skaars en geen proefpersele in óf Mpumalanga of die Noord-Kaap is gevind nie. Verdere proewe sal onder projek 1061, wanneer persele beskikbaar kom, uitgevoer word.

3.5.4 FINAL REPORT: Evaluation of a Villa Metaldehyde/Carbaryl snail bait pellet formulation and Nordox (Metallic copper) 86 WG against the brown garden snail, the white dune snail and the small pointed snail in citrus

Project 10/2013 (September 2012 – March 2013) by Sean Moore and Wayne Kirkman (CRI)

Summary

CRI was contracted by Villa Crop Protection to test an experimental metaldehyde/carbaryl snail bait in citrus orchards in the Eastern Cape in comparison with a standard registered bait. Trials were conducted in two orchards – a Nadorcott Mandarin orchard in the Gamtoos River Valley and a Bahianinha orchard in the Sundays River Valley. Additionally, the effect of Nordox (cuprous oxide) sprays on snails was measured. Three species of snails were evaluated in the trials – brown garden snails, *Helix aspersa*, white dune snails, *Theba pisana* and tower (or small pointed) snails, *Cochlicella (Prietocella) barbara*. Trials were applied in October and November 2012 and evaluated weekly for 6 and 10 weeks. The Villa bait was at least as effective as the standard bait – Sluggem – and there were indications that its effectiveness may be superior and particularly that it endured at a higher level for longer than Sluggem. Although the highest rate of the Villa bait (100 g/m²) was initially superior in a couple of cases, this was not sustained and there is no apparent reason to register this bait at a rate higher than 50 g/m². What was clear is that none of the treatments came close to eliminating the snail problem. This is not unique to the treatments used in this study, but is a general problem with all registered modes of snail control. Unfortunately, all treatments appeared ineffective in controlling tower snails. Nordox was essentially ineffective in reducing numbers of all snail species in trees.

Opsomming

CRI is deur Villa Crop Protection gekontrakteer om 'n eksperimentele metaldehyd/carbaryl slaklokaas in sitrus boorde in die Oos-Kaap te toets, in vergelyking met 'n standaard geregistreerde lokaas. Proewe is in twee boorde uitgevoer – 'n Afourer Mandaryn boord in die Gamtoosriviervallei en 'n Bahianinha boord in die Sondagsriviervallei. Boonop is die effek van Nordox (koperoksied) bespuitings op slakke gemeet. Drie slak spesies is in die proewe geëvalueer – bruin-tuinlakke, *Helix aspersa*, wit-duinlakke, *Theba pisana* en toringslakke, *Cochlicella (Prietocella) barbara*. Proewe is in Oktober en November 2012 toegedien en weekliks vir 6 en 10 weke geëvalueer. Die Villa lokaas is minstens gelyk in doeltreffendheid aan die standaard lokaas, Sluggem, en daar is aanduidings dat sy doeltreffendheid dalk selfs beter was, veral omdat sy werking langer as Sluggem s'n voortgeduur het. Alhoewel die hoogste dosis van die Villa lokaas (100 g/m²) in 'n paar gevalle oorspronklik beter gelyk het, is dit nie oplopend nie en dus is daar geen oënskynlike rede om hierdie lokaas teen 'n hoër dosis as 50 g/m² te registreer nie. Dit was duidelik dat nie een van die behandelings slakke kon uitwis nie. Hierdie is nie uniek vir die behandelings wat in hierdie studie gebruik is nie, maar is 'n algemene probleem met al die geregistreerde slakbeheer metodes. Ongelukkig kon nie een van die behandelings toringslakke suksesvol beheer nie. Nordox kon hoofsaaklik amper glad nie die getalle van enige van die slakspesies in die bome afbring nie.

Introduction

Citrus Research International was contracted by Villa Crop Protection to conduct field trials with an experimental metaldehyde/carbaryl snail bait in citrus orchards in the Eastern Cape. Simultaneously, the use of Nordox sprays to rid trees of snails was tested, as apparently practiced in Spanish citrus orchards. One orchard in the Sundays River Valley and one orchard in the Gamtoos River Valley were selected for the trial. Orchards were selected in which three species of snails (brown garden snails, *Helix aspersa*, White dune snails, *Theba pisana* and tower (or small pointed) snails, *Cochlicella (Prietocella) barbara*, were present.

Stated objectives

To test and compare the efficacy of baits and sprays at different rates, for control of snail pests in citrus orchards.

Materials and methods

Trial sites

Two sites were selected. The first was an orchard of 4 year old Afourer (Nadorcott) Mandarin trees, on Gonnakop Farm (33°46'57"S 24°49'54"E) in the Gamtoos River Valley, in which the trees were spaced at 6 m x 2 m (rows x trees). Trees were irrigated using a dripper system. The trees were sufficiently separated to

prevent snails from moving from the foliage of one tree to the foliage of the adjacent tree, thus buffer trees were not required. White dune snails were abundant in the trees; brown garden (or brown) snails were present in lower numbers. The trunks of the trees were infested with tower snails. Weeds on both sides of the rows of data trees were not treated with herbicide, unlike the remainder of the orchard, as it was speculated that this might reduce the snail pressure by removing this refuge.

The second site was an orchard of 5 year old Bahianina Navel orange trees on Far Away Farm (33°29'12"S 25°40'32"E) in the Sundays River Valley, in which the trees were spaced at 6 m x 3 m (rows x trees) and irrigated with drippers. As with the first orchard, no buffer trees were required. Infestation of the trees by all three snail species was visible.

Trial layout

The trials were laid out in a single-tree randomised block format, replicated 10 times. Nordox treatments (treatments 6 & 7, Table 3.5.4.1) were applied using a Janisch handgun spray machine, at 15 bar pressure using 1.75 mm nozzle orifices. Thereafter the bait treatments were applied (treatments 2 – 5, Table 3.5.4.1) on the soil around the trunks of the trees. All snails, barring the tower snails, were then removed from all trees, except the Nordox-treated trees, and placed beneath the tree canopy. This ensured that all baited and control trees were comparable at the start of the trial with negligible numbers of snails in trees and that snails would encounter the baits when returning to the trees. It was impractical to try and remove the tower snails as they were too numerous and are extremely small.

Treatment application

Treatments were applied at Gonnakop Farm on 25 October 2012. The Nordox sprays were applied at an average rate of 3.0 L per tree. Treatments were applied at Far Away Farm on 1 November 2012. Here the Nordox sprays were applied at an average rate of 5.25 L per tree.

Table 3.5.4.1. Various treatments applied for the control of three species of snails in citrus orchards in the Gamtoos and Sundays River Valleys

Tr. No.	Active ingredient	Formulation type	Application rate
1	Untreated Control	-	-
2	Sluggem (Metaldehyde/Carbaryl)	050 RB	50 g/10m ²
3	Villa Metaldehyde/Carbaryl	050 RB	50 g/10m ²
4	Villa Metaldehyde/Carbaryl	050 RB	70 g/10m ²
5	Villa Metaldehyde/Carbaryl	050 RB	100 g/10m ²
6	Nordox (Cuprous Oxide)	86 WG	175 g/hL
7	Nordox (Cuprous Oxide)	86 WG	200 g/hL

Trial evaluation

The treatments were evaluated at 7, 14, 21, and 28 days after application, and then every 14 days thereafter. For the brown snail and the white dune snail, the number of live snails of each species present in each tree, as well as the number of dead snails of each species beneath the tree, were counted. For the tower snail, only the live snails on the trunk were counted, as no dead snails of this species were observed on the ground.

All Nordox-sprayed trees were assessed each week for any signs of phytotoxicity. Particularly young flush and twigs and fruit were inspected and compared with those of untreated trees.

Daily rainfall preceding the initiation of trials and during the full duration of trial evaluation was recorded.

Statistical analysis

Mean percentage mortality of brown and white dune snails at each site was determined each week. Mortality recorded for each treatment was then corrected according to Abbott's formula (Abbott, 1925). Corrected percentage mortality measurements were normalised through an arcsine transformation and then subjected to an ANOVA and means were compared using Duncan's New Multiple Range Test at p = 0.05. For tower snails, mean numbers recorded were compared as described above, bar correction for control mortality.

Results and discussion

Once snails had been removed from trees, many of them very rapidly headed straight back to the tree trunk and began climbing into the tree again. It was noted that a number of snails encountered the bait surrounding the trunk and began to feed. Others seemed to ignore the bait, crawling straight past it to the tree trunk.

Trees which had been sprayed with Nordox were inspected for any signs of phytotoxicity on a weekly basis. Brown copper residues were conspicuous on leaves. However, there were no signs of burn, even on younger leaves and no distortion or retardation of growth. There was also no burn speckling on fruit as is sometimes the case with copper sprays.

Gonnakop trial – Gamtoos River Valley

Due to good rains leading up to the trial (125 mm in 17 days), the soil was moist at the time of application (25 October 2012) of the trial (Fig. 3.5.4.1). Two days after application a further 3.8 mm of rain was recorded. Rain fell periodically in small amounts during the 10 week period of the trial (total of 65 mm). It was already clear at two weeks after application that the integrity (structure) of the Villa bait pellets was maintained well in comparison with the Sluggem pellets. Even at the final evaluation, there was still notable structure to the Villa bait pellets.

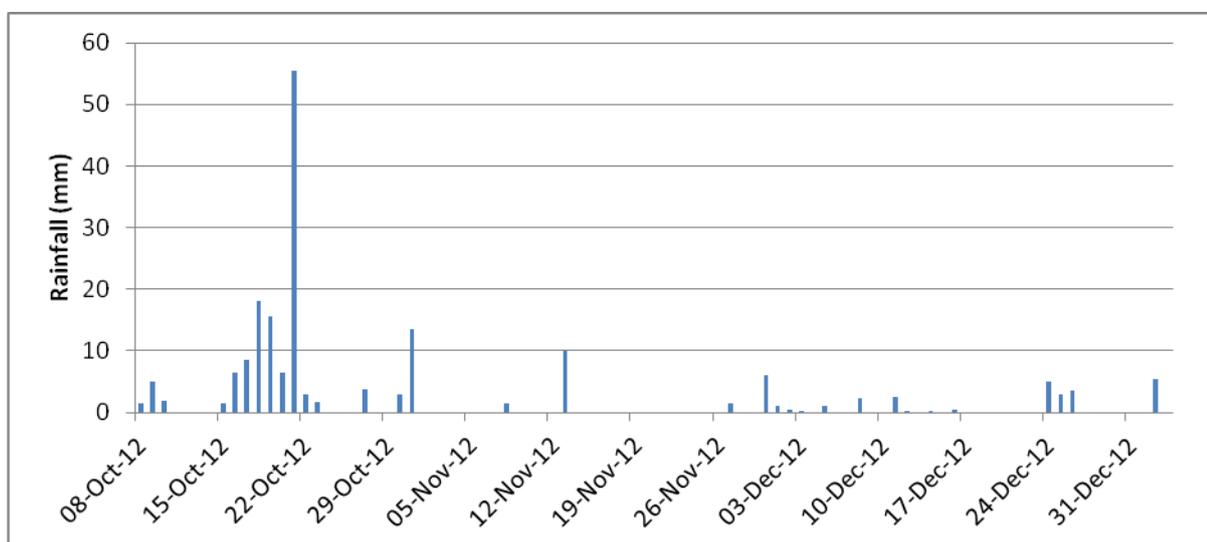


Fig. 3.5.4.1. Daily rainfall recorded at Patensie (Gamtoos River Valley) before and during the trial (8 October 2012 to 3 January 2013; application 25 October 2012; final evaluation 3 January 2013)

An observation made at the first assessment, one week after application, was the extent of variation in levels of snail infestation on trees within a treatment. This emphasised the importance of using a good number of replicates per treatment.

Another important observation was the effect of the Nordox sprays on the snails. The copper sprays resulted in very little mortality – slightly higher with the higher dosage. However, Nordox caused a conspicuous repellent response. Very few snails were recorded on the flush and young branches of the tree, whereas the vast majority of snails congregated in the centre of the tree, most probably where the copper residue was less. It is possible that the volume of Nordox applied at Gonnakop was a bit low.

Bait treatments were more effective against brown snails than white dune snails. Mortality of brown snails exceeded 60%, 70% and even 80% with certain treatments on occasion (Table 3.5.4.1 & Fig 3.5.4.2). Whereas, mortality of white dune snails did not reach even 50% with any of the treatments at any stage (Table 3.5.4.2 & Fig 3.5.4.3). At no time was there a statistically significant difference between the efficacy of the three Villa bait dosages against brown snail (Table 3.5.4.1). Efficacy of Sluggem also remained similar, until 6 weeks after application, at which time mortality in the Sluggem was significantly lower than that in the three Villa bait treatments. Initial efficacy against white dune snails was statistically superior with the highest dosage of Villa bait (1 and 3 weeks after application) (Table 3.5.4.2). However, this difference diminished over time. Efficacy of the lowest Villa bait dosage only significantly surpassed that of Sluggem at 10 weeks after application.

At no stage after application was there any significant difference between the efficacy of any of the treatments against the tower snail (Table 3.5.4.3). Although all treatments appeared to have some effect relative to the untreated control (Fig 3.5.4.4), only on a few occasions for a few treatments was there any significant difference to the control in numbers of live snails per tree (Table 3.5.4.3).

Table 3.5.4.1. Mortality (corrected for control mortality) of brown snails for various treatments at Gonnakop Farm in the Gamtoos River Valley (application 25 October 2012; final evaluation 3 January 2013).

Treatment	% Mortality ¹						
	1 WAT ²	2 WAT	3 WAT	4 WAT	6 WAT	8 WAT	10 WAT
Sluggem 50 g/10m ²	35.70b±4.25	40.20a±4.59	40.10a±6.11	68.50a±5.72	37.30b±8.01	42.00ab±12.94	35.80a±10.87
Villa 50 g/10m ²	39.60ab±5.02	50.60a±2.28	27.90ab±7.17	63.10a±10.10	64.50a±11.71	68.00a±9.88	39.30a±10.12
Villa 70 g/10m ²	39.20ab±4.69	44.00a±3.99	32.00a±6.16	69.80a±3.83	84.20a±4.54	71.60a±10.04	47.80a±9.38
Villa 100 g/10m ²	51.10a±1.45	52.70a±2.80	30.00a±7.40	59.30a±9.33	76.20a±9.37	63.80a±11.78	37.00a±10.23
Nordox 175g/hL	6.30d±3.57	10.20c±5.68	2.70c±2.20	10.90b±5.34	3.20c±2.26	7.90c±4.76	8.60b±6.83
Nordox 200g/hL	19.00c±5.31	26.00b±6.87	10.90bc±5.49	17.20b±8.16	31.80b±12.54	23.80bc±12.86	6.80b±6.80

¹Values in the same week (column) followed by the same letter are not significantly different (P>0.05).

²WAT=weeks after treatment.

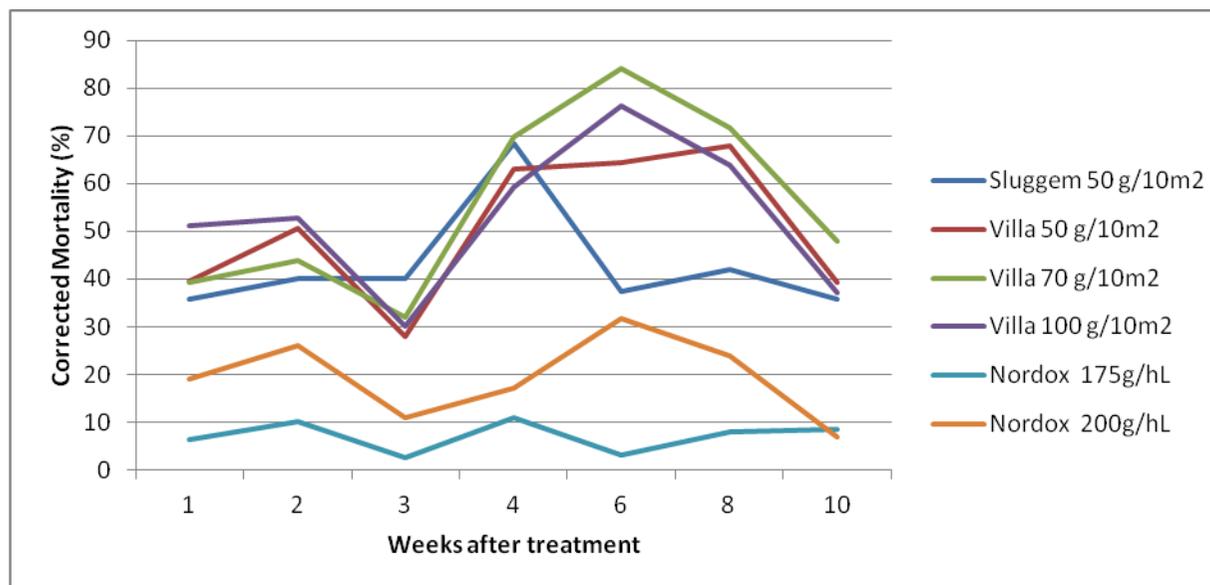


Fig. 3.5.4.2. Mortality (corrected for control mortality) of brown snails for various treatments at Gonnakop Farm in the Gamtoos River Valley (application 25 October 2012; final evaluation 3 January 2013).

Table 3.5.4.2. Mortality (corrected for control mortality) of white dune snails for various treatments at Gonnakop Farm in the Gamtoos River Valley (application 25 October 2012; final evaluation 3 January 2013).

Treatment	% Mortality ¹						
	1 WAT ²	2 WAT	3 WAT	4 WAT	6 WAT	8 WAT	10 WAT
Sluggem 50 g/10m ²	22.70b±3.54	32.60a±3.43	12.20b±3.77	19.40bc±3.86	28.70b±5.32	17.10bc±4.42	14.30cd±2.98
Villa 50 g/10m ²	22.60b±3.26	36.30a±3.45	10.60b±5.28	31.50ab±7.01	40.50ab±7.19	26.20ab±6.84	30.20a±5.68
Villa 70 g/10m ²	22.70b±4.36	34.20a±3.25	13.90b±3.15	30.60ab±5.35	38.20ab±4.98	35.70a±6.18	23.80bc±3.66
Villa 100 g/10m ²	38.90a±3.48	42.80a±2.24	30.30a±3.58	36.70a±5.49	48.10a±6.90	34.10a±6.28	24.20ab±5.00
Nordox 175g/hL	0.20d±0.20	6.40c±3.88	2.80b±2.80	0.80d±0.80	0.40c±0.30	3.60c±1.28	2.20d±0.85
Nordox 200g/hL	11.80c±3.85	17.10b±4.99	2.70b±1.98	7.90cd±4.74	1.80c±1.21	5.20c±3.13	10.50cd±5.33

¹Values in the same week (column) followed by the same letter are not significantly different (P>0.05).

²WAT=weeks after treatment.

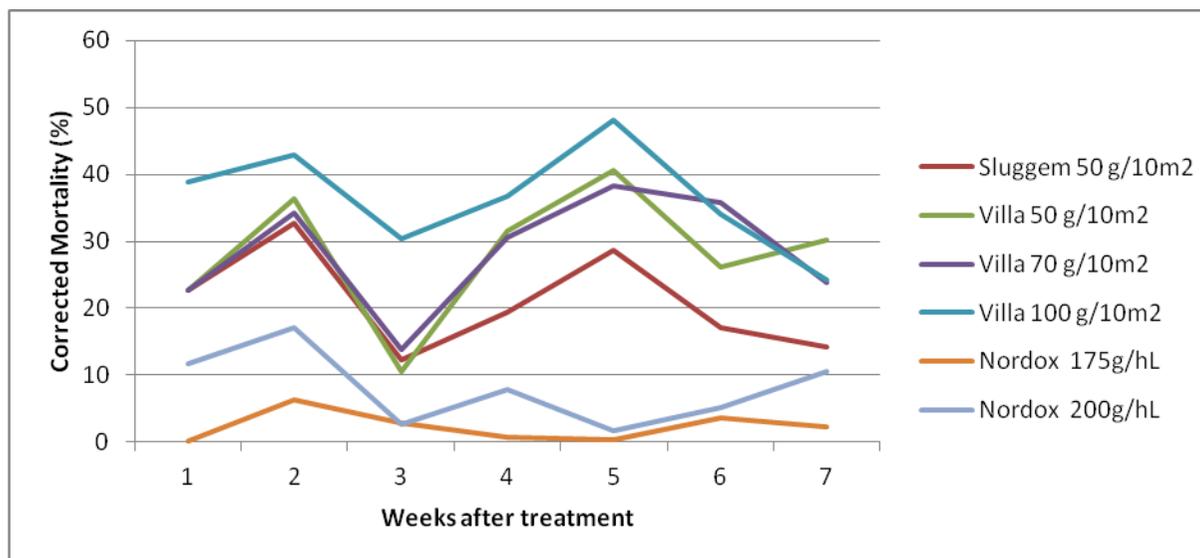


Fig. 3.5.4.3. Mortality (corrected for control mortality) of white dune snails for various treatments at Gonnakop Farm in the Gamtoos River Valley (application 25 October 2012; final evaluation 3 January 2013).

Table 3.5.4.3. Mean number of live tower snails per tree for various treatments at Gonnakop Farm in the Gamtoos River Valley (application 25 October 2012; final evaluation 3 January 2013).

Treatment	% Mortality						
	1 WAT	2 WAT	3 WAT	4 WAT	6 WAT	8 WAT	10 WAT
Untreated control	19.80a±3.51	57.20b±7.44	35.00b±6.32	33.00b±5.78	69.50a±12.92	65.00b±12.32	59.00a±10.05
Sluggem 50 g/10m ²	14.60a±3.28	23.30a±2.84	14.50a±4.31	20.50ab±5.19	38.00a±8.79	33.00ab±9.31	47.00a±10.01
Villa 50 g/10m ²	17.40a±3.33	44.50ab±12.78	22.00ab±6.25	23.00ab±6.11	43.00a±10.75	57.00ab±12.39	44.00a±8.84
Villa 70 g/10m ²	25.20a±7.97	43.10ab±10.43	16.50ab±4.09	17.50ab±4.03	54.50a±11.12	39.50ab±11.12	46.00a±9.68
Villa 100 g/10m ²	18.40a±3.30	24.70a±5.06	19.00ab±5.42	13.50a±4.09	36.00a±5.81	27.50a±6.29	41.00a±7.81
Nordox 175 g/hL	14.60a±2.20	31.10a±7.17	18.50ab±5.53	15.50a±4.37	55.50a±12.96	28.50a±9.49	39.00a±5.47
Nordox 200 g/hL	24.50a±4.54	36.40ab±3.87	21.00ab±5.21	18.00ab±4.10	35.00a±8.85	41.00ab±10.69	40.00a±8.16

¹Values in the same week (column) followed by the same letter are not significantly different (P>0.05).

²WAT=weeks after treatment

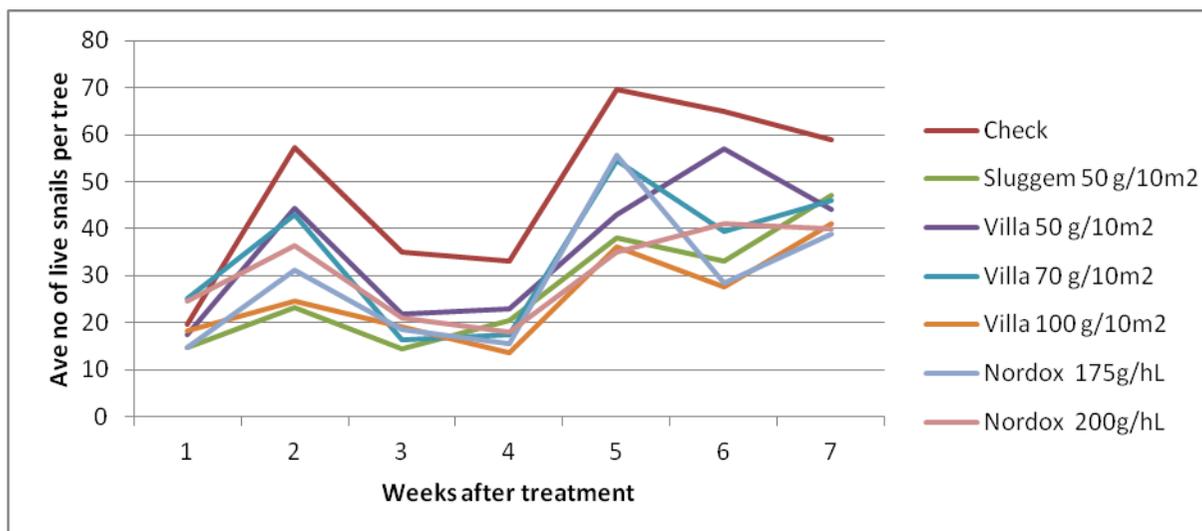


Fig. 3.5.4.4. Mean number of live tower snails per tree for various treatments at Gonnakop Farm in the Gamtoos River Valley (application 25 October 2012; final evaluation 3 January 2013).

Far Away trial – Sundays River Valley

As with the Gamtoos River Valley trial site, there were good rains leading up to the trial (120 mm during four weeks), resulting in the soil being moist at the time of application (1 November 2012) (Fig. 3.5.4.5). Rain only fell again 12 days after application. During the six week period of the trial only a meagre 8 mm of rain was recorded. However, as with the previous trial, the Villa bait pellets maintained their structure better than did the Sluggem pellets.

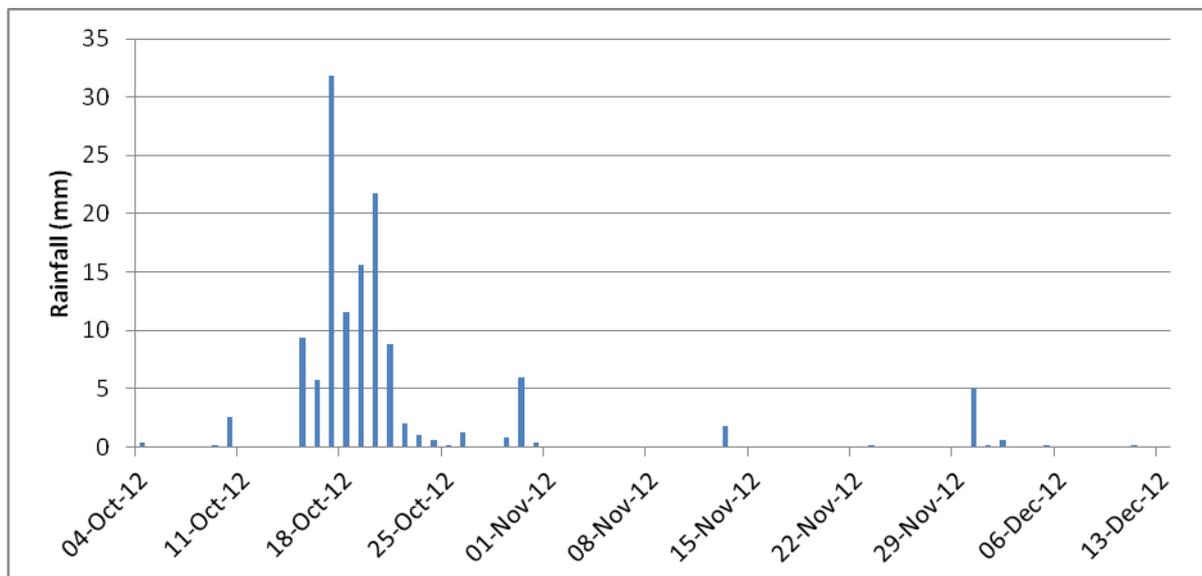


Fig. 3.5.4.5. Daily rainfall recorded at Addo (Sundays River Valley) before and during the trial (4 October to 13 December 2012; application 1 November; final evaluation 13 December 2013).

Overall efficacy at this trial site appeared to be slightly less against the brown snail but slightly better against the white dune snail than at the Gamtoos River Valley site. Efficacy against the brown snail only exceeded 60% with one treatment on one occasion (Table 3.5.4.4), whereas efficacy against the white dune snail exceeded 50% efficacy on four occasions (Table 3.5.4.5). The other noticeable thing was that treatments at this site appeared to lose efficacy sooner than at the Gamtoos site. Assessments had to be terminated at 6 weeks after treatment, as the grower applied his own snail bait underneath all trial trees, despite being reminded not to do so. At 4 weeks after treatment, no dead brown snails were found, despite dead white dune snails being recorded. This was peculiar and could not be explained.

As at the first trial site, the Nordox treatments were relatively ineffective in reducing numbers of live snails (Tables 3.5.4.4 & 3.5.4.5 and Figs 3.5.4.6 & 3.5.4.7).

Although initial efficacy with a couple of the Villa bait treatments was superior against brown snails to that of Sluggem (Table 3.5.4.3), this difference was not maintained and the trial may not have continued for long enough to be able to note differences in duration of efficacy before notable breakdown. Similarly, no such difference was recorded between Sluggem and the Villa bait against white dune snails (Table 3.5.4.5).

Initially there was no difference in efficacy between the three rates of Villa bait against the brown snail (Table 3.5.4.4). However, at 2 weeks after application, efficacy with the two higher rates was superior to that with the lowest rate. There was no significant difference in efficacy between the three rates, against white dune snails (Table 3.5.4.5).

Table 3.5.4.4. Mortality (corrected for control mortality) of brown snails for various treatments at Far Away Farm in the Sundays River Valley (application 1 November; final evaluation 13 December 2013).

Treatment	% Mortality ¹				
	1 WAT ²	2 WAT	3 WAT	4 WAT ^{3,4}	6 WAT ⁵
Sluggem 50 g/10m ²	33.00b±6.67	64.00a±2.67	41.00a±10.69	0.00±0.00	0.00±0.00
Villa 50 g/10m ²	47.00a±1.53	44.00b±9.79	52.00a±7.57	0.00±0.00	0.00±0.00
Villa 70 g/10m ²	44.00ab±3.40	61.00a±5.47	57.00a±6.83	0.00±0.00	0.00±0.00
Villa 100 g/10m ²	46.00a±2.21	60.00a±3.65	42.00a±7.27	0.00±0.00	0.00±0.00
Nordox 175g/hL	10.00c±5.58	2.00c±2.00	16.00b±7.63	-	0.00±0.00

Nordox 200g/hL	5.00c±4.01	2.00c±2.00	12.00b±8.14	-	0.00±0.00
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¹Values in the same week (column) followed by the same letter are not significantly different (P>0.05).

²WAT=weeks after treatment

³No dead brown snails

⁴Nordox treatments not evaluated at 4 WAT as it was concluded that there was no meaningful efficacy

⁵Grower had cleared under trees, therefore no dead snails

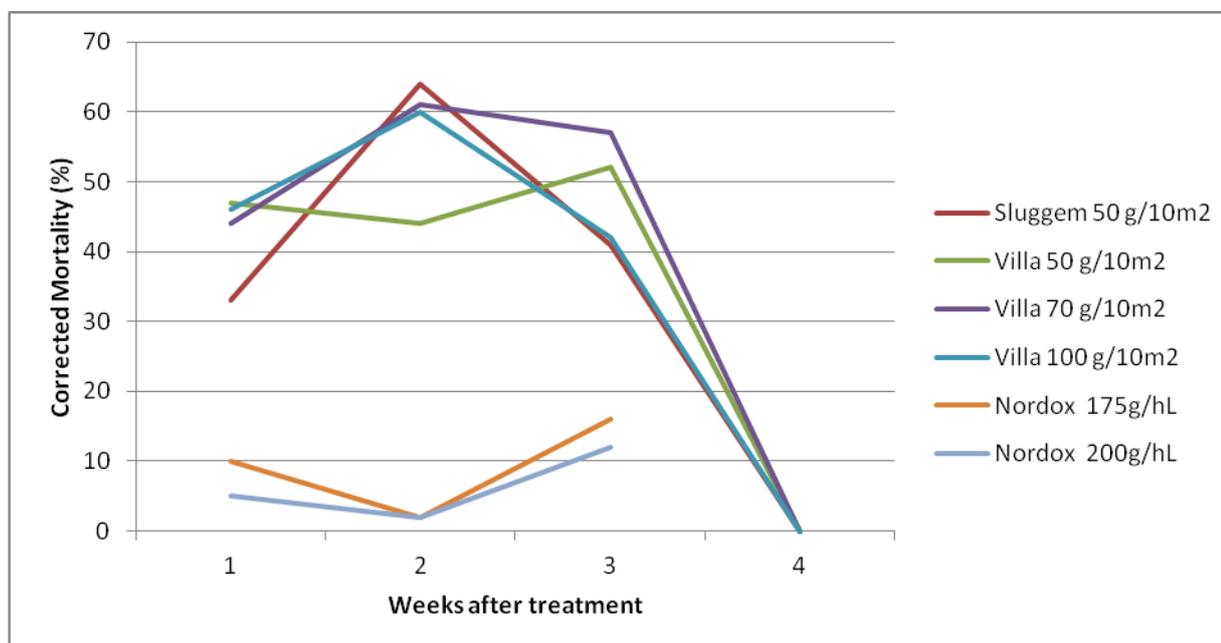


Fig. 3.5.4.6. Mortality (corrected for control mortality) of brown snails for various treatments at Far Away Farm in the Sundays River Valley (application 1 November; final evaluation 13 December 2013). (Results from 6 WAT have been excluded, as the grower removed dead snails before the evaluation was conducted. Nordox treatments were not evaluated at 4 WAT).

Table 3.5.4.5. Mortality (corrected for control mortality) of white dune snails for various treatments at Far Away Farm in the Sundays River Valley (application 1 November; final evaluation 13 December 2013).

Treatment	% Mortality ¹				
	1 WAT ²	2 WAT	3 WAT	4 WAT ³	6 WAT ³
Sluggem 50 g/10m ²	33.00ab±8.70ab	51.00a±12.42	27.00a±6.67	15.00ab±7.19	0.00±0.00
Villa 50 g/10m ²	46.00a±7.33	41.00ab±12.69	24.00a±6.86	19.00ab±7.67	0.00±0.00
Villa 70 g/10m ²	53.00a±6.51	45.00ab±11.76	35.00a±5.62	25.00a±7.92	0.00±0.00
Villa 100 g/10m ²	51.00a±7.37	67.00a±9.43	19.00ab±5.47	26.00a±6.86	0.00±0.00
Nordox 175 g/hL	17.00bc±8.31	15.00bc±8.97	6.00bc±4.99	-	0.00±0.00
Nordox 200 g/hL	9.00c±3.79	5.00c±4.01	0.00c±0.00	-	0.00±0.00

¹Values in the same week (column) followed by the same letter are not significantly different (P>0.05).

²WAT=weeks after treatment.

³Nordox treatments not evaluated at 4 WAT as it was concluded that there was no meaningful efficacy

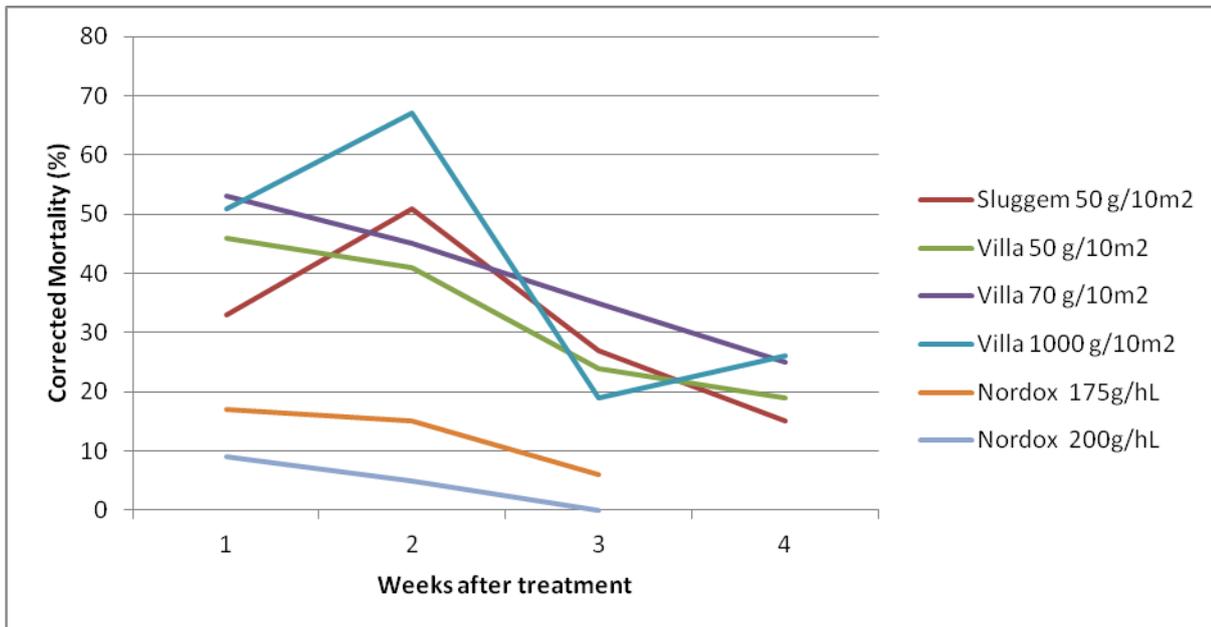


Fig. 3.5.4.7. Mortality (corrected for control mortality) of white dune snails for various treatments at Far Away Farm in the Sundays River Valley (application 1 November; final evaluation 13 December 2013). (Results from 6 WAT have been excluded, as the grower removed dead snails before the evaluation was conducted. Nordox treatments were not evaluated at 4 WAT).

At the Sundays River trial site the treatments appeared to have even less effect against the tower snail (Fig. 3.5.4.8) than at the Gamtoos River trial site (Fig. 3.5.4.4). There were some statistical differences between treatments at 4 weeks after treatment. However, as there were no differences before and after this time, this probably did not mean much.

Table 3.5.4.6. Mean number of live tower snails per tree for various treatments at Far Away Farm in the Gamtoos River Valley (application 1 November; final evaluation 13 December 2013).

Treatment	% Mortality ¹				
	1 WAT ²	2 WAT	3 WAT	4 WAT ³	6 WAT
Untreated control	29.00a±10.32	28.50a±10.49	38.00a±8.92	41.50ab±8.50	65.50a±10.34
Sluggem 50 g/10m ²	34.00a±12.45	25.00a±10.33	19.00a±5.42	21.50a±6.37	37.00a±11.72
Villa 50 g/10m ²	26.90a±10.11	32.50a±10.36	39.00a±9.33	39.00ab±11.94	55.00a±13.17
Villa 70 g/10m ²	28.00a±12.16	16.50a±6.01	30.50a±12.39	24.50a±9.32	50.00a±12.18
Villa 100 g/10m ²	19.00a±6.86	29.00a±7.22	34.00a±9.88	56.00b±10.46	48.50a±10.28
Nordox 175 g/hL	33.00a±10.23	29.50a±9.44	25.00a±9.31	-	42.50a±11.60
Nordox 200 g/hL	28.50a±12.76	38.50a±14.22	20.50a±6.69	-	56.50a±10.90

¹Values in the same week (column) followed by the same letter are not significantly different (P>0.05).

²WAT=weeks after treatment.

³Nordox treatments not evaluated at 4 WAT as it was concluded that there was no meaningful efficacy

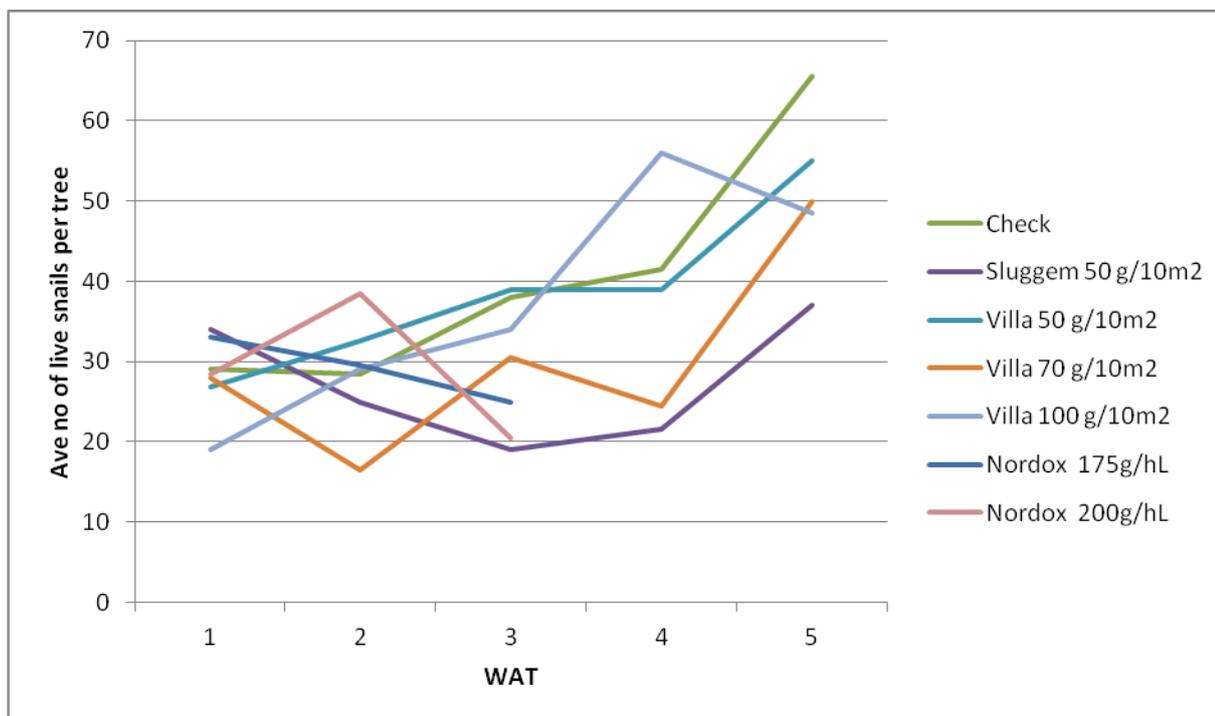


Fig. 3.5.4.8. Mean number of live tower snails per tree for various treatments at Far Away Farm in the Gamtoos River Valley (application 1 November; final evaluation 13 December 2013). (Nordox treatments were not evaluated at 4 WAT).

Conclusion

The Villa bait was at least as effective as the standard bait – Sluggem – and there were indications that its effectiveness may be superior and particularly that it endured at a higher level for longer than Sluggem. Although the highest rate of the Villa bait (100 g/m²) was initially superior in a couple of cases, this was not sustained and there is no apparent reason to register this bait at a rate higher than 50 g/m². What was clear is that none of the treatments came close to eliminating the snail problem. This is not unique to the treatments used in this study, but is a general problem with all registered modes of snail control. Unfortunately, all treatments appeared ineffective in controlling tower snails. This is unfortunate, as a number of growers, particularly in the Gamtoos River Valley, have reported unacceptable late season damage on Nadorcott Mandarin fruit, caused by tower snails. Nordox was essentially ineffective in reducing numbers of all snail species in trees.

Future research

No further research on this project is planned.

Technology transfer

As these results belong to Villa, they will make decisions on dissemination of the information.

References cited

Abbott, W.S., 1925: Method of computing the effectiveness of insecticides. *J. Econ. Entomol.* 18: 265-267.

3.6 PROGRAMME: NON-PHYTOSANITARY KEY PESTS

Programme Coordinator: Tim G. Grout (CRI)

3.6.1 Programme summary

The 2011/2 season was unusual in that no research was conducted on citrus thrips due to most research being focussed on market access pests and the need to delay some planned research on thripicides until new isolates of entomopathogenic fungi are available. However, we were able to conclude 7 years of research on the development of an original ant bait that is effective against the two most important ants in citrus orchards and is now ready to be commercialised (3.6.2). The other area of research in this

programme that is still in progress is to determine whether sublethal levels of imidacloprid in fruit are stimulating citrus moth pests to produce more offspring than normal. This is particularly important for false codling moth because we are trying to control this pest as effectively as possible, but it may also explain recent increases in the abundance of other lepidopteran pests that were previously considered of minor importance, such as the lemon borer moth (3.6.3).

Programopsomming

Die 2011/2 seisoen was ongewoon omdat geen navorsing op sitrus blaaspootjies gedoen is nie omdat die meeste navorsing op marktoegangsplae gefokus het en van die beplande navorsing op blaaspootjiedoders uitgestel moes word totdat nuwe isolate van entomopatogeniese swamme beskikbaar is. Ons was egter in staat om 7 jaar se navorsing te voltooi oor die ontwikkeling van 'n nuwe mierlokmiddel wat doeltreffend teen die twee mees belangrike miere in sitrusboorde is en nou gereed vir kommersialisering is (3.6.2). Die ander area van navorsing in hierdie program waarmee daar nog voortgegaan word is om te bepaal of subletale vlakke van imidacloprid op vrugte, sitrusmot-plae stimuleer om meer nageslag as normaalweg te produseer. Dit is veral belangrik vir valskodlingmot omdat ons poog om hierdie plaag so doeltreffend as moontlik te beheer, maar dit mag ook onlangse toenames in die voorkoms van ander lepidoptera plae verduidelik wat voorheen as van mindere belang beskou is, soos die suurlemoen boordermot (3.6.3).

3.6.2 FINAL REPORT: Development of ant baits

Project 857 (2006/7-2012/3) by Tim G. Grout and Kim C. Stoltz (CRI)

Summary

Earlier research resulted in the development of an attractant that both the pugnacious ant *Anoplolepis custodiens* and the brown house ant *Pheidole megacephala* would consistently feed on and it was named Saga. Research then focused on the development of a suitable toxicant to be combined with Saga that would be effective against both ant species. Toxicants that were evaluated included imidacloprid, fipronil, pyriproxyfen and hydramethylnon. Fipronil was extremely effective against the pugnacious ant but not against the brown house ant. Imidacloprid was repellent at high dosages and ineffective at low dosages. Pyriproxyfen was slow-acting but often effective at 0.25%, but we decided not to use this because of the widespread use of this active ingredient in citrus and possible detrimental effects on predatory beetles on the trunk. Hydramethylnon at 0.9% was found to be effective against both ant species and successful results were obtained in 7 out of 7 large-scale trials on brown house ant and in 6 out of 6 large-scale trials against the pugnacious ant, in three different provinces. In these trials the product was used on every second tree at a rate of approximately 3 kg/ha. No chemicals are registered against the pugnacious ant but this bait treatment was found to be more effective than a trunk spray of chlorpyrifos as registered against some ants. Saga plus hydramethylnon was as effective as the registered treatment Siege for brown house ant when only applying Saga to every second tree. It was found that Saga plus hydramethylnon had no effect on *Camponotus* sp., an ant that is sometimes found in citrus orchards in Limpopo. The addition of a preservative did not reduce the efficacy of the ant bait and no phytotoxic effects have been found on the bark. This research has now terminated and the bait will now be commercialised.

Opsomming

Vroeëre navorsing het tot die ontwikkeling van 'n lokmiddel vir beide die malmier, *Anoplolepis custodiens*, en die bruin huismier, *Pheidole megacephala*, gelei, waarop hul konstant kan voed, en dit is Saga genoem. Navorsing het toe op die ontwikkeling van 'n geskikte gifstof om met Saga te kombineer gefokus, wat teen beide mierspesies effektief sou wees. Gifstowwe wat geëvalueer is, sluit imidacloprid, fipronil, pyriproxyfen en hydramethylnon in. Fipronil was baie doeltreffend teen die malmier maar nie teen die bruin huismier nie. Imidacloprid was 'n afweermiddel teen hoë dosisse en oneffektief teen lae dosisse. Pyriproxyfen het 'n stadige maar dikwels effektiewe aksie teen 0.25% gehad, maar ons het besluit om dit nie te gebruik nie weens die wye gebruik van hierdie aktiewe bestanddeel in sitrus, en die moontlik nadelige uitwerking op predatoriese besies op die stam. Hydramethylnon teen 0.9% was teen beide mierspesies effektief, en suksesvolle resultate is in 7 uit 7 grootskaalse proewe op die bruin huismier en in 6 uit 6 grootskaalse proewe teen die malmier, in drie verskillende provinsies, verkry. In hierdie proewe is die produk op elke tweede boom teen 'n dosis van ongeveer 3 kg/ha gebruik. Geen chemikalieë is teen die malmier geregistreer nie, maar hierdie lokmiddelbehandeling blyk meer effektief as 'n stambespuiting van chlorpyrifos, wat teen sommige miere geregistreer is, te wees. Saga plus hydramethylnon was so effektief as die geregistreerde behandeling Siege vir bruin huismier, wanneer Saga slegs aan elke tweede boom toegedien is. Daar is gevind dat Saga plus hydramethylnon geen effek op *Camponotus* sp., 'n mier wat somtyds in sitrusboorde in Limpopo gevind word, gehad het nie. Die byvoeging van 'n preserveermiddel het nie die

effektiwiteit van die mierlokmiddel verminder nie en geen fitotoksiese effek is op die bas gevind nie. Hierdie navorsing is nou gestaak en die lokmiddel gaan gekommersialiseer word.

Introduction

Although some new products have been registered for the control of certain citrus pests, corrective options for red scale, soft scales and mealybugs are largely dependent on old chemistry that may not be available in the near future. It therefore remains essential to maximise biocontrol of these pests during summer and for this, ants must be kept out of the trees. Other than ant bands (Samways and Buitendag 1986), which some people consider too labour intensive, there is no registered chemical treatment for the pugnacious ant (PA) *Anoplolepis custodiens* Smith which is common in northern citrus orchards. Previously an attractant called Saga was developed by the authors that attracted both PA and the brown house ant (BHA, *Pheidole megacephala* (F.)) (Grout et al. 2008). Some preliminary work was conducted with BHA using Regent (fipronil 200 g/l SC) as a possible toxicant with Saga at 0.03% Regent by weight. This was equivalent to 0.006% a.i. compared with 1×10^{-5} % a.i. used by Hooper-Bui and Rust (2000) for *Linepithema humile*, 0.0001% a.i. used by Vega and Rust (2003) for the same ant, 0.0015% a.i. used by Collins and Callcott (1998) for *Solenopsis invicta* and the 0.05% used by Ulloa-Chacón and Jaramillo (2003) for *Tapinoma melanocephalum*. However, the results after 6 and 7 days showed no significant impact (Grout et al. 2008). Between 2008 and 2013 further research was conducted to find a toxicant that would be effective in Saga against both these dominant ant species when using the same dosage. Once a suitable toxicant was found, large scale trials were conducted in different parts of the country and finally some different preservatives were screened for acceptability by both ants. This research is all covered in this final report.

Stated objective

Develop an ant bait that will be effective in controlling both the brown house ant and the pugnacious ant.

Materials and methods

2008-9

In order to establish the level of BHA activity, peanut butter (Yum-Yum brand) was placed in small petri dishes (39 mm diam.) close to BHA nests and numbers feeding after 30 min exposure determined before selecting nests for a trial. Nests were always separated by at least 10 m. Bait mixtures were then placed in similar petri dishes for 24 or 48 h with ant feeding being determined at the bait after this period. Post-treatment evaluations were again conducted using peanut butter as an attractant in case ants that had received a sub-lethal dosage of toxicant were repelled by the bait.

The technique used for PA differed in that the petri dishes were filled with fish paste (Redro brand) and were each placed on top of a vertical dowel rod (46 cm long), within 50 cm of a nest opening to prevent hyper-active ants from running into the food and disturbing other ants. This meant that it took longer (numbers feeding determined 2 h after setting up fish paste) for the ants to find the attractant but that all ants that were visiting the bait were doing so for feeding purposes and not by chance. With PAs, fish paste was used before and after presenting the bait mixture to the ants because these ants are not attracted to peanut butter.

The first small BHA trial was started on 22 April 2008 at 12 nests on the CRI – Nelspruit grounds. Dosages of Regent (fipronil) 200 SC of 0.05, 0.1, 0.25, 0.5 and 1.0% by mass (0.01, 0.02, 0.05, 0.1 and 0.2% a.i., respectively) were used with two replicates each plus two controls that received Saga without Regent. Treatments were evaluated by counting ants visiting the bait per minute using Saga after 2 and 24 h to determine whether any of the higher dosages were repelling the ants. Maximum temperatures were in the low 20s°C. On 23 April a trial with PA at the Lowveld Agricultural College was initiated using the same range of Regent dosages. After determining numbers of ants feeding on fish paste per minute, the Saga bait was placed and evaluated after 2.5 h and 24 h with two controls.

Another trial was started at CRI – Nelspruit against BHA on 12 May 2008 using Regent at 0.05 and 0.1% by mass (0.01 and 0.02% a.i.) and a control comprising Saga alone. Four replicates of each treatment were used. Evaluations of activity towards Saga per minute were conducted after 24 and 48 h and towards peanut butter after 7 and 14 d. A similar trial was conducted with PA at the Lowveld Agricultural College starting on 13 May. However, although there was a lot of ant activity on the ground on this day, none of the ants visited the bait stations during the set up evaluation using fish paste. The baits were still placed but evaluations on Saga after 24 and 48 h still showed virtually no activity so the trial was discontinued. It was concluded that the night temperatures had become too cold for this species and it was no longer foraging. This was the last trial with PA until summer.

On 26 May a further BHA trial was initiated at CRI in Nelspruit with the same design as the previous trial but with Regent at 0.25% and 0.5% by mass (0.05 and 0.1% a.i.). Activity was evaluated 30 min after setting up using peanut butter. Counts were made of the number of ants feeding per bait station per minute after 24 and 48 h on Saga bait, then after 7 and 14 d on peanut butter.

In July, two trials were conducted at CRI with Confidor 700 WG as a toxicant in Saga against BHA. In the first trial the same technique was used as before with the use of peanut butter to evaluate nest activity, presentation of the baits for 48 h with evaluation of feeding activity after 24 and 48 h, then further evaluation of activity with peanut butter after 7 and 14 d. Confidor WG was used at 1.4 g/kg (0.14% formulated or 0.098% a.i.) in 4 replicates and at 0.14 g/kg (0.014% formulated or 0.0098% a.i.) in another 4 replicates. There were also 4 control replicates that received Saga alone. The trial was started on 1 July and the last evaluation conducted 14 d later.

The second trial with Confidor against BHA was started on 23 July but fewer active nests were available by this time so only a single rate of 3 g/kg (0.3% formulated or 0.06% a.i.) was compared with a control using 3 replicates each. The last evaluation was conducted 5 d later.

Activity at most BHA nests at CRI then declined and no further work was conducted there during 2008. However, due to the decline of the nests an investigation of all the treatments used at each nest was made to see whether the decline could be due to certain low dosages of Regent that had not appeared toxic at the time but may have had an effect over several weeks.

During summer 2008-9, Confidor was evaluated against PA at two different locations (Litchi orchard and storm drain) at the Lowveld College of Agriculture near Nelspruit. Two dosages of Confidor WG in Saga were used and Saga alone as a control. The dosages were 0.071 g Confidor WG/kg Saga (0.005% a.i.) and 0.71 g Confidor WG/kg Saga (0.05% a.i.). Five replicates were used per dosage and control, using dowel rods to raise the bait stations above the ground. An upward facing petri dish was placed on top of the rod as before with a hole or two to let water out. A roof over this dish formed by a larger petri dish was used to prevent rain from getting in the bait. A space of at least 1 cm between the bottom petri dish and the roof was left so that the small dish containing bait could be slipped in and removed easily and the ants had ready access.

Nests were identified and numbered that were at least 10 m apart. A rod was pushed into the ground approximately 50 cm from the nest opening. When the ants had calmed down, a small petri dish of known weight containing fish paste (6 g) was placed in the feeding chamber. After 1 h the number of ants feeding on the fish paste in 1 minute was determined. 22 h later, each bait dish was removed and weighed. The nests were ranked according to feeding and weight removal, then treatments assigned so that the average activities or bait removals were similar for each treatment. The weighed baits were then placed on the appropriate rods. A feeding count was conducted after 22 h when the baits were also weighed. The feeding chamber was then left empty and after 6 days a weighed fish paste bait dish was inserted and the number of ants feeding per minute after 1 h determined. The fish paste was left for 22 h, then removed and weighed. This was repeated 14, 21 and 28 d after placing the Saga bait mixtures. The days when the Saga bait mixtures were placed at these sites were 19 January 2009 in the litchi orchard and 27 January 2009 in the storm drain.

Two additional sites were monitored for four weeks in April but PA activity was not suitable for further trials due to low soil temperature.

2009-10

The previous work indicated that fipronil 0.001% a.i. and imidacloprid 0.005% a.i. should now be used as toxicants in larger efficacy trials. The former dosage was close to the 0.0015% a.i. fipronil used by Collins and Callcott (1998) for *Solenopsis invicta* control while the imidacloprid dosage was considered a minimum for delayed toxicity in the Argentine ant, *Linepithema humile*, by Rust et al. (2004).

Research was delayed by frequent rainfall in spring but four citrus orchards were finally chosen for the large scale trials at Golden Frontiers Citrus, Hectorspruit. Two of these orchards comprised young trees with high populations of PA and two with mature trees had high infestation levels of BHA.

The treatments evaluated were as follows:

Untreated control

Siege granular bait with hydramethylnon 0.73% a.i. at 10 g/tree in an ant bait station (Grout 2008)

Dursban WG 2.5 kg/hl spray of trunk and soil at base of tree

Saga with fipronil 0.001% at 10 g/tree in an ant bait station

Saga with imidacloprid 0.005% at 10 g/tree in an ant bait station

Trials against PAs were initiated at both orchards on 2 December 2009. Trials against BHA were initiated at both orchards on 11 Jan 2010. Bait station tubes (Grout 2008) were placed at the base of every tree. Treated blocks of trees were separated by at least 15 m borders or 3 untreated rows. The blocks themselves were at least 6 rows wide and 14 trees long. The centre 8 trees in each of the centre 4 rows were used for data. A small plastic lid (40 mm diameter and 3 mm deep) containing peanut butter (Yum-Yum brand) for BHA and fish paste (Redro brand) for PA was used to determine pre-treatment ant feeding activity per minute on the ground between trees at two points per data row (8 counts per block). The dishes were placed close to the tree trunks on the ground, 30 to 60 min before the count was made to allow ants to find the attractant. Post-treatment evaluations were conducted at the same points and the same time of day once a month for 4 months after the treatments were applied in the PA trials and for 3 months in the BHA trials.

When the PA trials were initiated in December 2009 the ants were very active and evenly distributed throughout the orchards. Actual numbers of ants feeding per minute could therefore be compared when evaluating treatments. In the BHA trial sites, there was much more variation in ant activity between blocks. We therefore averaged the two counts per data-row and expressed the post-treatment ant foraging scores as a proportion of the pre-treatment scores. Even so, non-parametric statistics had to be used to compare treatments because transformations did not normalize the data sufficiently.

2010-1

On 30 November and 1 December 2010 two BHA trials were set up at Golden Frontiers Citrus, Hectorspruit in two different Valencia orchards (11G and 11E). Treatment blocks were 6 rows wide and 14 trees long with the centre 8 trees in each of the centre 4 rows being used for data collection. Once again, small petri dishes containing peanut butter were used to determine pre-treatment activity levels. Two of these dishes were placed between the trees in each of the 4 rows of data trees, giving 8 feeding stations per treatment. The dishes were left for at least 30 min before counts of feeding ants were made. Ant activity was very variable on 30 November so the exercise was repeated on 1 December and an average level of activity determined from the two counts. Due to low ant activity and frequent rain, treatments were only applied on 13 December 2010 when 10 g of each bait were placed in bait station tubes at the base of each tree trunk. The following treatments were compared:

Untreated control

Siege granular bait containing hydramethylnon 0.73% a.i.

Saga plus hydramethylnon 0.9% a.i.

Saga plus pyriproxyfen 0.25% a.i.

The treatments were evaluated using petri dishes with peanut butter in the same way that the pretreatment ant activity was determined. Evaluations were conducted on 13 January, 14 February, 17 March, 12 April and 9 May 2011 and counts were transformed to square root ($x+0.5$) before analysis of variance.

Another two similar trials were conducted with PA at Riverside Farms, Malelane using the same layout in 2 different orchards of Star Ruby grapefruit trees where there were approximately 400 trees per hectare. Pretreatment levels of ant foraging activity were determined on 24 and 25 January 2011 and a mean of the two counts per replicate used. Eight petri dishes containing fish paste were placed on the ground in the centre 4 rows of each block and the numbers of PA feeding after at least 1 h determined. The treatments were presented to the ants in tubular bait stations at the base of each tree trunk on 31 January 2011 with the exception of the fipronil 2 kg/ha dose that was only applied to every second tree. The following treatments were compared.

Untreated control

Saga + fipronil 0.001% a.i. at 10 g per tree (4 kg/ha)

Saga + fipronil 0.001% a.i. at 10 g every second tree (2 kg/ha)

Saga + pyriproxyfen 0.25% a.i. at 10 g per tree

Treatments were evaluated using petri dishes with fish paste in the same way that the pre-treatment ant activity was determined. Evaluations were conducted on 28 February, 14 April and 11 May 2011. After this

the ant activity in all treatments declined due to low soil temperatures. Counts were transformed to square root ($x+0.5$) before one way analysis of variance.

2011-2

We found that PA was still active at Letaba Estates near Letsitele in winter due to the warmer soil temperatures so some trials were conducted there in June 2011. Initially we were planning to evaluate Saga plus pyriproxyfen at 0.5% but this dosage of toxicant repelled PA and no ants were found feeding when a few petri dishes were placed near active ant nests. Due to the common use of pyriproxyfen in citrus orchards for red scale we thought it would be better to evaluate hydramethylnon as a toxicant with Saga against PA. In a Midnight Valencia orchard at Letaba Estates with high numbers of trees infested with PA we laid out 3 blocks each comprising 140 trees (14 rows x 10 trees with 3 m spaces between trees in the row and 6 m between the rows) with the central block untreated. Eight active nest openings in the central part of each block, separated by approximately 10 m, were each marked with a plastic peg and a small dish of fish paste placed within 1 m of the peg at 12h00 on 21 June 2011. Numbers of ants feeding at these dishes 2 h later were recorded as pre-treatment activity assessments. Treatments were applied in the crotch of every second tree, above the microsprinklers and away from other non-target organisms on the orchard floor. A scoop that delivered 12 g bait was used to apply this amount of bait per treated tree later the same afternoon. The 2 treatments that were compared were Saga plus fipronil 0.001% a.i. and Saga plus hydramethylnon 0.9%. The first evaluation was conducted 1 month later on 18 July 2011. Small dishes of fish paste were placed near the 8 plastic pegs in each block at 12h00 and left until 14h00 before the numbers of PA feeding at each dish were determined. A similar evaluation was conducted 2 months after treatment on 17 August 2011 and another after 3 months on 20 September 2011. Counts were transformed to square root ($x+0.5$) before one way analysis of variance.

Prior to August 2011, Saga was made with constituents that would not be cost effective for mass production. We therefore changed the protein source to one that was commercially available in large quantities and conducted a small feeding comparison with PA to see whether the new mixture was as attractive as the old. The comparison between Saga 5 and Saga Bulk was made at Letaba Estates, Letsitele on 17 August 2011. Two small dishes with approximately 5 g of each attractant (without toxicant) were placed approximately 20 cm from 10 active PA nest openings at 13h30 on a cloudy day with a temperature of 21°C. After 75 min the numbers of ants feeding on each bait at each nest opening were determined and Student's t-test used to determine whether there were any differences between the mixtures.

The design used in the first large scale trial where Saga was used with hydramethylnon as described above was used in further trials in different parts of the country for registration purposes. Ant foraging activity, as indicated by feeding on fish paste (PA) or peanut butter (BHA), was used as the more reliable method of comparing efficacy but the presence of ants on the tree trunk or main framework of the tree was also used as an indication of ant infestation. However, the latter approach was dependent on infestations of honeydew-producing insects which can change with time so was less reliable than the foraging on the ground. In all large-scale trials where bait was applied to hundreds of trees, observations were made to see whether there were any signs of bark cracking or phytotoxicity.

Large scale orchard trials in Limpopo

A trial was conducted with PA in a Valencia orchard (C73) at Letaba Estates, Letsitele, Limpopo Province where Saga with hydramethylnon 0.9% was compared with a very concentrated spray of chlorpyrifos on the tree trunk, and an untreated control. Although there were a lot of PA in the road next to the orchard the numbers in the orchard were low. However, the orchard had recently received a lime application on the soil so we thought this may have temporarily reduced the numbers of PA and we proceeded with setting up the trial. Each treatment block comprised 368 trees (8 rows by 46 trees) and was separated from adjacent treatments by 3 untreated rows. Five feeding stations were used per treatment comprising plastic dishes (40 mm diameter and 3 mm deep) completely filled with Redro fish paste. These were placed by approximately every 5th tree in the central 2 rows of each treatment, starting 5 trees in from the border. A pre-treatment evaluation of foraging activity was attempted on 24 October 2011 but ant numbers were very low so the dishes were left overnight and recharged the next morning when another count was made. A treatment of Saga plus hydramethylnon 0.9% (10 g) was applied late on 24 October to every second tree in the block in the crotch of the tree using a plastic spoon. Trunk sprays of chlorpyrifos (Dursban 480 EC at 30 ml/L) were applied by use of a knapsack sprayer to the bottom 40 cm of the trunk on 24 October. This is equivalent to 2 kg/hl of the 750 WG formulation that is registered for BHA and was chosen as an alternative treatment because nothing is registered for the PA and this was at least registered for some other ant species. Evaluations of ant activity using feeding stations as above and by inspecting the framework of 20 trees for a

period of 10 s and recording the trees as infested or not, were conducted on 29 November 2011, 4 January and 25 January 2012. Counts were transformed to square root ($x+0.5$) before one way analysis of variance.

A second trial was conducted at Letaba Estates in Midnight Valencia orchard K17 at the same time as the above trial. This orchard had high numbers of PA in the orchard so a pre-treatment evaluation of foraging activity on fish paste was conducted on 24 October 2011 using 5 feeding stations per treatment. A chlorpyrifos trunk spray and a Saga plus hydramethylnon bait application to the trunk of every second tree as described above were applied on 24 October. Evaluations using 5 feeding stations per treatment and inspecting 20 trees per treatment were conducted on 29 November 2011, 4 January and 25 January 2012. Counts were transformed to square root ($x+0.5$) before one way analysis of variance.

Large scale orchard trials in Mpumalanga

A trial was conducted on PA in a Delta Valencia orchard at Riverside Farms, Malelane, Mpumalanga where 140 trees were used per treatment block (7 rows by 20 trees). The same technique as described above was used with rows 3 and 5 being inspected for ant infestation and 5 feeding stations being placed on the ground along row 4 to determine foraging activity on the ground. The treatments included an untreated control, Saga plus hydramethylnon 0.9% at 10 g in the crotch of every second tree, and a trunk spray of chlorpyrifos 480 EC at 30 ml/L on every tree. The pre-treatment evaluations were conducted on 5 December 2011 and the treatments applied shortly afterwards. Post-treatment evaluations were conducted on 5 January, 6 February and 5 March 2012. Counts were transformed to square root ($x+0.5$) before one way analysis of variance.

Two trials were conducted with BHA in Valencia orchards (denoted A and B) at Golden Frontiers Citrus, Hectorspruit. Treatment block sizes were 175 trees (7 rows by 25 trees) and Siege (containing hydramethylnon 0.73%) was used as a standard to compare with Saga plus hydramethylnon 0.9%. Ant activity was determined on the ground by using 5 feeding stations (plastic lids 40 mm in diameter and 3 mm deep) filled with peanut butter evenly spaced along the central row in the block. All 25 trees in each of the adjacent rows were inspected for ant activity and recorded as infested or not. Siege was applied at 10 g per tree by scattering the granules around the base of the tree, whereas Saga was applied at 10 g to the crotch of every second tree. The pre-treatment evaluations were conducted on 15 December 2011 and the treatments applied shortly afterwards. Post-treatment evaluations in both orchards were conducted on 24 January, 21 February and 15 March 2012. Counts were transformed to square root ($x+0.5$) before one way analysis of variance.

Another trial with PA was established at Ryton Estates near Ngodwana on 8 May 2012 using the same treatments as described above for Riverside Farms but by the time of the first evaluation in June the weather had become too cold and the ants were no longer foraging so this trial was abandoned.

Large scale orchard trials in the Eastern Cape

Four trials were conducted in the Sundays River Valley on different farms between Addo and Kirkwood. Two trials were conducted on PA and 2 on BHA.

One trial on PA was conducted on Huguenot Farm on the eastern end of the valley near Addo. The orchard comprised old navel orange trees with a lot of ground cover on the orchard floor. PA was very abundant in the road next to the orchard so all blocks were allocated along this road with 12 rows each and 8 trees per row. Between the time of choosing the site and evaluating pre-treatment levels of ants the farmer sprayed cypermethrin on the trees and all the ants disappeared. Pre-treatment evaluations were therefore not possible but the ant treatments were applied, even though the ant bait may have been exposed to the elements for a week before the ants recovered enough to start feeding on it. The treatments were applied on 7 December 2011 using Saga plus hydramethylnon 0.9% at 10 g in the crotch of every second tree in the row and a spray of chlorpyrifos 480 EC at 30 ml/L on every tree trunk. Post-treatment evaluations were conducted on 11 January, 8 February and 8 March 2012 using 5 feeding stations containing Redro fish paste as described above and inspecting the framework of 8 trees in each of the central 5 rows of the block for ant infestation. Numbers of ants foraging on the feeding stations after 1 h were transformed to square root ($x+0.5$) before one way analysis of variance.

Another trial with PA was conducted in a mature navel orange orchard (Orchard 4) at Halaron farm near Sunland in the Sundays River Valley. The layout was very similar to the above trial because once again there was a lot of ant activity in the dirt road alongside the orchard but very little in the orchard itself. Blocks of 12 rows by 10 trees per row were therefore arranged alongside the orchard border. A pre-treatment evaluation of foraging activity on the ground was conducted on 7 December 2011 using 5 feeding stations filled with

Redro fish paste in each block. Very few trees appeared to be infested at this time so no evaluation of tree infestation was conducted. Treatments were applied the following morning as for the trial at Huguenot Farm. Post-treatment evaluations were conducted on 11 January, 8 February and 8 March 2012 as described above and data analysis was also as above.

A trial with BHA was conducted in a navel orange orchard (Retief) near Hermitage in the Sundays River Valley using 240 trees per treatment (10 rows by 24 trees) where trees were 2 m apart in the row and the inter-row distance was 5 m. Ant activity was determined on the ground by using 5 feeding stations (plastic lids 40 mm in diameter and 3 mm deep) filled with peanut butter (Yum-Yum) evenly spaced along the central 2 rows (5 and 6) in the block. Twenty trees in each of the adjacent rows (4 and 7) were inspected for ant activity and recorded as infested or not. Siege was applied at 10 g per tree by scattering the granules around the base of the tree, whereas Saga was applied at 10 g to the crotch of every second tree. The pre-treatment evaluations were conducted on 8 December 2011 after the feeding stations had been left overnight and the treatments were applied shortly afterwards. Post-treatment evaluations in both orchards were conducted on 11 January, 8 February and 8 March 2012 with the feeding stations being on the ground for at least 2 h before evaluation. Counts were transformed to square root ($x+0.5$) before one way analysis of variance.

A second trial was conducted with BHA near Sunland in the Sundays River Valley in a mandarin orchard at Bethel Farm. Treatment block size was 192 trees (8 rows of 24 trees) with row 4 being used for placement of feeding stations and rows 3 and 5 being used for tree infestation assessment on 20 trees per row. Treatments and evaluations were identical to the trial near Hermitage. A pre-treatment evaluation was conducted on 8 December 2011 shortly before the treatments were applied and post-treatment evaluations were conducted on 11 January, 8 February and 8 March 2012. Data were analysed as above.

Inclusion of a preservative

Once it was clear that the Saga plus hydramethylnon gave effective control of both species of ants in 3 different provinces research focused on what preservative should be incorporated in the bait in order to improve shelf-life. It is known that methylparaben (Nipagin-M) at concentrations of 0.6% (Voss 2000) and even as low as 0.26% (Martin 2004) are detrimental to insect growth so we were concerned that a preservative may have repellent effects. Methylparaben was evaluated at concentrations of 0.1 and 0.2% and 2 preservatives for cosmetics from Savannah Fine Chemicals, Euxyl K320 (containing parabens) and Euxyl K510 were evaluated at recommended concentrations of 0.5% for K320, and 0.05 and 0.1% for K510.

Saga plus hydramethylnon 0.9% was blended with the various concentrations of preservatives on 21 November 2012 and subdivided into glass baby-food jars for future shelf life testing. The jars would be opened every 3 months to check for any signs of fungal growth or bacterial deterioration of the product. These mixtures were then also used in the following trials against both ant species.

Two trials against BHA were conducted in grapefruit orchards at Golden Frontiers Citrus, Hectorspruit, Mpumalanga. Treatment blocks comprised 168 trees (7 rows by 24 trees) and the 3 central rows were used for data collection. Pre-treatment evaluations were based on the use of small plastic lids filled with peanut butter and counting the numbers of ants feeding on them after 2 h. Five feeding stations were evenly spaced out along each of the data rows. The different mixtures of Saga plus hydramethylnon and preservative were applied to the crotch of every second tree at a rate of 10 g per tree. An untreated control was not used but a treatment without a preservative was included. The pre-treatment evaluations were conducted on 10 December 2012 and the treatments applied on 11 December. Evaluations were conducted after 9 days and after 1 month using the same technique as for the pre-treatment evaluations. Counts were transformed to square root ($x+0.5$) before one way analysis of variance.

Two small-scale trials were conducted with PA to ascertain whether any of the preservatives were repellent. These were conducted at Riverside Farms, Malelane where small dishes of Saga plus hydramethylnon plus preservative were placed close to active nest openings and after an hour the presence of feeding ants was recorded. The distribution of the feeding stations was completely randomised in a grapefruit orchard. The first trial was conducted on 30 January 2013 and the second trial on 5 February 2013. In the second trial the whole trial was duplicated in another part of the same orchard.

The results of the small-scale PA trials led to one final large scale trial where Saga with hydramethylnon but no preservative was compared with the same bait with K510 at 0.1% and both were compared to an untreated control. A young citrus orchard was used for this purpose so the baits were applied at the base of the trees as the crotch size could not support 10 g bait. Treatment blocks comprised 7 rows by 24 trees. This orchard had extremely high levels of PA infestation. Pre-treatment evaluation of ground foraging was

done by placing plastic lids with fish paste at 6 positions in each of the rows straddling the centre row in the block and counting the numbers of ants feeding after 2 h. In the centre row of each block, all 24 trees were rated for the presence or absence of ants. The treatments were applied to every second tree on 11 February 2013 but that night it rained so they may have been partially washed away. Evaluations were conducted on 25 February, 12 March and 22 April 2013. Counts were transformed to square root ($x+0.5$) before one way analysis of variance.

Results and discussion

2008-9

All results proved difficult to interpret due to various factors influencing ant activity. This has been a continual challenge with all ant work because worker activity is influenced by needs in the nest. This was why an attractant had to be developed that satisfied both carbohydrate and protein requirements. However, rainfall results in nest repairing activity that may last for a few days. During this time ants do not feed much. PA is also very sensitive to ground temperature. If it becomes too hot the ants stay in the nest. As the soil temperature cools in winter they also become less active until they stop foraging all together in May.

Imidacloprid and fipronil have been used by other researchers in ant baits and there are some commercial products available that use these toxicants. However, the concentrations of these toxicants in the commercial baits vary considerably depending on how much is consumed and the target ant species. Stringer et al. (1964) maintained that a suitable toxicant for an ant bait should cause less than 15% mortality after 24 h but more than 89% mortality after 20 d. However, by 7 d most suitable toxicants should be showing a significant degree of mortality. Ants could not be retrieved in these trials so actual mortality levels could not be determined. This meant that efficacy had to be determined by the numbers of ants foraging after exposure to the bait.

The first trial with BHA and Regent involved a range of dosages to see whether certain dosages would be repellent. This appeared to be the case with fewer ants feeding on the Saga bait mixes at the higher dosages after 24 h (Table 3.6.2.1). However, further trials with dosages in this range did not seem to have much effect in lowering ant numbers by 7 or 14 d after treatment (Table 3.6.2.1). Perhaps all these dosages were too high.

Table 3.6.2.1. Mean numbers of BHA feeding per minute after feeding on Saga bait with various dosages of Regent at CRI – Nelspruit

Treatments	Trial started 22 April	Trial started 12 May	Trial started 26 May
	Mean ants on Saga bait per min 24 h after treatment	Mean ants on peanut butter per min 7+14 d after treatment	Mean ants per min 7+14 d after treatment
Saga only	1150 b	1300 a	464 a
Saga + Regent 0.05%	25 a	785 a	-
Saga + Regent 0.10%	20 a	451 a	-
Saga + Regent 0.25%	14 a	-	650 a
Saga + Regent 0.50%	6 a	-	463 a
Saga + Regent 1.0%	4 a	-	-

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

Results of two trials with Regent against PA in April and May 2008 were inconclusive (Table 3.6.2.2). In the second trial, although the ants appeared active, they were not attracted to the bait as they were probably preparing for winter.

Table 3.6.2.2. Number of PAs feeding per minute after feeding on Saga bait with various dosages of Regent at Lowveld College of Agriculture

Treatments	Trial started 23 April	Trial started 13 May
	Mean ants per min 24 h after treatment	Mean ants per min 48 h after treatment
Saga only	43	1
Saga + Regent 0.05%	0	0
Saga + Regent 0.10%	6.5	0
Saga + Regent 0.25%	0.5	0

Saga + Regent 0.50%	0	0
Saga + Regent 1.0%	0	0

Table 3.6.2.3. Mean numbers of BHA feeding per minute after feeding on Saga bait with various dosages of Confidor WG for 48 h at CRI – Nelspruit

Treatments	Trial started 1 July	Trial started 23 July
	Mean ants per min 7+14 d after treatment	Mean ants per min 48 h after treatment
Saga only	174 a	107 a
Saga + Confidor WG 0.014%	241 a	-
Saga + Confidor WG 0.14%	494 a	-
Saga + Confidor WG 0.3%*	-	233 a

There were no significant differences between treatments ($P > 0.05$)

*This dosage was noticeably repellent at times.

Two trials with BHA and Confidor in Saga showed no apparent toxic effect of a range of dosages from 0.014 to 0.3% formulation (Table 3.6.2.3), although the 0.3% rate was noticeably repellent at times. After all these trials at CRI – Nelspruit, many of the BHA nests became inactive and could not be used in the last July trial. It is therefore possible that some of the treatments used ultimately weakened the nests. Unfortunately, when it appeared that a treatment had no effect on ants from a specific nest, that nest was re-used in another trial. It is therefore difficult to determine which dosages ultimately had an effect or whether certain combinations of treatments were responsible for the collapse of these colonies (Table 3.6.2.4). One possible fault with the experimental design was the removal of the bait after 24 or 48 h. In the future the bait should be left in position and replenished as necessary, with an alternative food source such as peanut butter or fish paste used for activity evaluations.

In the last two Confidor trials against PA in summer, the extra step of weighing the bait and evaluation food after 22 h provided a valuable back up for feeding activity. These trials used an extra low dosage of Confidor and were evaluated for a long period. In the litchi orchard trial (Table 3.6.2.5) it appeared that the low rate of Confidor at 0.0071% formulated or 0.005% a.i. had an effect until 8 d after the 22 h feeding period (highlighted in table). The amount of Saga bait removed at T1 was not different from the control so the dosage was not repelling them in any way; in fact the higher concentration was removed more rapidly. After 2 d there were no ants feeding and the remaining test food was significantly more than the control or the other treatment. The numbers feeding after 7 d were still significantly lower than in the control and the remaining test food mass after 8 d was significantly higher. Thereafter, no further treatment effect was noticeable. Unfortunately the results from the second Confidor trial with PAs in a storm drain (Table 3.6.2.6) were too variable and there were no significant differences. However, the mass of the Saga baits after feeding for 22 h were very similar for all three treatments so there was no evidence of repellency with these rates of Confidor.

While this research was being conducted, several commercial ant baits appeared on the market in other countries that contained either fipronil or imidacloprid as toxicants. Two liquid ant baits are produced by Bayer CropScience that probably have a sugar solution attractant. Maxforce for home use contains imidacloprid at 0.005% (as we concluded above for PA) and Vitis for agricultural use contains imidacloprid at 0.001%. The latter product is aimed at Argentine ant but also has BHA on the label. These baits will not work for PAs because the attractant is sugar. Bayer CropScience also now produces a bait in a bait station called Maxforce FC that contains 0.01% fipronil while they also have a granular bait for fire ant (*Solenopsis invicta*) that contains only 0.00015% fipronil. Our dosages of fipronil that probably ultimately led to the collapse of two-thirds of the BHA nests that we used were at the upper end of this dosage range and did seem to cause some repellency (Table 3.6.2.1), so further large scale trials should be conducted with fipronil in Saga at 0.001%.

Table 3.6.2.4. History of treatments applied near BHA nests at CRI and ant-feeding activity per minute

NEST	25 Mar	AVG 6+7d	22 Apr	1 DAT	12 May	AVG 7+14 d	26 May	AVG 7+14d	1 Jul	AVG 7+14d	23 Jul	5d	Comments on nest
1	Regent 0.03%	500.5	Regent 1.0%	0	Regent 0.1%	625	Regent 0.5%	16	Control	35.5	Inactive	-	Possible effect of Regent 0.03, 1, 0.1 and 0.5%
2	Regent 0.03%	15.5	Regent 0.5%	7	Control	1600	Regent 0.25%	1100	Confidor 0.14%	400	Inactive	-	No effect Regent 0.5%
3	Regent 0.03%	193.5	Regent 0.25%	20	Regent 0.05%	1450	Control	800	Confidor 0.014%	50	Inactive	-	No effect Regent 0.05, 0.25%
4	Regent 0.03%	450	Control	1100	Regent 0.05%	1150	Control	451.5	Control	0	Inactive	-	Possible effect of Regent 0.03 and 0.05% only.
5	Regent 0.03%	97.5	Regent 0.25%	7	Regent 0.05%	140	Control	320	Confidor 0.014%	900	Inactive	-	No effect Regent 0.03, 0.25 and 0.05%
6	Regent 0.03%	400	Regent 0.05%	50	Regent 0.1%	450	Regent 0.25%	175	Control	60	Inactive	-	Regent 0.03, 0.05, 0.1, 0.25%
7	Regent 0.03%	50	Regent 0.1%	40	Control	98.5	Regent 0.5%	60	Confidor 0.014%	0.5	Confidor 0.3%	0	Low activity throughout
8	Regent 0.03%	350	Regent 0.05%	0	Regent 0.05%	400	Control	285	Confidor 0.014%	12.5	Inactive	-	Regent 0.03 and 0.05% followed by Confidor 0.014%.
9	Regent 0.03%	232.5	Regent 1.0%	8	Control	1750	Regent 0.5%	800	Confidor 0.14%	350	Confidor 0.3%	700	No effect Regent 0.03 or 1%
10	Regent 0.03%	192.5	Regent 0.5%	5	Regent 0.1%	500	Regent 0.25%	850	Confidor 0.14%	700	Control	70	No effect of Regent 0.03 or 0.5, 0.1 or 0.25%
11	Regent 0.03%	65.5	Regent 0.1%	0	Regent 0.1%	230	Regent 0.25%	475	Control	600	Inactive	-	No effect of Regent 0.25%
12	Regent 0.03%	654	Control	1200	Control	1750	Regent 0.5%	975	Confidor 0.14%	525	Confidor 0.3%	0	No effect of Regent 0.03 or 0.5%
Days between treatments and evaluations:													
	Treated			29 d		59 d		73 d					
			Treated			31 d		45 d					
					Treated			25 d		61 d			
							Treated			47 d			

Table 3.6.2.5. PAs feeding per minute in a litchi orchard at the Lowveld College of Agriculture and the mean mass of fish paste remaining after 22 h when tested at various periods in days after 24 h exposure to Confidor bait

Statistic	Treatments applied 19 Jan 2009		
	Control	Confidor 0.0071%	Confidor 0.071%
Ants feeding T1*	26.4 b	5.4 a	70 c
Mass of bait T1*	2.66 b	2.36 b	0.86 a
Ants feeding T2	162 c	0 a	27.2 b
Mass of bait T2	2.5 a	4.32 c	3.42 b
Ants feeding T7	60 b	0.2 a	27 ab
Ants feeding T8	10.6 a	2.8 a	13.2 a
Mass of bait T8	0.76 a	3.44 b	1.08 a
Ants feeding T15	17 a	13.4 a	30 a
Mass of bait T15	1.48 a	1.68 a	0 a
Ants feeding T22	2.2 a	2 a	6.2 a
Mass of bait T22	1.4 b	1.5 b	0 a
Ants feeding T29	9.8 a	3.6 a	23.4 a
Mass of bait T29	0.78 ab	1.98 b	0 a
Ants feeding T90	1.2 a	0.2 a	5.2 a
Mass of bait T90	1.88 a	0.92 a	1.32 a

*On Saga bait, whereas feeding after this was always on fish paste.

Means in the same row followed by the same letter were not significantly different ($P>0.05$ SNK).

Table 3.6.2.6. PAs feeding per minute in a storm drain at the Lowveld College of Agriculture and the mean mass of fish paste remaining after 22 h when tested at various periods in days after an initial 24 h exposure to Confidor bait

Statistic	Treatments applied 27 Jan 2009		
	Control	Confidor 0.0071%	Confidor 0.071%
Ants feeding T1*	9.4	0.2	3.6
Mass of bait T1*	4.42	4.58	4.82
Ants feeding T7	49.0	35.2	25.4
Mass of bait T7	1.18	1.58	2.08
Ants feeding T14	4.6	6.2	0.4
Mass of bait T14	1.46	No data	No data
Ants feeding T21	14.8	4.2	17.6
Mass of bait T21	0.6	0.06	0.66
Ants feeding T28	16.6	43.0	12.6
Mass of bait T28	0.34	0.96	0.24
Ants feeding T82	0.6	3.0	8.8
Mass of bait T82	1.62	2.18	1.7

*On Saga bait, whereas feeding after this was always on fish paste.

No means in the same row were significantly different ($P>0.05$ SNK).

2009-10

As expected, neither Siege nor trunk sprays of Dursban caused any significant reduction in PA activity and in fact Dursban sprays increased ant activity relative to the untreated control at both sites with this being significant ($P<0.05$) at one site (Tables 3.6.2.7 and 8). A similar increase in ant activity seemed to occur with the Saga plus imidacloprid treatments at both sites. Saga plus fipronil was the only effective treatment against PA and virtually eliminated them from both treatments (Tables 3.6.2.7 and 8). Unfortunately this ant disappeared completely from all treatments in both orchards and in untreated orchards in the area at around the time that the 3-month evaluation was due. After 4 months there was still no sign of PAs and BHA started moving into the orchards.

Table 3.6.2.7. Treatment effects on PA foraging at Site 1, Hectorspruit

Treatments applied 2 Dec 2009	Ants feeding per minute on fish paste			
	Pre-treatment	After 1 month	After 2 months	After 3 and 4 months
Untreated	8.6	8.6 b	3.9 a	0
Siege 10 g/tree	7.0	7.5 b	22.5 b	0
Dursban WG 2.5 kg/hl trunk spray	9.3	14.1 b	14.9 b	0
Saga + fipronil 0.001% at 10 g/tree	8.0	0.0 a	0.5 a	0
Saga + imidacloprid 0.005% at 10 g/tree	7.8	20.9 b	26.5 b	0

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

Table 3.6.2.8. Treatment effects on PA foraging at Site 2, Hectorspruit

Treatments applied 2 Dec 2009	Ants feeding per minute on fish paste			
	Pre-treatment	After 1 month	After 2 months	After 3 and 4 months
Untreated	7.5	17.5 b	24.6 ab	0
Siege 10 g/tree	12.0	32.4 b	25.9 ab	0
Dursban WG 2.5 kg/hl trunk spray	9.3	30.3 b	58.1 b	0
Saga + fipronil 0.001% at 10 g/tree	15.4	0.0 a	2.6 a	0
Saga + imidacloprid 0.005% at 10 g/tree	13.6	33.5 b	91.4 b	0

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

The results with BHA were very different to those with PA and the Saga-fipronil combination was found to be ineffective (Tables 3.6.2.9 and 10). At both sites, Siege was the most effective treatment and kept the ants at very low levels for at least 3 months. The Dursban stem sprays were also quite effective for about 2 months. Once again there were signs that Saga plus imidacloprid may have been stimulating ant reproduction or activity with significantly higher numbers in this treatment at one site than in the control after 3 months (Table 3.6.2.10).

Table 3.6.2.9. Treatment effects on BHA foraging at Site 1, Hectorspruit

Treatments applied 11 Jan 2010	Feeding activity as a proportion of pretreatment activity on peanut butter			
	Pre-treatment	After 1 month	After 2 months	After 3 months
Untreated	731.3	0.67 b	2.42 b	1.08 a
Siege 10 g/tree	192.8	0.08 a	0.01 a	0.30 a
Dursban WG 2.5 kg/hl trunk spray	92.6	0.22 ab	0.46 ab	1.42 a
Saga + fipronil 0.001% at 10 g/tree	931.3	0.46 ab	0.61 ab	0.74 a
Saga + imidacloprid 0.005% at 10 g/tree	831.3	0.41 b	0.49 ab	0.48 a

Means in the same column followed by the same letter were not significantly different ($P>0.05$) (Kruskal-Wallis test).

Table 3.6.2.10. Treatment effects on BHA foraging at Site 2, Hectorspruit

Treatments applied 11 Jan 2010	Feeding activity as a proportion of pretreatment activity on peanut butter			
	Pre-treatment	After 1 month	After 2 months	After 3 months
Untreated	1277.5	0.58 b	0.60 b	0.39 b
Siege 10 g/tree	225.0	0.01 a	0.002 a	0.002 a
Dursban WG 2.5 kg/hl trunk spray	88.8	0.09 ab	0.05 a	0.37 abc
Saga + fipronil 0.001% at 10 g/tree	1906.3	0.45 ab	0.68 ab	0.68 bc
Saga + imidacloprid 0.005% at 10 g/tree	346.3	0.75 b	2.88 b	1.92 c

Means in the same column followed by the same letter were not significantly different ($P>0.05$) (Kruskal-Wallis test).

Further research with ant baits will focus on reducing the number of bait stations required to control PA when using Saga plus fipronil 0.001% a.i. and on using a different active ingredient in Saga for the control of BHA.

2010-1

The results from Orchard 11G at Hectorspruit (Table 3.6.2.11) showed that all 3 treatments were capable of reducing BHA foraging activity for at least 1 month but later evaluations after this showed no significant differences between any of the treatments and the activity in the control declined. At the second site (11E) the numbers of BHA in the control 1 month after treatment dropped considerably (Table 3.6.2.12) and the pyriproxyfen treatment was not significantly different ($P>0.05$) from the control, but from there onwards all treatments were significantly better than the control ($P<0.05$) for up to 5 months after a single treatment. Both these trials therefore showed that Saga plus hydramethylnon 0.9% was equivalent to Siege and that although pyriproxyfen was slower acting, it ultimately had a similar effect.

The trials in grapefruit orchards at Malelane with PA showed no significant results at one site with ant numbers declining slowly in all treatments as the weather cooled and BHA eventually taking over the trial site (Table 3.5.2.13). At the other site, all treatments were effective against PA for 3 months and there was no difference between 2 or 4 kg/ha of Saga plus fipronil (Table 3.6.2.14).

Table 3.6.2.11. Treatment effects on BHA foraging at Orchard 11G, Golden Frontiers Citrus, Hectorspruit

Treatments applied 13 Dec 2010	Mean number of ants feeding per minute on peanut butter					
	Pre-treatment 30 Nov + 1 Dec 2011	After 1 month 13 Jan 2011	After 2 months 14 Feb 2011	After 3 months 17 Mar 2011	After 4 months 12 Apr 2011	After 5 months 9 May 2011
Untreated	25.2 a	23.8 a	44.3 a	105.6 a	18.4 a	33.8 a
Siege 10 g/tree	72.8 a	0.0 b	46.4 a	26.5 b	33.4 a	11.3 a
Saga + hydramethylnon 0.9% at 10 g/tree	76.6 a	0.0 b	58.0 a	67.3 ab	51.9 a	10.4 a
Saga + pyriproxyfen 0.25% at 10 g/tree	69.7 a	2.5 b	15.1 a	45.0 ab	45.6 a	33.1 a

Means in the same column followed by the same letter were not significantly different ($P>0.05$) (Tukey's HSD test).

Table 3.6.2.12. Treatment effects on BHA foraging at Orchard 11E, Golden Frontiers Citrus, Hectorspruit

Treatments applied 13 Dec 2010	Mean number of ants feeding per minute on peanut butter					
	Pre-treatment 30 Nov + 1 Dec 2011	After 1 month 13 Jan 2011	After 2 months 14 Feb 2011	After 3 months 17 Mar 2011	After 4 months 12 Apr 2011	After 5 months 9 May 2011
Untreated	203.4 a	17.5 a	170.8 a	558.1 a	263.1 a	132.5 a
Siege 10 g/tree	396.3 a	0.6 b	45.6 b	71.9 b	69.1 b	17.8 b
Saga + hydramethylnon 0.9% at 10 g/tree	105.1 a	0.0 b	13.1 b	10.0 b	59.4 b	38.4 b
Saga + pyriproxyfen 0.25% at 10 g/tree	334.4 a	13.1 ab	8.4 b	71.0 b	18.4 b	11.9 b

Means in the same column followed by the same letter were not significantly different ($P>0.05$) (Tukey's HSD test).

Table 3.6.2.13. Treatment effects on PA foraging at Orchard 1, Riverside Farms, Malelane

Treatments applied 31 Jan 2011	Ants feeding per minute on fish paste			
	Pre-treatment 24+25 Jan 2011	After 1 month 28 Feb 2011	After 2 months 14 Apr 2011	After 3 months 11 May 2011
Untreated	28.4 a	85.0 a	34.7 a	1.4 a
Saga + fipronil 0.001% at 10 g per tree (4 kg/ha)	8.4 a	3.0 a	5.0 a	4.4 a
Saga + fipronil 0.001% at 10 g every second tree (2 kg/ha)	17.9 a	6.1 a	6.6 a	2.7 a
Saga + pyriproxyfen 0.25% at 10 g per tree	24.6 a	50.3 a	5.0 a	17.3 a

Means in the same column followed by the same letter were not significantly different ($P>0.05$) (Tukey's HSD test).

Table 3.6.2.14. Treatment effects on PA foraging at Orchard 2, Riverside Farms, Malelane

Treatments applied 31 Jan 2011	Ants feeding per minute on fish paste			
	Pre-treatment 24+25 Jan 2011	After 1 month 28 Feb 2011	After 2 months 14 Apr 2011	After 3 months 11 May 2011
Untreated	17.5 a	45.0 a	185.7 a	101.4 a
Saga + fipronil 0.001% at 10 g per tree (4 kg/ha)	6.1 a	0.0 b	0.1 b	1.4 b
Saga + fipronil 0.001% at 10 g every second tree (2 kg/ha)	7.1 a	0.1 b	12.7 b	1.6 b
Saga + pyriproxyfen 0.25% at 10 g per tree	9.1 a	2.6 b	4.4 b	15.7 b

Means in the same column followed by the same letter were not significantly different ($P>0.05$) (Tukey's HSD test).

Both toxicants with Saga were extremely effective against PA at Letaba estates in June and no feeding activity occurred in either treatment for 3 months (Table 3.6.2.15). These results led to hydramethylnon being chosen as the preferred toxicant for both ant species because although pyriproxyfen was also effective it is frequently used in citrus orchards and applications on tree trunks may be detrimental to predatory beetles.

Table 3.6.2.15. Large scale comparison of fipronil and hydramethylnon in Saga for control of PA in Midnight block K17, Letaba Estates, Letsitele

Treatments applied 21 June 2011	Mean numbers of ants feeding on fish paste after 2 h			
	Pre-treatment 21 Jun 2011	After 1 month 18 Jul 2011	After 2 months 17 Aug 2011	After 3 months 20 Sep 2011
Untreated	35.4 a	21.9 a	16.3 a	23.9 a
Saga + fipronil 0.001% at 12 g every 2 nd tree	32.8 a	0.0 b	0.0 b	0.0 b
Saga + hydramethylnon 0.9% at 12 g every 2 nd tree	33.9 a	0.0 b	0.0 b	0.0 b

Means in the same column followed by the same letter were not significantly different ($P > 0.05$) (Tukey's HSD test).

The comparison of Saga with the bulk recipe of Saga showed no significant differences for PA with the mean numbers of ants feeding per station with Saga being 16.6 and the mean for Saga bulk being 18.6 ($t = 1.335$; $df = 9$; $P = 0.215$).

Large scale orchard trials in Limpopo

In the Valencia trial at Letaba Estates, PA never appeared in the orchard and only *Camponotus* sp. (a large ant like PA) and BHA were seen on the tree trunks. However, the trial was evaluated as planned to see what the impact on these species would be. The only significant ($P < 0.05$) treatment effect in this trial was an increase in numbers of *Camponotus* sp. feeding on fish paste on the ground 4 weeks after treatment (Table 3.6.2.16). This may have been due to the ants being repelled from the trees and doing more foraging on the ground because tree infestation was lower after 4 weeks than at other times (Fig. 3.6.2.1). Saga plus hydramethylnon did not have a significant impact on this species. This trial was also evaluated for BHA infestation which increased in the control with time (Table 3.6.2.17). Both the trunk spray and Saga bait treatments had significantly lower ($P < 0.05$) BHA foraging activity on the ground than the control after 9 and 12 weeks. However, tree infestation in the control remained around 17% and remained even lower in the treatments (data not shown). This trial can be used as a successful field trial for BHA.

Table 3.6.2.16. Large scale comparison of Saga plus hydramethylnon with chlorpyrifos trunk sprays for control of *Camponotus* sp. in Valencia block C73, Letaba Estates, Letsitele

Treatments applied 24 Oct 2011	Mean numbers of ants feeding on fish paste after 2 h			
	Pre-treatment 24 Oct 2011	After 4 weeks 29 Nov 2011	After 9 weeks 4 Jan 2012	After 12 weeks 25 Jan 2012
Untreated control	1.2 a	3.6 b	0.0 a	6.4 a
Chlorpyrifos (Dursban 480 EC at 30 ml/L) applied to the bottom 40 cm of each trunk	0.4 a	18.2 a	5.6 a	3.6 a
Saga + hydramethylnon 0.9% at 10 g every 2 nd tree in the crotch	1.4 a	0.8 b	4.2 a	0.2 a

Means in the same column followed by the same letter were not significantly different ($P > 0.05$) (Tukey's HSD test).

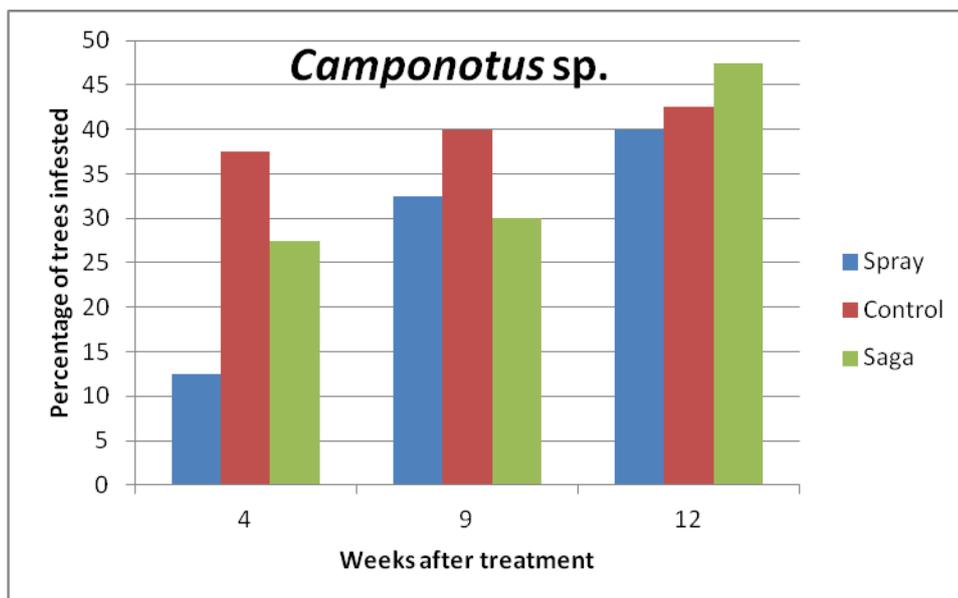


Figure 3.6.2.1. Percentage of trees infested with *Camponotus* sp. at Letaba Estates after spraying trunks with chlorpyrifos or applying Saga plus hydramethylnon to every second tree.

Table 3.6.2.17. Large scale comparison of Saga plus hydramethylnon with chlorpyrifos trunk sprays for control of BHA in Valencia block C73, Letaba Estates, Letsitele

Treatments applied 24 Oct 2011	Mean numbers of ants feeding on fish paste after 2 h			
	Pre-treatment 24 Oct 2011	After 4 weeks 29 Nov 2011	After 9 weeks 4 Jan 2012	After 12 weeks 25 Jan 2012
Untreated control	4.4 a	14.6 a	72.6 a	57.8 a
Chlorpyrifos (Dursban 480 EC at 30 ml/L) applied to the bottom 40 cm of each trunk	3.6 a	0.0 a	12.0 b	2.0 b
Saga + hydramethylnon 0.9% at 10 g every 2 nd tree in the crotch	0.0 a	0.0 a	3.4 b	10.8 b

Means in the same column followed by the same letter were not significantly different ($P > 0.05$) (Tukey's HSD test).

In the PA trial in Midnights at Letaba, Saga plus hydramethylnon was extremely effective and gave total control of PA for up to 9 weeks and significantly better control than the chlorpyrifos trunk spray in every evaluation for up to 12 weeks after a single application (Table 3.6.2.18). These trends were supported by the tree infestation levels (Fig. 3.6.2.2).

Table 3.6.2.18. Large scale comparison of Saga plus hydramethylnon with chlorpyrifos trunk sprays for control of PA in Midnight block K17, Letaba Estates, Letsitele

Treatments applied 24 Oct 2011	Mean numbers of ants feeding on fish paste after 2 h			
	Pre-treatment 24 Oct 2011	After 4 weeks 29 Nov 2011	After 9 weeks 4 Jan 2012	After 12 weeks 25 Jan 2012
Untreated control	13.0 a	9.8 a	0.6 ab	6.0 a
Chlorpyrifos (Dursban 480 EC at 30 ml/L) applied to the bottom 40 cm of each trunk	13.8 a	4.2 b	3.2 a	5.2 a
Saga + hydramethylnon 0.9% at 10 g every	14.4 a	0.0 c	0.0 b	1.0 b

2 nd tree in the crotch				
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Means in the same column followed by the same letter were not significantly different ($P>0.05$) (Tukey's HSD test).

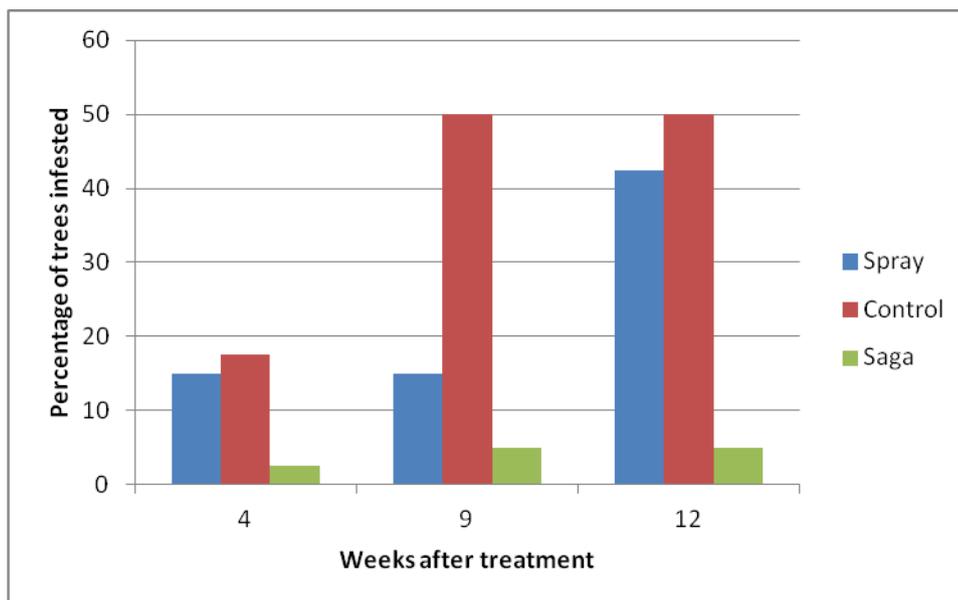


Figure 3.6.2.2. Percentage of trees infested with PA at Letaba Estates after spraying trunks with chlorpyrifos or applying Saga plus hydramethylnon to every second tree.

Large scale orchard trials in Mpumalanga

The trial near Malelane on PA showed that Saga plus hydramethylnon resulted in significantly lower ($P<0.05$) ant activity than in the control after 4 weeks and again after 12 weeks (Table 3.6.2.19). The 8 week control counts had too much variation to show significant differences. The chlorpyrifos trunk spray was only better than the control on the 4-week evaluation but was reducing tree infestation after 12 weeks (Fig. 3.6.2.3) so perhaps more ants were foraging on the ground at that time. The tree infestation levels in the Saga treatments (Fig. 3.6.2.3) followed the ground foraging activity results in Table 3.6.2.19, indicating that Saga reduced the whole PA population in that treatment and didn't just repel the ants from the trees for a while.

Table 3.6.2.19. Large scale comparison of Saga plus hydramethylnon with chlorpyrifos trunk sprays for control of PA in Delta Valencia block 16B, Riverside Farms, Malelane

Treatments applied 5 Dec 2011	Mean numbers of ants feeding on fish paste after 2 h			
	Pre-treatment 5 Dec 2011	After 4 weeks 5 Jan 2012	After 8 weeks 6 Feb 2012	After 12 weeks 5 Mar 2012
Untreated control	11.0 a	46.6 a	70.4 a	156.0 a
Chlorpyrifos (Dursban 480 EC at 30 ml/L) applied to the bottom 40 cm of each trunk	4.8 a	2.0 b	6.2 a	20.0 ab
Saga + hydramethylnon 0.9% at 10 g every 2 nd tree in the crotch	14.0 a	0.6 b	2.0 a	1.2 b

Means in the same column followed by the same letter were not significantly different ($P>0.05$) (Tukey's HSD test).

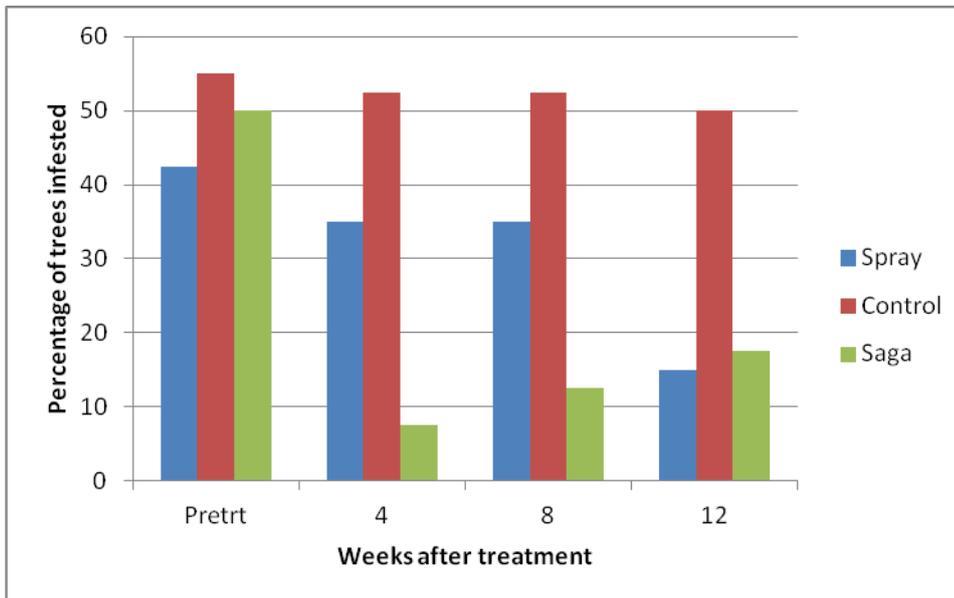


Figure 3.6.2.3. Percentage of trees infested with PA at Riverside Farms, Malelane after spraying trunks with chlopyrifos or applying Saga plus hydramethylnon to every second tree.

In orchard A at Golden Frontiers Citrus, Hectorspruit, foraging activity on the ground in the Saga block before treatments were applied was significantly lower than in the other two treatment blocks so the square root transformed numbers were increased by a factor of 2.531. This may have resulted in artificially high numbers by the 12-week post-treatment evaluation but it had to be consistently applied (Table 3.6.2.20). Tree infestation levels declined with time in all treatments (Fig. 3.6.2.4) so that may have led to the increased foraging activity on the ground. Although Saga did lower ground foraging activity to zero 5 weeks after treatment, this was not significantly different due to large variation between feeding stations in the other treatments.

Table 3.6.2.20. Large scale comparison of Saga plus hydramethylnon on every second tree with Siege bait on every tree for control of BHA in Valencia orchard A at Golden Frontiers Citrus, Hectorspruit

Treatments applied 15 Dec 2011	Mean numbers of ants feeding on peanut butter after 2 h			
	Pre-treatment 15 Dec 2011	After 5 weeks 24 Jan 2012	After 9 weeks 21 Feb 2012	After 12 weeks 15 Mar 2012
Untreated control	86.0 a	36.0 a	47.0 ab	138.0 ab
Siege with hydramethylnon 0.73% at 10 g per tree base	67.0 a	121.0 a	18.0 b	78.0 b
Saga + hydramethylnon 0.9% at 10 g every 2 nd tree in the crotch	68.7 a*	0.0 a*	211.8 a*	391.3 a*

Means in the same column followed by the same letter were not significantly different ($P > 0.05$) (Tukey's HSD test).

*Saga pre-treatment levels were significantly lower so all transformed values were adjusted by the same factor and these numbers are back-transforms of the means.

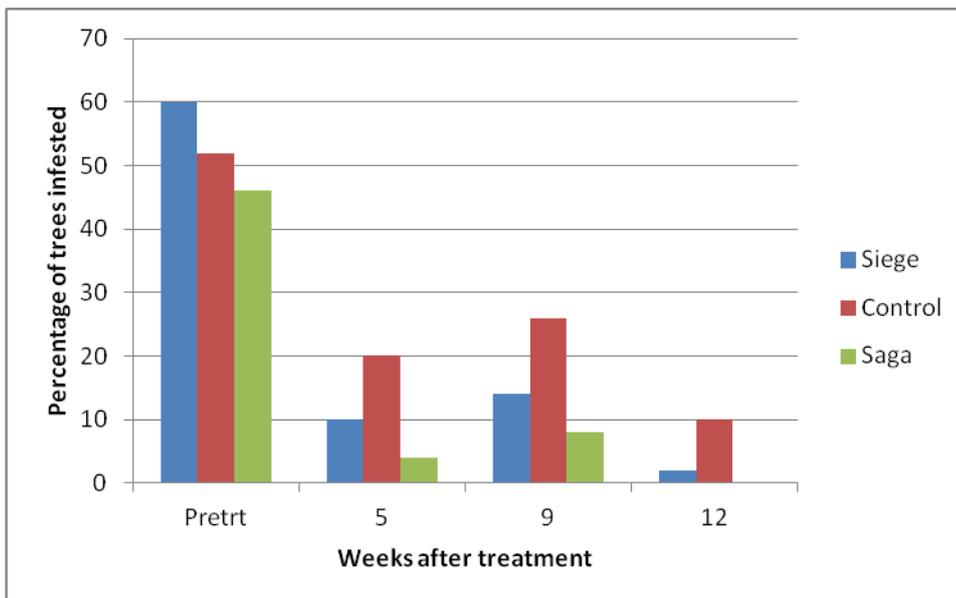


Figure 3.6.2.4. Percentage of trees infested with BHA in orchard A at Golden Frontiers Citrus, Hectorspruit after applying Siege to every tree or Saga plus hydramethylnon to every second tree.

In Valencia orchard B at Golden Frontiers Citrus, Hectorspruit, levels of ant activity on the ground were not significantly different between treatment blocks before the treatments were applied so no adjustments were required before analysis, besides the square root transformation. The only significant difference ($P < 0.05$) between treatments when considering foraging on the ground was between the Siege treatment and the control after 12 weeks (Table 3.6.2.21), although numbers of foraging ants in the Saga treatment were considerably lower than in the control at this time. Saga results did not differ significantly from those with Siege at any time. Tree infestation (Fig. 3.6.2.5) also declined with time as seen in orchard A. This also occurred in the control so perhaps it was due to a declining source of honeydew in the trees and not a treatment effect.

Table 3.6.2.21. Large scale comparison of Saga plus hydramethylnon on every second tree with Siege bait on every tree for control of BHA in Valencia orchard B at Golden Frontiers Citrus, Hectorspruit

Treatments applied 15 Dec 2011	Mean numbers of ants feeding on peanut butter after 2 h			
	Pre-treatment 15 Dec 2011	After 5 weeks 24 Jan 2012	After 9 weeks 21 Feb 2012	After 12 weeks 15 Mar 2012
Untreated control	123.0 a	33.0 a	61.0 a	456.0 a
Siege with hydramethylnon 0.73% at 10 g per tree base	83.0 a	19.0 a	58.0 a	1.0 b
Saga + hydramethylnon 0.9% at 10 g every 2 nd tree in the crotch	90.0 a	12.0 a	28.0 a	272.0 ab

Means in the same column followed by the same letter were not significantly different ($P > 0.05$) (Tukey's HSD test).

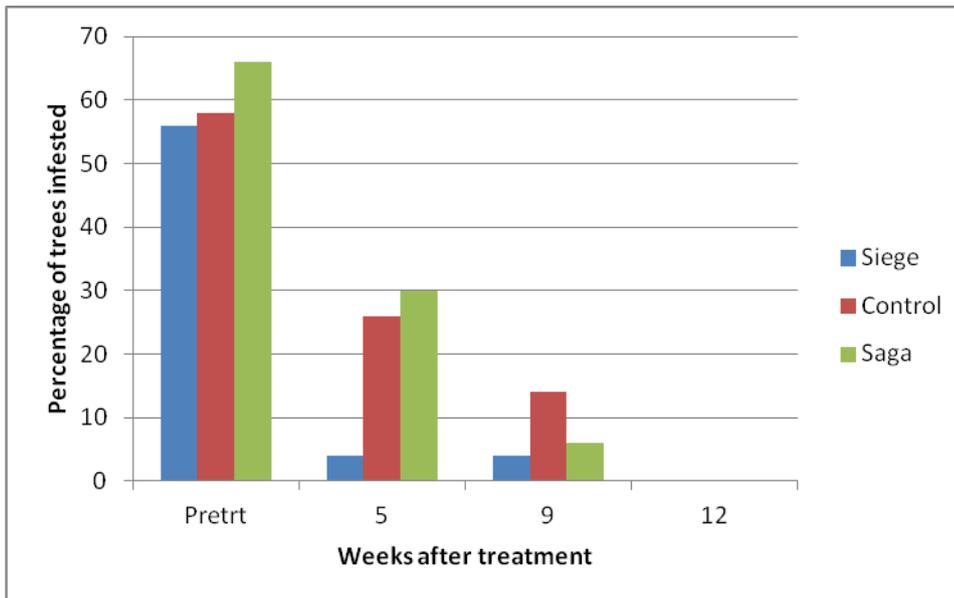


Figure 3.6.2.5. Percentage of trees infested with BHA in orchard B at Golden Frontiers Citrus, Hectorspruit after applying Siege to every tree or Saga plus hydramethylnon to every second tree.

At Huguenot Farm, numbers of PA on the ground were low 5 weeks after application and there were no significant differences between treatments (Table 3.6.2.22), although tree infestation was highest in the control (Fig. 3.6.2.6). At the later evaluations both Saga and the trunk sprays had significantly less ant activity on the ground.

Table 3.6.2.22. Large scale comparison of Saga plus hydramethylnon with chlorpyrifos trunk sprays for control of PA in navel oranges at Huguenot Farm, Sundays River Valley

Treatments applied 7 Dec 2011	Mean numbers of ants feeding on fish paste after 1 h		
	After 5 weeks 11 Jan 2012	After 8 weeks 8 Feb 2012	After 12 weeks 8 Mar 2012
Untreated control	4.6 a	17.8 a	15.4 a
Chlorpyrifos (Dursban 480 EC at 30 ml/L) applied to the bottom 40 cm of each trunk	0.8 a	0.0 b	2.8 b
Saga + hydramethylnon 0.9% at 10 g every 2 nd tree in the crotch	0.8 a	4.0 b	3.0 b

Means in the same column followed by the same letter were not significantly different ($P>0.05$) (Tukey's HSD test).

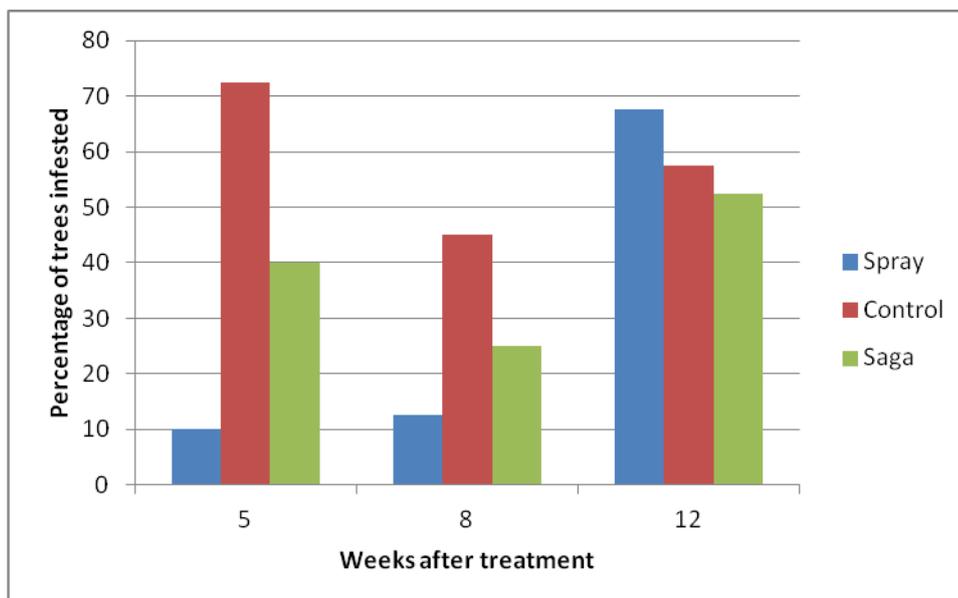


Figure 3.6.2.6. Percentage of trees infested with PA at Huguenot Farm, Sundays River Valley after spraying trunks with chlorpyrifos or applying Saga plus hydramethylnon to every second tree.

At Halaron Farm, PA numbers in the orchard remained low and Saga bait was only significantly better than the control in the the 5-week evaluation (Table 3.6.2.23). The highest level of tree infestation in the control was 10% and this declined with time to zero after 12 weeks so these results were of no value and are not presented.

Table 3.6.2.23. Large scale comparison of Saga plus hydramethylnon with chlorpyrifos trunk sprays for control of PA in navel oranges at Halaron Farm, Sundays River Valley

Treatments applied 7 Dec 2011	Mean numbers of ants feeding on fish paste after 1 h			
	Pre-treatment 7 Dec 2011	After 5 weeks 11 Jan 2012	After 8 weeks 8 Feb 2012	After 12 weeks 8 Mar 2012
Untreated control	8.2 a	1.4 a	14.6 a	0.0 a
Chlorpyrifos (Dursban 480 EC at 30 ml/L) applied to the bottom 40 cm of each trunk	8.2 a	0.4 ab	0.2 a	0.0 a
Saga + hydramethylnon 0.9% at 10 g every 2 nd tree in the crotch	16.2 a	0.0 b	0.2 a	0.0 a

Means in the same column followed by the same letter were not significantly different ($P>0.05$) (Tukey's HSD test).

At Hermitage, both treatments against BHA were extremely effective in reducing foraging activity as measured on the ground with peanut butter and counts were significantly lower ($P<0.05$) for both treatments at all evaluations for 3 months (Table 3.6.2.24). Tree infestation levels dropped to zero in the treatments at the 5-week evaluation but increased slowly in the later evaluations (Fig. 3.6.2.7).

Table 3.6.2.24. Large scale comparison of Saga plus hydramethylnon on every second tree with Siege bait on every tree for control of BHA in a navel orange orchard near Hermitage, Sundays River Valley

Treatments applied 8 Dec 2011	Mean numbers of ants feeding on peanut butter after 2 h			
	Pre-treatment 8 Dec 2011	After 5 weeks 11 Jan 2012	After 8 weeks 8 Feb 2012	After 12 weeks 8 Mar 2012
Untreated control	126.0 a	20.2 a	135.2 a	244.0 a
Siege with hydramethylnon 0.73% at 10 g per tree base	142.6 a	0.0 b	0.0 b	10.0 b

Saga hydramethylnon 0.9% at 10 g every 2 nd tree in the crotch	+	198.4 a	0.0 b	0.0 b	4.0 b
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Means in the same column followed by the same letter were not significantly different ($P>0.05$) (Tukey's HSD test).

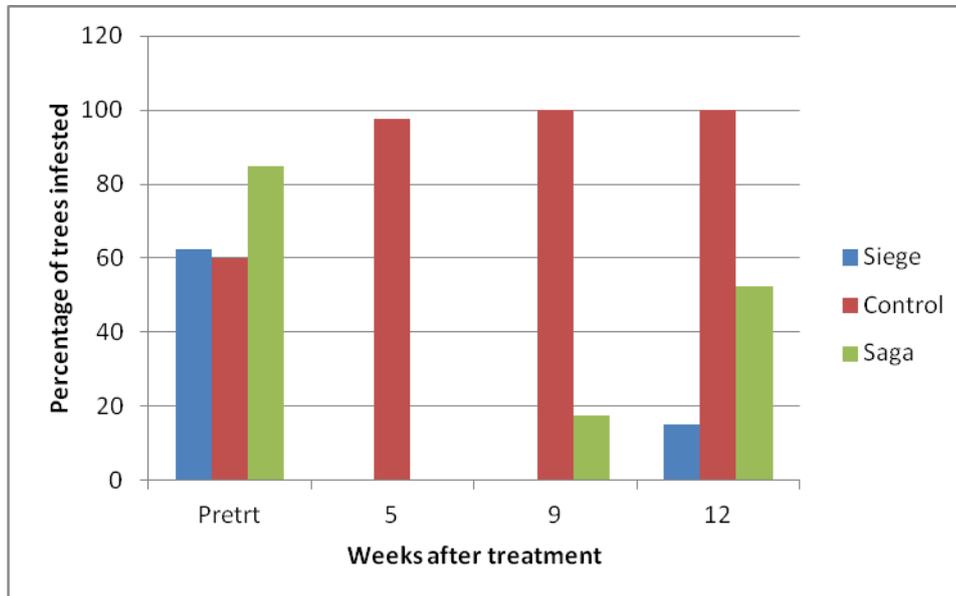


Figure 3.6.2.7. Percentage of trees infested with BHA in a navel orange orchard near Hermitage after applying Siege to every tree or Saga plus hydramethylnon to every second tree.

Near Sunland the efficacy of Saga for BHA on every second tree was similar to that of Siege on every tree and both treatments were significantly better ($P<0.05$) than the control 5 weeks after treatment (Table 3.6.2.25). In later evaluations, ant foraging in the treated areas remained lower than the control but not significantly so. Tree infestation in the control remained close to 100% for the duration of the trial but was only around 20% in the treated areas after 12 weeks (Fig. 3.6.2.8).

Table 3.6.2.25. Large scale comparison of Saga plus hydramethylnon on every second tree with Siege bait on every tree for control of BHA in a mandarin orchard near Sunland, Sundays River Valley

Treatments applied 8 Dec 2011	Mean numbers of ants feeding on peanut butter after 2 h			
	Pre-treatment 8 Dec 2011	After 5 weeks 11 Jan 2012	After 8 weeks 8 Feb 2012	After 12 weeks 8 Mar 2012
Untreated control	35.6 a	292.6 a	12.8 a	150.0 a
Siege with hydramethylnon 0.73% at 10 g per tree base	36.2 a	0.0 b	0.0 a	48.0 a
Saga + hydramethylnon 0.9% at 10 g every 2 nd tree in the crotch	30.0 a	0.0 b	1.0 a	45.6 a

Means in the same column followed by the same letter were not significantly different ($P>0.05$) (Tukey's HSD test).

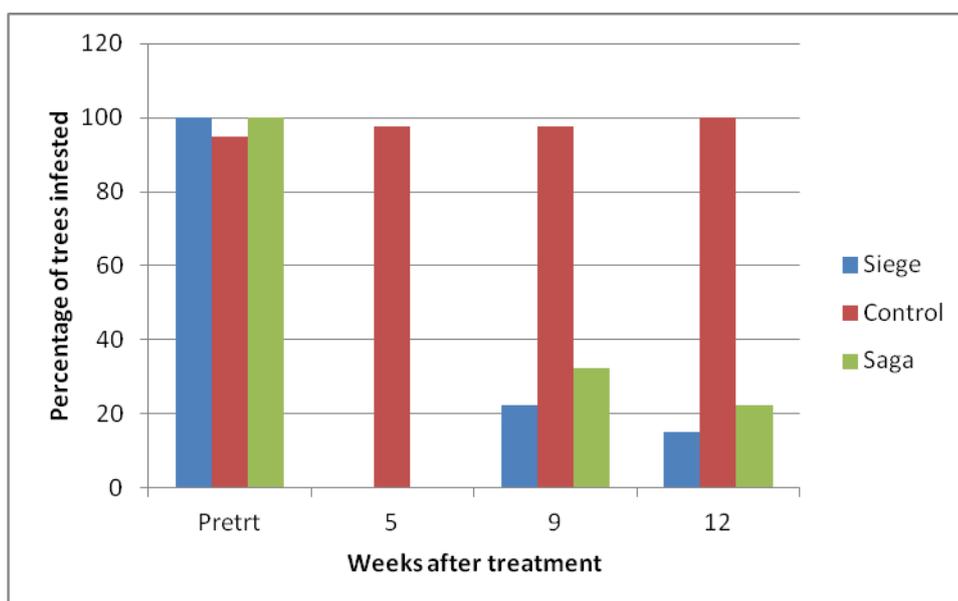


Figure 3.6.2.8. Percentage of trees infested with BHA in a mandarin orchard near Sunland after applying Siege to every tree or Saga plus hydramethylnon to every second tree.

Inclusion of a preservative

The first trial at Hectorspruit to try Saga with various preservatives against BHA showed that after 9 days, Saga without a preservative and Saga with K510 at 0.1% were giving the best control and this was significantly better ($P < 0.05$) than Saga plus Nipagin 0.1% (Table 3.6.2.26). After 1 month there were no significant differences between treatments and all had reduced the ant numbers to very low numbers. In the second trial at the same location, there were no significant differences between treatments 9 days after treatment (Table 3.6.2.27) but after 1 month the Saga plus K320 treatment had significantly more ants than Saga without preservative, Saga plus K510 at 0.1% or Saga plus Nipagin 0.1%. It therefore appeared that K510 at 0.1% was a good option for BHA control.

Table 3.6.2.26. BHA's response to Saga plus hydramethylnon with different preservatives on every second tree in a grapefruit orchard at Golden Frontiers Citrus, Hectorspruit

Treatments applied 11 Dec 2012	Mean numbers of ants feeding on peanut butter after 2 h		
	Pre-treatment 10 Dec 2012	After 9 days 20 Dec 2012	After 4 weeks 14 Jan 2013
Saga + hydramethylnon 0.9% (Saga-H) at 10 g every 2 nd tree in the crotch	18.8 a	0.0 b	0.1 a
Saga-H plus Nipagin-M 0.1%	27.0 a	0.3 a	3.1 a
Saga-H plus Nipagin-M 0.2%	15.2 a	0.1 ab	2.7 a
Saga-H plus Euxyl K320 0.5%	23.5 a	0.0 ab	0.1 a
Saga-H plus Euxyl K510 0.05%	19.6 a	0.2 ab	3.0 a
Saga-H plus Euxyl K510 0.1%	18.8 a	0.0 b	0.6 a

Means in the same column followed by the same letter were not significantly different ($P > 0.05$) (Tukey's HSD test).

Table 3.6.2.27. BHA's response to Saga plus hydramethylnon with different preservatives on every second tree in another grapefruit orchard at Golden Frontiers Citrus, Hectorspruit

Treatments applied 11 Dec 2012	Mean numbers of ants feeding on peanut butter after 2 h		
	Pre-treatment 10 Dec 2012	After 9 days 20 Dec 2012	After 4 weeks 14 Jan 2013
Saga + hydramethylnon 0.9% (Saga-H) at 10 g every 2 nd tree in the crotch	8.9 a	0.5 a	0.0 b
Saga-H plus Nipagin-M 0.1%	6.1 a	1.3 a	0.0 b
Saga-H plus Nipagin-M 0.2%	11.0 a	0.5 a	18.1 ab
Saga-H plus Euxyl K320 0.5%	19.9 a	0.8 a	41.1 a

Saga-H plus Euxyl K510 0.05%	4.5 a	0.5 a	8.5 ab
Saga-H plus Euxyl K510 0.1%	5.8 a	0.5 a	0.0 b

Means in the same column followed by the same letter were not significantly different ($P>0.05$) (Tukey's HSD test).

The first small-scale trial with PA at Malelane in January 2013 (Table 3.6.2.28) was conducted when the ants were not foraging much but it clearly showed that no ants went to the bait with K320 and both baits with Nipagin were not visited much. These results were confirmed in the 2 trials conducted in February (Table 3.6.2.28) when the ants were more active and the best responses were found with the K510 preservative. This preservative was then compared with bait without preservative in the large scale trial at Golden Frontiers Citrus, Hectorspruit.

Table 3.6.2.28. PA's response to Saga plus hydramethylnon with different preservatives in feeding stations on the ground in grapefruit orchards at Riverside Farms, Malelane

Treatments offered to PA in feeding stations	Stations with foraging ants after 2 h (%)		
	Malelane 30 Jan 2013	Malelane A 5 Feb 2013	Malelane B 5 Feb 2013
Saga + hydramethylnon 0.9% (Saga-H) at 10 g every 2 nd tree in the crotch	60	100	100
Saga-H plus Nipagin-M 0.1%	20	70	90
Saga-H plus Nipagin-M 0.2%	20	70	90
Saga-H plus Euxyl K320 0.5%	0	0	0
Saga-H plus Euxyl K510 0.05%	50	100	100
Saga-H plus Euxyl K510 0.1%	40	90	100

In the large-scale trial with BA at Hectorspruit, both Saga treatments with and without the preservative K510 reduced ant numbers significantly ($P<0.05$), despite the rainfall shortly after treatment (Table 3.6.2.29). Upto 4 weeks after treatment, Saga plus K510 was significantly more effective than Saga without the preservative but by 10 weeks after treatment it was the other way around. Tree infestation levels did not follow any pattern and there were always low numbers of ants in the trees with an average infestation level of around 70% (data not shown).

Table 3.6.2.29. Large scale comparison of Saga plus hydramethylnon with and without a preservative K510 for control of PA in a young citrus orchard at Golden Frontiers Citrus, Hectorspruit

Treatments applied 11 Feb 2013	Mean numbers of ants feeding on fish paste after 1 h			
	Pre-treatment 11 Feb 2013	After 2 weeks 25 Feb 2013	After 4 weeks 12 Mar 2013	After 10 weeks 22 Apr 2013
Untreated control	110.0 a	120.8 a	90.4 a	99.2 a
Saga + hydramethylnon 0.9% at 10 g every 2 nd tree on the ground	89.2 a	61.7 b	28.3 b	7.8 c
Saga + hydramethylnon 0.9% + K510 0.1% at 10 g every 2 nd tree on the ground	89.2 a	20.8 c	11.5 c	24.2 b

Means in the same column followed by the same letter were not significantly different ($P>0.05$) (Tukey's HSD test).

Conclusion

After evaluating imidacloprid, fipronil, pyriproxyfen and hydramethylnon as toxicants for the ant attractant Saga, hydramethylnon at 0.9% was chosen as the preferred toxicant that was effective against both important ant species. This treatment successfully controlled the pugnacious ant in 6 out of 6 large scale orchard trials in 3 provinces and also controlled the brown house ant in 7 out of 7 large scale trials. The addition of the preservative K510 at 0.1% had no detrimental effect on treatment efficacy for both ant species. No phytotoxic effects were observed on bark where bait had been placed. This bait must now be registered and commercialised as no other product is registered against the pugnacious ant.

Future research

Periodically some product will be examined for shelf tests but no further research is planned at this time. Some product may be provided to researchers in the Western Cape to evaluate against the Argentine ant.

Technology transfer

Apart from the publication below on the use of a bait station, a poster on ant bait research was presented at the Citrus Research Symposium in 2012 and a talk at the Entomological Society of Southern Africa congress in Potchefstroom in July 2013. A refereed scientific publication is planned and once the product is registered its use will be explained to growers at regional meetings.

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3.6.3 PROGRESS REPORT: The effect of systemically-applied imidacloprid on lepidopteran pests of citrus

Project 954 (2010/1 – 2011/2) by Sean Moore, Wayne Kirkman (CRI) and Rachel van der Walt (NMMU)

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 11 years fruit damage attributed to the lemon borer moth or citrus flower moth (*Prays citri*), has 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 significantly heightened juvenile hormone levels in FCM adult females which had developed from imidacloprid-treated fruit, confirming results from the previous two years' trials. In a field trial, there was no indication that imidacloprid application resulted in elevated levels of FCM, unlike the previous season. However, this could be explained by a)

imidacloprid residues being present even in the untreated blocks, b) imidacloprid residues were lower than during the previous season, and c) no data for the previous season from the trial orchards were available in order to make an inter-seasonal comparison of FCM levels. This study is not yet complete. Further ovarian protein, juvenile hormone and fecundity trials are being conducted with fruit samples collected in subsequent months. Field trial evaluation will also continue for a few more weeks.

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 betekenisvolle verhoogde jeughormoon getoon in volwasse VKM wyfies wat op imidacloprid behandelde vrugte ontwikkel het. Hierdie het die vorige twee seisoen se proefresultate bevestig. Boonop was eierlegging van motte wat in imidacloprid behandelde vrugte ontwikkel het, hoër as die van motte wat in onbehandelde vrugte ontwikkel het, al was die verskil nie betekenisvol nie. In 'n veldproef was daar geen aanduiding dat imidacloprid toediening tot verhoogde vlakke van VKM gelei het nie, in teenstelling met die vorige seisoen. Nietemin, kon hierdie soos volg verduidelik word: a) imidacloprid residue is selfs in die onbehandelde blokke gekry, b) imidacloprid residue is laer as gedurende die vorige seisoen, en c) geen data vir die vorige seisoen van die proefboorde is beskikbaar om 'n interseisoen vergelyking van VKM vlakke te maak nie. Hierdie studie is nog nie voltoei nie. Verdere ovarium proteïen, jeughormoon en fekunditeits proewe word tans uitgevoer met vrugmonsters wat in die eersvolgende maande versamel is. Veldproef evaluasie sal vir nog 'n paar weke uitgevoer word.

4 PORTFOLIO: DISEASE MANAGEMENT

4.1 PORTFOLIO SUMMARY

By P.H. Fourie (Portfolio Manager: Disease Management)

Most projects in the Disease Management programme are showing very good progress and most grower priorities are addressed in projects designed to meet certain short-, medium- and long-term strategic objectives. The progress of the 2012-13 reporting period is briefly summarised below.

Apart from pure research projects, the Graft Transmissible Diseases programme continues to provide essential services for the Citrus Improvement Scheme (CIS) through re-indexing of mother 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. 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 and mechanisms of CTV cross-protection are studied at Pretoria University and CRI-Nelspruit. 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* subspecies, 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. A project investigating the seasonal population fluctuation of *Trioza erytreae* and infection with the greening organism, *Candidatus Liberibacter africanus* and coinciding transmission of greening to citrus was concluded. Positive *T. erytreae* individuals were found throughout most of the season even though peaks in infectivity were found. The study underscores the importance of the current triozid (psyllid) control recommendations to control the spread of African Greening by limiting population build-up during the first flush period.

In the Soilborne Diseases programme, 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 pre-plant fumigated sites remain nematode-free and trees perform significantly better than post-plant treated sites. Multivariate statistical analyses of large datasets of variables that were collected from trees in various stages of decline is used to determine principal components and interactions between factors that will give researchers a better understanding of tree decline and potential early indicators of tree decline. Various citrus rootstocks are studied to elucidate the biochemical mechanisms involved in resistance / tolerance of citrus rootstocks against *Phytophthora* root rot. One book chapter was submitted for publication.

In the Citrus Black Spot programme, three important papers were published that will be invaluable in continued market access negotiations with Europe. Researchers are also involved in a collaborative project with USA, Brazilian and Argentinian researchers to develop a probabilistic model to quantitatively predict the risk of fruit as a pathway for CBS. CBS epidemiology is being studied in this project, as well as in the Eastern Cape, Limpopo and Mpumalanga provinces through spore trapping and weather monitoring. The global population structure of the CBS pathogen is being studied. Spray programmes are continuously being improved to manage fungicide resistance, improve formulations, to make it more cost effective and to register new active ingredients. Important research has also been concluded to indicate that the CBS pathogen has a lower risk of developing resistance against the strobilurin fungicide group. Five articles and one book chapter was published.

In the Fruit and Foliar Diseases programme, new control options for *Alternaria* brown spot (ABS) are 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; a research paper with these findings was published. Anomalous findings following adjuvant research have necessitated additional fundamental research to be conducted. Control of *Botrytis* blossom blight and fruit drop in lemons is also being studied. Suitable fungicides were identified, but the optimal timing of application needs to be determined.

The Postharvest Diseases programme remains a very high priority and several projects were directly aimed at improving postharvest disease management in packhouses. Potential alternative fungicides and sanitisers are continuously screened, and certain products evaluated in 2012 showed promise. Imazalil, thiabendazole and pyrimethanil residue loading following application in drench, fungicide bath, wax, as well as the JBT heated flooder application and subsequent bio-efficacy against sensitive and resistant *Penicillium* strains were studied, giving valuable insight into the optimal use of these postharvest fungicides. Study of the practical impact of imazalil resistance clearly showed that this very effective fungicide was rendered ineffective when applied against resistant isolates. Resistance management is therefore vitally important, and methods to determine resistance frequencies in packhouses are presently being evaluated. Integration of preharvest silicon, and postharvest heat and biocontrol against green mould are also being studied. Two research papers were published.

The Diagnostic Centre (DC) continues to perform a sterling service to 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 programme. The DC also continued providing quality control analyses for River Bioscience. In total, a staggering 8559 samples were analysed by one diagnostician, a technician and assistant.

In general, good progress was made in Disease Management. Apart from excellent 'non-research', such as support for biosecurity, improvement scheme, market access, and formal and *ad hoc* extension activities, the quality and quantity of tangible research outputs has improved through consolidated and focused research. In 2012-13, 10 scientific papers (8x articles and 2x book chapters) were published.

Portefeuljeopsomming

Meeste van die projekte in die Siektebestuurprogram toon baie goeie vordering en die meeste produsente-prioriteite word aangespreek in projekte wat ontwerp word om sekere kort-, medium- en langtermyn strategiese doelwitte te bereik. Die vordering vir die 2012-13 verslagperiode word kortliks hieronder opgesom.

Die Ent-oordraagbare Siekte program het, afgesien van suiwer navorsingsprojekte, voortgegaan om noodsaaklike dienste aan die Sitrusverbeteringskema (SVS) te verskaf, deur her-indeksering van moederblossoms, 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. 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 en meganismes van CTV kruisbeskerming word by Pretoria Universiteit en CRI-Nelspruit bestudeer. Hierdie studies en gevolglike insig omtrent kruisbeskermingsverlies sal grondleggend 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 blyk dit asof elke genus sy eie unieke Liberibacter huisves. Basispaarvolgorde-bepaling ("sequencing") van die *capensis* sub-spesie, asook van die vergroeningspatogeen, "*Candidatus Liberibacter africanus*", is in samewerking met VSA navorsers, onderweg. Twee moontlike 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. 'n Projek wat die seisonale populasie-fluktuasie van *Trioza erythrae* en infeksie met die vergroeningsorganisme, *Candidatus Liberibacter africanus*, en gepaardgaande oordrag van vergroening na sitrus, ondersoek, is afgesluit. Positiewe *T. erythrae* individue is deur die grootste deel van die seisoen gevind, hoewel pieke in infektiwiteit gevind is. Die studie onderstreep die belang van die huidige bladvlooi beheer-aanbevelings om die verspreiding van Afrika Vergroening te beheer, deur die opbou van die populasie gedurende die eerste stuwingsperiode te beperk.

Verskeie proewe in die Grondgedraagde Siekte program het die beheer van nematodes met alternatiewe, meer omgewingsvriendelike produkte, ondersoek. 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. Uitkomstes uit hierdie navorsing is waardevol vir die sitrusbedryf, veral ná 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 vóór-plant beroekte persele steeds vry van nematodes en bome vaar betekenisvol beter in vergelyking met ná-plant behandelde persele. Veelvoudige veranderlike statistiese analises van groot datastelle wat vanaf bome in verskeie stadia van agteruitgang versamel is, word gebruik om die hoofkomponente en interaksies tussen faktore te bepaal, wat navorsers beter insig kan

gee in boom-agteruitgang en moontlike vroeë indikatore van boom-agteruitgang. Verskeie sitrus-onderstamme word bestudeer ten einde biochemiese meganismes betrokke in weerstand/bestandheid van sitrus-onderstamme teen *Phytophthora* wortelvrot, te ontrafel. Een boekhoofstuk is vir publikasie ingedien.

In die Sitrus Swartvlek (SSV) program is drie belangrike artikels gepubliseer, wat van onskatbare waarde in voortgesette marktoegang onderhandelinge met Europa sal wees. Navorsers is ook betrokke in 'n gesamentlike projek met die VSA, Brasiliaanse en Argentynse navorsers ten einde 'n waarskynlikheidsmodel te ontwikkel om kwantitatief die risiko van vrugte as 'n verspreidingsweg vir SSV te voorspel. SSV-epidemiologie word in hierdie projek deur spoorvangstudies en weermonitering bestudeer, asook in die Oos-Kaap, Limpopo en Mpumalanga provinsies. Die globale populasie-struktuur van die SSV-patogeen word bestudeer. Suiweprogramme word voortdurend verbeter ten einde funksiesweerstand en formulasies te verbeter, dit meer koste-effektief te maak, en om nuwe aktiewe bestanddele te registreer. Belangrike navorsing is afgehandel en gepubliseer was daarop dui dat die SSV patogeen 'n laer risiko van weerstandsontwikkeling teen die strobilurin swamdodergroep het. Vyf artikels en een boekhoofstuk is gepubliseer.

In die Vrug- en Blaarsiekte program, is nuwe beheer-opsies van *Alternaria* bruinvlek (ABV) bestudeer. Navorsing fokus ook op die verbetering van spuittoediening deur optimale gebruik van spuitmasjiene of byvoegmiddels. Flourisensie-pigment drempelwaardes wat effektiewe ABS-beheer toon, is bepaal, en word gebruik om spuitbedekkingsresultate beter te interpreteer. 'n Navorsingsartikel met hierdie bevindinge is gepubliseer. Teenstrydige resultate uit benatter-werk het daartoe gelei dat fundamentele navorsing hieroor gedoen sal moet word. 'n Nuwe eksperiment is geïnisieer om beheer van *Botrytis* bloeiselsversenging en vrugval in suurlemoene, te bestudeer. Geskikte funksiesdes is geïdentifiseer, maar die optimale tyd vir toediening moet egter nog bepaal word.

Die Na-oes siektes program bly 'n baie hoë prioriteit en verskeie projekte is direk gerig op die verbetering van na-oes siektebestuur in pakhuse. Moontlike alternatiewe funksiesdes en saniteerders word deurlopend geëvalueer, en sekere produkte wat in 2012 geëvalueer is, toon belofte. Imazalil, thiabendazole en pyrimethanil residu-lading, volgende op doop, funksiesbad- en wakstoedienings, asook die JBT vloedtoediening, en gevolglike bio-effektiwiteit teen sensitiewe en weerstandbiedende *Penicillium* isolate, is bestudeer, en het waardevolle insig in die gebruik van ons belangrikste na-oes funksiesde gegee. Studies oor die praktiese impak van imazalil-weerstand het duidelik getoon dat hierdie baie effektiewe funksiesde oneffektief raak wanneer teen weerstandbiedende isolate toegedien word. Weerstandbestuur is gevolglik van kardinale belang, en metodes om die weerstandsfrekwensies in pakhuse te bepaal, word tans geëvalueer. Integrasie van voor-oes silikon, en na-oes hitte en biobeheer teen groenskimmel, word ook tans bestudeer. Twee navorsingsartikels is gepubliseer.

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 8559 monsters analiseer, en dit met slegs een diagnostikus, een tegnikus en een assistent.

Goeie vordering is in die algemeen in Siektebestuur gemaak. Afgesien van voortgesette 'nie-navorsing' dienste, soos ondersteuning vir biosekuriteit, verbeteringskema, marktoegang, en formele en *ad hoc* voorligtingsaktiwiteite, het die kwaliteit en kwantiteit van tasbare navorsingsuitsette deur gekonsolideerde en gefokusde navorsing verbeter. In 2012-13 is 10 wetenskaplike artikels gepubliseer (8 x artikels en 2 x boekhoofstukke).

4.2 **PROGRAMME: GRAFT TRANSMISSIBLE DISEASES**

Programme coordinator: G. Cook (CRI)

4.2.1 **Programme summary**

Outbreaks of citrus diseases such as Psorosis, Exocortis and Cachexia are unheard of in the South African commercial context today. Young researchers in our country learn of these diseases in text books and specimen stumps preserved from a previous era. The virtual elimination of these graft transmissible pathogens in the commercial environment is due to focused research efforts and the establishment of the Citrus Improvement Scheme (CIS). Tree decline due to *Citrus tristeza virus* (CTV) and African Greening are two diseases which are no longer seen as the major production limiting factors that they were in what seems a distant past. In the background of this present experience are vector and pathogen management strategies. CTV cross-protection has been applied by the CIS since its inception. The selection of sources that are used for this management practice were based on years of field work and glasshouse trials. Grapefruit orchards especially, have much longer lifespans since the introduction of CTV cross-protection.

Breakdown in protection ability of the initial pre-immunising source GFMS12 was addressed by the replacement of GFMS 35. Current field trials on grapefruit support this replacement, yet we did not understand the underlying mechanisms.

Research of CTV dynamics and cross-protection mechanisms are conducted in project 885 at Pretoria University and project 1056 at CRI-Nelspruit. This has shown that the virus is a complex of various strains. It is only within the last few years that this understanding and the subsequent development of diagnostics for strain identification have become available. This insight now enables an understanding of which strains are associated with disease. In addition, a strain selection by the different citrus types occurs. The complexity of a mixture of strains and host selection complicates the understanding of the mechanism involved in cross-protection and ultimately why our industry experienced breakdown of this protection in certain circumstances. Within project 1056 [4.2.11], we have expanded on a published CTV strain testing system and have tested various maintenance sources of the GFMS12 and GFMS35 pre-immunisation sources (4.2.2). Segregation of strains is noted in different maintenance sources which gives insight into the cross-protection breakdown in the field. This project aims also to investigate the host selection of the CTV strains and the influence on symptom expression of specific strains. The ability to identify strains in a mixed population enables correlations to be made with symptom expression in the field and the CTV strain complement within the plant. Pre-immunisation sources applied in grapefruit trials generally show consistency between trials with regard to tree health, but ultimate selection of these sources should also be based on their influence on fruit production and quality. These field trials investigate cross-protection in a number of citrus types and are vital for selection of pre-immunising sources (Projects 679 [4.2.3], 738 [4.2.4], 739 [4.2.5], 742 [4.2.6], 789 [4.2.7] and 968 4.2.8).

Further understanding of citrus greening epidemiology is gained in two projects (886 [4.2.10], 988 [4.2.9]). Indigenous plants of the citrus family *Rutaceae* are evaluated for their ability to host "*Candidatus Liberibacter africanus*" (Laf) the African Greening pathogen (4.2.10) (Project 886). Laf has not been detected in any indigenous host yet and findings to date indicate they do not play a role in the epidemiology of citrus greening. Some Rutaceae genera have now been shown to harbour Liberibacters differing from Laf and include *Zanthoxylum*, *Vepris*, *Clausena* and *Calodendrum*. Full genome sequences of the different Liberibacter species, including the Laf, are available and will assist in understanding differences between these pathogens and their plant interactions. Significant progress has also been made towards sequencing of LafC genome, the Liberibacter found in *Calodendrum*.

The other epidemiological investigation of Greening, studied the seasonal population fluctuation of its vector, *Trioza erytreae*, and the seasonal fluctuations in the infectivity of triozaid populations with Laf (4.2.9) (Project 988). Results support the importance of the current triozaid control recommendations to control the spread of African Greening by limiting early population build-up during the first flush period.

Field evaluation of sweet orange, embryo-rescued clones, selected for resistance/tolerance to greening is on-going. No greening was detected in these trees after the fifth year in the field. The third crop was harvested and fruit quality and production compare favourably to a standard commercial variety (4.2.2) (Project 815). Resistant varieties to Greening are seen as the ultimate control mechanism and the potential of these selections hold much promise.

Programopsomming

Uitbrake van sitrussiektes soos Psorose, Eksokortis en Cachexia is vandag ongehoord in die Suid-Afrikaanse kommersiële konteks. Jong navorsers in Suid Afrika leer van hierdie siektes in handboeke en bewaarde voorbeelde van 'n vorige era. Die uitskakeling van ent-oordraagbare siektes in die kommersiële omgewing is te danke aan doelgerigte navorsingpogings en die totstandkoming van die Sitrus Verbeteringskema (SVS). Boom terugsterwing as gevolg van *Citrus tristeza virus* (CTV) en Afrika vergroeningsiekte is twee siektes wat nie meer beskou word as die belangrikste produksie beperkende faktore tot die mate wat hulle in die verlede ervaar was nie. In die agtergrond van die huidige ervaring is vektor en patoëen bestuur strategieë. CTV kruis-beskerming is toegepas deur die SVS sedert sy ontstaan. Die keuse van bronne wat vir hierdie bestuurspraktyk gebruik word, is gebaseer op jare se veld- en glashuisproewe. Pomelo boorde veral, het 'n baie langer kommersiële leeftyd sedert toepassing van CTV kruis-beskerming. Die verlies in beskermingsvermoë van die aanvanklike pre-immuniseringsbron, GFMS12, is aangespreek deur die vervanging met die GFMS35 bron. Huidige pomelo veldproewe ondersteun hierdie vervanging, maar ons verstaan nog nie die onderliggende meganismes nie.

Navorsing van CTV dinamika en kruisbeskermingmeganismes word in projek 885 by Pretoria Universiteit en projek 1056 [4.2.11] by CRI-Nelspruit gedoen. Hierdie werk van die CTV virus het getoon dat dit uit 'n kompleks van verskillende rasse bestaan. Hierdie begrip en die daaropvolgende ontwikkeling van

diagnostiese toetse vir ras identifikasie het onlangs beskikbaar geword. Hierdie insig maak dit nou moontlik om te bepaal watter rasse met siekte toestande geassosieer word. Daarbenewens is daar 'n CTV ras seleksie deur die verskillende sitrus kultivars. Die kompleksiteit van 'n mengsel van rasse en die gasheer bemoeilik die begrip van die meganisme wat betrokke is by kruis-beskerming en waarom ons bedryf afnames in die beskermingsvermoë ervaar onder sekere omstandighede. In projek 1056 [4.2.11], het ons uitgebrei op 'n gepubliseerde CTV rastoets tegniek en verskeie instandhoudingsbronne van GFMS12 en GFMS35 getoets (4.2.2). Segregasie van rasse is waargeneem in die verskillende instandhoudingsbronne wat 'n moontlike verduideliking is vir die verlies van hul kruisbeskermingvermoë in die veld. Hierdie projek het ook ten doel om die gasheer seleksie van die CTV rasse en die invloed daarvan op simptoom uitdrukking van elke ras te ondersoek. Die vermoë om CTV rasse te identifiseer in 'n gemengde bevolking stel ons in staat om korrelasies met simptoom uitdrukking en die CTV ras populasie onder boordtoestande te doen. Pre-immuniseringsbronne wat in pomelo proewe aangewend is, wys oor die algemeen konsekwentheid tussen proewe met betrekking tot boom gesondheid, maar die uiteindelige keuse van hierdie bronne moet gebaseer wees op die invloed daarvan op vrugproduksie en gehalte. Hierdie veldproewe ondersoek kruis-beskerming in 'n aantal sitrus tipes en is noodsaaklik vir die keuse van pre-immuniseer bronne (Projekte 679 [4.2.3], 738 [4.2.4], 739 [4.2.5], 742 [4.2.6], 789 [4.2.7] en 968 [4.2.8]).

Verdere begrip van Sitrus vergroeningsepidemiologie word ingewin binne twee projekte (886 [4.2.10], 988 [4.2.9]). Inheemse plante van die sitrus familie *Rutaceae* word ondersoek vir hul vermoë om as alternatiewe gasheer vir "*Candidatus Liberibacter africanus*" (Laf), die Afrika-vergroening patogeen, te dien [4.2.10] (Projek 886). 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*, huisves wel soortgelyke Liberibacters maar verskil van Laf.

Volledige genoom volgordebepaling van die verskillende Liberibacter spesies, insluitend die van Laf, is beskikbaar en dit sal die insig van hierdie patogene en hulle plant interaksies bevorder. Vordering is ook gemaak met die genoom volgordebepaling van LafC, die Liberibacter wat in *Calodendrum* gevind is [4.2.10], Projek 886.

Die ander epidemiologiese ondersoek van hierdie siekte word na die seisoenale bevolkingfluktuasie van die insek vector, *Trioza erytrae*, en moontlike seisoensverandering in sitrusbladvlooi populasies se besmettingsvlakke met Laf gekyk (4.2.9) (Exp 988). Resultate ondersteun die belangrikheid van die huidige vektor beheer aanbevelings om die verspreiding van vergroeningsiekte te beperk gedurende die vroeë bevolkingstoename tydens die eerste groeistuwingsyklus van die bome.

Veld evaluering van embryo-herwinningsklone vanaf soetlemoen wat potensiële weerstand / toleransie teen vergroening toon, is voortgesit. Geen vergroening is in hierdie bome na die vyfde jaar in die veld opgespoor nie. Die derde oes vanaf hierdie bome dui dat vruggehalte vergelykbaar is met 'n standaard kommersiële kultivar (4.2.2) (Exp 815). Bestandheid teen vergroeningsiekte word beskou as die uiteindelige beheermeganisme en die potensiaal van hierdie klone hou groot belofte in.

4.2.2 **FINAL REPORT: Dynamics of citrus tristeza virus mild and severe strains in mild strain cross-protection strategies.**

Project 885 (April 2007 – April 2013) by Gerhard Pietersen (ARC-PPRI and UP), D. Read (UP), O. Zablocki (UP) and J. Lubbe (UP)

Summary

This project was initially planned to determine the reasons for cross protection breakdown to citrus tristeza virus (CTV) in grapefruit. However, the subsequent watershed study of Foliminova et al. (2010) helped to clarify why breakdown was probably occurring, and defined objectives which would improve on the grapefruit cross protection strategy used. These objectives were therefore also included in this project. Mild strains or candidate mild strains, and some single aphid transfer (SAT) isolates of these (eg. 12-1 to 12-9 from GFMS12) already identified (van Vuuren and coworkers in numerous publications) were studied initially by characterizing these populations to genotype level and assessing the homogeneity of the sub-isolates. In early tests this was done using genotype-specific PCRs (Hilf et al., 2005), but the specificity of the test (unknown genotypes would not be detected), mixed genotype nature of the local CTV populations observed, and potentially confounding occurrence of inter-genotype recombination events, sub-genomic particles and potentially defective particles of CTV, made results ambiguous and difficult to interpret. This technique only indicated presence/absence of specific genomic regions of genotypes and was unable to determine the genotype that is predominant in the population, and hence most likely to be isolated by SATs. Therefore a new approach was utilized where amplicons of at least two (A-fragment and p23 gene) but sometimes four

(also the F-fragment and the CP gene) genomic regions of sub-isolated were prepared and cloned, and multiple clones sequenced. The amplicons were derived from primers which were designed against conserved regions within the CTV genotypes known at that stage (Rubio et al., 2001) but amplified sequences with sufficiently variability to identify genotypes. Using this technique we identified a number of genotypes predominant in various CTV populations both from pre-immunizing sources, including GFMS12 sub-isolates 12-7, 12-8 and 12-9, as well as the field. However, subsequent to the start of this project additional CTV genotypes were described internationally, some with the target primer binding sites on some genes containing potentially important sequence differences. Even under the non-stringent conditions employed during the study amplification of these genotypes may be selected against. Identity of genotypes with this approach is also based on just a few regions of the genome. Next generation sequencing (NGS) platforms were therefore evaluated for whole genome sequence characterization of genotypes, determination of the homogeneity of sources, determination of genotype composition of viral populations, detection of new and novel genotypes and possibly use in the analysis of multiple samples in parallel (for example for use in surveys). Three different templates of CTV (dsRNA replicative intermediate form, total RNA, and virus particles obtained by a simple immune-capture procedure) were assessed for the characterization of the GFMS12 sub-isolates 12-7, 12-8 and 12-9 and to confirm that they were in fact single genotype sources. All three templates were useful but each creates a bias of its own. For example, dsRNA templates represent genotypes actively replicating at the time of sampling and would have an over-representation of the 3' end genes. Yield of CTV-specific reads were relatively low with all three templates (6-7% of reads) but were sufficient for genotype identification, whole genome characterization, and homogeneity assessment of CTV in 12-7, 12-8 and 12-9. Based on whole genome sequences generated, it was demonstrated that all three sub-isolates contained the same predominant CTV strain, a novel strain called CTV-ZA1, CTV-ZA2 and CTV-ZA3 from 12-7, 12-9 and 12-8, respectively. This strain is most closely related to the T68-1 isolate of CTV, and clusters within the HA16-5 genotype clade (our results). T68 has recently (Harper, 2013) been proposed as a new genotype. This virus is present in all three sources in an overwhelming majority, however low numbers of reads of sequences unique to various other genotypes are present in all three populations. We are unsure at this stage whether this represents mutation- or recombination-induced variations within specific genes of the CTV-ZA isolates themselves or the presence of low levels of other genotypes in the population or artifacts of the short reads induced by the Illumina NGS platform. We anticipate greater clarity in interpreting NGS data as we gain experience. In conclusion, the highly defined objectives implicit in the results of Folimonova et al., (2010), embarked upon in this project, and now also advocated by Folimonova (2013), in combination with the superior identification and characterization abilities afforded by the NGS technique, serves as an excellent foundation for the subsequent project to progress significantly towards improved cross protection of grapefruit. Difficulties in isolating pure sources of the genotypes however will hamper progress and this still needs some attention in the subsequent project.

Opsomming

Hierdie projek het oorspronklik ten doel gehad om uit te vind hoekom kruisbeskerming teen sitrus tristeza virus (CTV) in pomelos afbreek oor tyd. Intussen het die opspraakwekende studie van Folimonova et al. (2010) help verduidelik hoekom dit moontlik gebeur, en het terselfdertyd ook goed gedefinieerde doelwitte daargestel om die kruisbeskerming van pommelos te verbeter. Hierdie doelwitte is dus ook ingesluit in dié projek. Milde rasse van CTV, of kandidaat milde rasse, en 'n paar enkel plantluis oordragings- (SAT) subisolate van hulle (bv. 12-1 tot 12-9 van GFMS12) is reeds vroeër deur van Vuuren en medewerkers in verskeie publikasies beskryf, en is dus die eerste bronne wat hier bestudeer is. Die genotipe samestelling van virus populasies en hulle homogeniteit is bepaal. In vroeëre toetse is dit gedoen deur middel van genotipe-spesifieke PKRe (Hilf et al., 2005), maar die onvermoë van die toetse om nuwe genotipes op te spoor, die veelvoudige genotipe samestelling van Suid-Afrikaanse bronne, en die moontlikheid van inter-genotipe rekombinasie van gene, sub-genomiese nuleïensuur produkte, en moontlike defektiewe nukleïensuur komponente, het gemaak dat die resultate ambivalent was en moeilik was om te interpreteer. Verder, omdat die tegniek net die teenwoordigheid al dan nie van CTV genotipes aandui en nie 'n aanduiding gee van die vlak van genotipes in die populasie nie, is dit moeilik om uit die data te voorspel watter genotipes waarskynlik geïsoleer sal kan word met SATs. 'n Nuwe benadering is toe later gebruik waar amplikone van ten minste twee (A-fragment en p23 geen) of vier (ook die F-fragment en die CP geen) subgenomiese gedeeltes van die virus sub-isolate voorberei, gekloneer en die nukleotiedvolgorde van verskeie klones bepaal. Die amplikone is afkomstige van stukke genoom geamplifiseer met voorvoegsels gemik teen die gekonserveerde nukleotied volgorde daardie tyd bekend van die CTV rasse (Rubio et al, 2001), en amplicone gedeeltes varieërbaar genoeg om tussen genotipes te onderskei. Met hierdie benadering het ons verskeie genotipes en hul vlakke in verskeie CTV bronne geïdentifiseer, insluitend GFMS12 se 12-7, 12-8 en 12-9 subisolate sowel as bronne uit kommersiële boorde. Sedert die begin van die projek is daar egter verskeie nuwe CTV genotipes internasionaal beskryf, waarvan van hulle verskille in die voorvoegsel teken nukleotied volgorde bevat. Selfs met die nie-streng bindings kondisies wat ons vir

PKR gebruik het, is dit moontlik dat van dié genotipes swak of glad nie sal amplifiseer nie. Verder word met hierdie benadering weereens slegs klein gedeeltes van die genoom bepaal. Nuwe geslag nukleotiedvolgordebepaling (NGS) was dus geëvalueer vir die heel genoom karakterisering van genotipes, bepaling van homogeniteit van bronne, bepaling van die bevolkingskomposisie, opsporing van nuwe of unieke genotipes en parallel opsporing (vir gebruik in opnames). Drie tipes begin materiaal is aanvanklik geëvalueer vanaf subisolaat 12-7 namens dubbelstring RNA (dsRNA), totale RNA, RNA vanaf virus partikels, en RNA verryk deur immunobinding van viruspartikels. Al drie begin materiaal is gevind om bruikbaar te wees, maar elkeen het spesieklike voorkeure en nadele. By voorbeeld, dsRNA verteenwoordig slegs virus wat aktief repliseer ten tye van monsterneming, en sal ook 'n oorvertenwoordiging van 3' end gene bevat. Die opbrengs van CTV-spesifieke sekvenslopie vir 12-7 met aldrie was redelik laag (6-7% van die totale sekvenslopie) maar was voldoende vir die identifikasie van genotipes, heelgenombepaling, en homogeniteit van die virusbevolking van subisolaat 12-7, 12-8 en 12-9 te verkry. Daar is bepaal dat al drie bronne bevat dieselfde dominante genotype en, gebaseer op die heelgenoom, dat hierdie 'n nuwe CTV ras is, wat bekend staan as CTV-ZA1, CTV-ZA2 en CTV-ZA3, uit bron 12-7, 12-9 en 12-8 respektiewelik. Hierdie ras is naverwant aan T68-1, en kom saam met die HA16-5 se vertakking van dendrograme voor (ons resultate). T68 is egter onlangs (Harper, 2013) as 'n nuwe genotype voorgestel. Hierdie virus is teenwoordig in al drie bronne in 'n oorweldigende meerderheid, maar alle bronne bevat egter ook lae vlakke van sekvenslopie ("reads") verwant aan ander genotipes. Ons is nie tans seker hoe om hierdie ander sekvenslopie se teenwoordigheid te interpreteer nie. Dit mag óf mutasie geïnduseerde verskille (kwasi-spesies) óf areas van rekombinasie verteenwoordig van die CTV-ZA virus self, of mag die teenwoordigheid van die ander genotipes teen lae vlakke verteenwoordig, óf self kwinkslae van die sekvenslopie geïnduseer deur die Illumina platform wees. Ons sal egter met ervaring en verder toetse leer om sulke inligting te interpreteer.

In opsomming, die hoogs gedefiniëerde doelwitte implisiet in die resultate van Foliminova et al. (2010) wat opgeneem is in dié projek, en wat nou pertinent deur Folimonova (2013) aanbeveel word, saam met die kragtige NGS tegnieke sal dien as 'n uitstekende fondasie van waar die opvolgende projek tot dié een kan vorder in die verbetering van die kruisbeskerming van pommelos. Probleme om enkelbronne van die genotipes te verkry sal egter 'n stremming op die vordering plaas en kort meer aandag in die opvolg projek.

4.2.3 PROGRESS REPORT: Evaluation of citrus material for greening resistance

Project 815 (2006 - 2015) by S.P. van Vuuren (CRI)

Summary

Attempts are made to obtain greening resistance by rescuing embryos from healthy chimeras on greening infected fruit and growing them on artificial medium. The little plants that are generated are micro-grafted on vigorous rootstocks. These clones are multiplied on healthy rootstocks and exposed to field triozids. 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 triozid 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 triozids that were used for challenging the plants were infected with the greening organism. A third clone, GTC-14, showed possible tolerance. These three clones have been multiplied on virus-free rootstocks and separately pre-immunised with two *Citrus tristeza virus* sources where after they were planted during 2007 in an orchard for field evaluations. After 5 years no greening symptoms were observed on the trees and PCR tests were also negative. The third crop was harvested from the trees and fruit quality compared favourably with the Midnight Valencia control. Clone GTC-E2 had the best production and was significantly better than the Midnight Valencia. Generally fruit size was smaller this year due to a higher yield. External and internal qualities were within the export requirements. No fruit with suitable chimeras were found during 2012 and therefore no new clones were generated. This part of the Project will be terminated. A new project will be registered when suitable chimeras are found again.

Opsomming

Daar word gepoog om vergroening weerstandbiedendheid te verkry deur embryo's uit gesonde chimeras van vergroende vrugte te verwyder en op kunsmatige medium te kweek. Die plantjies wat genereer word, word op groeikragtige onderstamme deur middel van mikro-enting gevestig. Hierdie klone word op onderstamme vermeerder en aan boord sitrusbladvlooië, 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 vergroening en sodoende die plante blootgestel het aan die organisme. Na 3 maande word die plante ge-evalueer vir die voorkoms van vergroeningsimptome 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 vergroenings-organisme 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 geïdentifiseer na blootstelling aan besmette insekte. PKR het getoon dat hulle vry van die organisme is. 'n Derde kloon, GTC-14, het moontlike toleransie getoon. Die drie klone is op onderstamme vermeerder en afsonderlik met twee *Citrus tristeza virus* bronne gepreïmmuniseer en gedurende 2007 in 'n boord uitgeplant vir verdere evaluasie. Na 5 jaar is nog geen vergroeningsimptome waargeneem nie en PKR toetse was ook negatief. Die derde oes is van die bome verkry en vruggehalte vergelyk goed met die Midnight Valencia kontrole. Kloon GTC-E2 het die beste produksie gelewer en was betekenisvol beter as die van Midnight Valencia. Vruggrootte was hierdie jaar oor die algemeen kleiner as gevolg van 'n hoër produksie. Uiterlike en inwendige gehalte het aan die uitvoer vereistes voldoen. Geen vrugte met geskikte chimeras is gedurende 2012 gevind nie en is daar geen nuwe klone gegenereer nie. Hierdie gedeelte van hierdie Projek word gesluit. 'n Nuwe projek sal geregistreer word wanneer geskikte chimeras weer gevind word.

4.2.4 FINAL REPORT: Cross-protection of Star Ruby grapefruit using Beltsville sub-isolates of Nartia mild strain

Project 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 Marsh trees were planted at Riversbend in the Nkwaleni Valley and the Star Ruby trees at Tambuti Estates in Swaziland during 2003. The citrus orchards at Riversbend, including the trial trees, were removed in 2009 and replaced with sugarcane. Limited yield data was obtained from the Marsh trial. Star Ruby trees were evaluated for yield and stem pitting for 9 years. General observations of the trial are: i) GFMS12 suppressed growth and was associated with severe stem pitting; ii) sub-isolate 12/7 inoculated trees also developed unacceptable stem pitting; iii) The current cross protector GFMS35, showed no stem pitting and is therefore considered superior to GFMS12 with regard to tree health performance. The MxT rootstock performed poorly in this trial and trees only produced acceptable yields late in the trial. Unfortunately the Star Ruby trees were hedged as part of the normal management practices of the farm, and smaller trees, due to the CTV infection, were not hedged. Yield data was thereafter not reliable for comparative yield assessment. Virus free trees started to show stem pitting 6 years after planting, which indicated that new CTV infections following aphid vectoring did occur. The trial was terminated as per the proposed schedule. Data of this trial will be evaluated in combination with similar grapefruit trials (project 742) on Marsh and Star Ruby. Recent molecular diagnostics now enables detection of CTV strains in mixed populations. To finalise this trial, windows will be cut in the bark to do an accurate stem pitting assessment and the inner cambium and phloem layers of each tree will be tested to determine the CTV strains present. A final report containing this additional information and the cumulative yield data for the duration of the trial will be presented in 2014.

Opsomming

Die Nartia CTV preïmmuniseringsbron (GFMS 12) is met GFMS 35 as 'n CTV-kruisbeskermingsbron vir rooi pomelos gedurende 1998 vervang, 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 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 gepreïmmuniseer, twee sub-

isolate van die LNR-ITSG (12/7, 12/9) en die GFMS12 en GFMS35 bronne. Virusvrye bome is as kontroles gebruik. Preïmmunisasie is deur middel van ELISA bevestig wat ook uitgewys het dat twee van die Beltsville sub-isolate nie voldoen aan die vereistes vir 'n goeie kruisbeskermingsbron nie; deurdat hulle 'n lae persentasie oordraagbaarheid het, asook stadig vermeerder en beweeg in die plant. Hierdie twee sub-isolate word nie verder geëvalueer nie. Die Marsh proef is gedurende 2003 by Riversbend in die Nkwaleni Valei geplant en die Star Ruby proef by Tambuti landgoed in Swaziland. Die sitrusboorde, insluitend die Marsh proef, is by Riversbend in 2009 verwyder en met suikerriet vervang. Beperkte opbrengs data is van die Marsh proef verkry. Star Ruby bome is vir opbrengs en stamgleuf ontwikkeling vir 9 jaar geëvalueer. Die volgende waarnemings is gemaak, wat betref boom gesondheid: i) GFMS 12 onderdruk groei en veroorsaak strawwe stamgleuf; ii) sub-isolaat 12/7 het ook onaanvaarbare stamgleuf veroorsaak; iii) die huidige kruisbeskermer GFMS35, presteer beter as GFMS12, die vorige beskermer deurdat geen stamgleuf waargeneem is tydens die proef nie. Die MxT onderstam het nie goed presteer nie en aanvaarbare oes data was eers laat in die proef tydperk verkry. Ongelukkig is die Star Ruby bome meganies gesnoei as deel van die boordbestuur van die plaas, en kleiner bome, as gevolg van die CTV-infeksie, is nie gesnoei nie. Opbrengs data was dus daarna nie vergelykbaar vir die opbrengs analise nie. Ses jaar na plant het virus-vry geplante bome stamgleuf ontwikkel wees nuwe CTV besmetting na plantluis oordraging. Dit wys dat daar wel infeksiedruk in die omgewing was. Die proef is binne die voorgestelde skedule beëindig. Data sal in kombinasie met soortgelyke pomelo proewe (projek 742) op beide Marsh en Star Ruby geëvalueer word. Onlangse molekulêre diagnose maak dit nou moontlik vir die opsporing van CTV rasse in gemengde populasies. Voor finalisering van hierdie proef, sal vensters in die bas van elke proef boom uitgesny word om 'n akkurate stamgleuf evaluering te doen en die binneste kambium en floëem lae van elke boom sal vir die spesifieke teenwoordigheid van CTV rasse getoets word. 'n Finale verslag met hierdie bykomende inligting en die kumulatiewe opbrengs data vir die duur van die proef sal in 2014 voorgelê word.

Introduction

Citrus tristeza virus (CTV) is the causal agent of one of the most destructive disease of citrus. The disease is spread by propagation material and various aphid species of which *Toxoptera citricida* is the most efficient. Many strains of CTV occur and co-exist as mixtures in individual field trees. Symptoms induced by CTV, range from mild with no noticeable effect on the host to severe stem pitting and decline, resulting in uneconomic production (Marais *et al.*, 1996). The only practical means of controlling CTV at present is by mild strain cross-protection (van Vuuren *et al.*, 1993). A breakdown in the protection offered by the Nartia A (GFMS 12) source owing to the probable presence of a severe component within the complex (van Vuuren *et al.*, 2000), motivated the separation of strains by single aphid transmission (SAT). Twenty SAT sub-isolates were obtained from two Nartia sources (Nartia A = GFMS 12 and Nartia C = GFMS 14; van Vuuren *et al.*, 1993) and the Mouton source that was collected by L.J. Marais (Outspan). These sub-isolates have undergone biological indexing to differentiate between the severe and mild forms. Some sub-isolates showed no potential as cross-protecting sources as titre and movement of the virus in the plant were poor and/or severe stem pitting was associated with them. Four of these sub-isolates will be evaluated in field trials as potential cross-protecting sources. Promising SAT sub-isolates of Nartia obtaining from the ARC-ITSC are also included in this experiment. As CTV exhibits host and climatic specificity, it is imperative that mild protective isolates be tested in the different production areas.

Objectives

To compare the cross-protecting ability of CTV sub-isolates to existing pre-immunising sources on Grapefruit under field conditions in Swaziland.

Performance evaluation is based on:

- Presence/severity of stem pitting
- Tree canopy volume
- Yield
- Fruit size

Materials and methods

The 20 SAT sub-isolates of the Nartia A and C CTV cross-protecting sources (A=GFMS 12 and C=GFMS 14) as well as those from the Mouton source were prepared at the quarantine facility in Beltsville MD, USA and were imported back to South Africa. In a greenhouse experiment, they were bud-inoculated separately onto CTV sensitive Mexican lime indicator plants to differentiate the sub-isolates according to their effects on the host. Growth and stem pitting ratings were determined and the virus titer was measured by means of enzyme-linked immunosorbent assay (ELISA) for each sub-isolate 6 months after inoculation. The four sub-

isolates with the best potential (GFMS 14 subs: B389-1, B389-4; Mouton subs: B390-3, B390-5) were used to pre-immunise virus-free Star Ruby grapefruit on MxT rootstocks that were prepared under insect-free conditions in the greenhouse. Their potential as cross-protectors are being compared to GFMS 12 (previous standard for white grapefruit), GFMS 35 (present standard for grapefruit), GFMS 12/7 and GFMS12/9 (ARC-ITSC single aphid transfer sub-isolates from GFMS 12) and control trees planted with a virus-free status. Pre-immunisation has been confirmed by ELISA 6 months after inoculation. In 2003 the Star Ruby grapefruit trees were planted in Swaziland at Tambuti Estates. The trees were planted in a randomised block design with 5 replications. Tree size, were measured and the canopy volumes calculated according to Burger *et al.* (1970), which uses a formula assuming the trees' form is half sphere *viz.* volume = $R^2(PIH-1.046R)$, where R = the radius of the tree and H = the height of the fruit bearing part, production and tree health are monitored on an annual basis.

Results and discussion

Objective / Milestone	Achievement
A. Evaluate horticultural performances:	
1. Canopy volume measurement	Assessed visually.
2. Yield and fruit size assessment	Achieved, see results below
3. Stem pitting development and decline assessment	Achieved, see results below
B. Report writing	

Tree size:

The trees were planted in a commercial block of Star Ruby and as of 2010 were pruned with all the other trees in the orchard. Visually, trees inoculated with GFMS 12 were much smaller than those of other treatments.

Production:

The trees were harvested and graded into the different fruit sizes (Table 4.2.4.1). GFMS12 and B389/4 had the highest yield per tree and the virus-free trees the lowest.

Tree health:

Trees were inspected for the occurrence of stem pitting and rated on a severity scale of 0 to 3, where 0 is a smooth trunk with no visible pits, and 3 is severe stem pitting accompanied by decline of twigs (Table 4.2.4.2). All trees pre-immunised with GFMS 12 displayed severe stem pitting, confirming that GFMS 12 contains a severe CTV component. Sub-isolate GFMS 12/7 also showed stem pitting.

Table 4.2.4.1. Average yield (kg/tree), of Star Ruby grapefruit trees pre-immunised with different CTV sources and sub-isolates, 9 years after planting at Tambuti.

Treatment	Fruit size (kg)						Total kg/tree
	27	32	36	40	48	64	
B389/1	3.1 a	3.1 ab	8.1 ab	11.2 ab	16.6 bcd	5.1 bcd	47.4 abc
B389/4	6.4 ab	6.3 b	16.8 c	24.6 c	24.7 cd	4.8 abcd	83.7 c
B390/3	10.3 bcd	5.9 b	12.9 bc	16.4 abc	16.2 bc	3.4 abc	65.1 bc
B390/5	9.5 bcd	3.8 ab	11.5 abc	8.0 a	7.5 ab	0.7 ab	40.9 ab
GFMS 12/7	14.4 d	5.3 b	11.7 abc	6.0 a	3.6 a	0.4 a	41.3 ab
GFMS 12/9	12.9 cd	6.3 b	11.2 abc	9.7 ab	5.9 ab	1.4 ab	47.2 abc
GFMS 12	6.6 ab	5.3 b	14.5 bc	19.3 bc	17.9 bcd	9.0d	72.5 bc
GFMS 35	1.5 a	1.8 a	8.0 ab	15.9 abc	29.0 d	7.5 cd	63.6 bc
Virus-free	1.9 a	3.9 ab	4.9 a	9.6 ab	5.5 ab	0.9 ab	26.7 a

Table 4.2.4.2. Percentage large and small fruit of trees pre-immunised with different CTV sources and sub-isolates.

Treatment	% large fruit (27-32)	% small fruit (40-64)
B389/1	30	70
B389/4	35	65
B390/3	45	55
B390/5	60	40
GFMS 12/7	76	24
GFMS 12/9	65	35
GFMS 12	36	64
GFMS 35	18	82
Virus-free	40	60

Table 4.2.4.3. Stem pitting rating of Star Ruby grapefruit trees pre-immunised with different CTV pre-immunising sources and sub-isolates, 9 years after planting at Tambuti*.

Treatment	Stem pitting rating**
B389/1	0 a
B389/4	0 a
B390/3	0.1 a
B390/5	0 a
GFMS 12/7	1.6 b
GFMS 12/9	0.7 a
GFMS 12	3 c
GFMS 35	0 a
Virus-free	0.6 a

* Figures in each column followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

** Stem pitting rating: 0 = Smooth trunk; 1 = Mild pitting; 2 = Moderate pitting; 3 = Severe pitting.

Conclusion

GFMS12 suppressed growth and severe stem pitting was associated with this source. Trees inoculated with sub isolate GFMS12/7 developed unacceptable stem pitting. Trees containing the current CIS cross protector for grapefruit, GFMS35 performed better than those inoculated with the previous protector GFMS12 where tree health is concerned. Sub isolate B389/4 gave the best average yield per tree this season. The trial was terminated as per the proposed schedule. Data of this trial will be evaluated in combination with similar grapefruit trials on Marsh and Star Ruby. Current molecular diagnostics enables detection of CTV strains in mixed populations. To finalise these trials windows will be cut in the bark to do an accurate stem pitting assessment and the inner cambium/phloem layer of each tree will be tested for the CTV strain complement. A final report containing this additional information and the cumulative yield data for the duration of the trial will be presented as the final report in 2014.

Technology transfer

1. Presentation at 7th Citrus Research Symposium; Citrus Tristeza Virus cross-protection of Star Ruby grapefruit: field trial results J.H.J. Breytenbach, S.P. van Vuuren and G.Cook

Further objectives and work plan

This project will terminate after the current financial year.

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4.2.5 **PROGRESS REPORT: Cross-protection of Star Ruby using Beltsville sub-isolates of Nartia mild strain for the Orange River Valley**

Project 738 (2004 - 2014) by G. Cook, S.P. van Vuuren and J.H.J. Breytenbach (CRI)

Summary

Indications of a possible severe *Citrus tristeza virus* component in the Nartia (GFMS 12) 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 GFMS 14 and 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 (GFMS 14: 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 GFMS 35 (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 and re-infection indicator. 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 areas (experiment 742). During this report period, tree sizes were measured at the Kakamas trial 8 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. Trees were also harvested and graded into export sizes. The average yield per tree of all the treatments did not differ significantly. Trees with B389/1 had significantly more small fruit than all the other treatments and the least large fruit while trees with GFMS 12/7 yielded the most large fruit and the least small fruit.

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 GFMS 14 en 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 (GFMS 14: 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. GFMS 12 (vorige kruisbeskermingsbron) en GFMS35 (huidige kruisbeskermingsbron) is as kontrole verwysings gebruik. Virusvrye Star Ruby boompies is in 'n glashuis voorberei en met die verskeie bronne gepreïmmuniseer. 'n Virusvrye behandeling is as kontrole ingesluit wat herbesmettings sal aandui. 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 is 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 word jaarliks vir boomgrootte, stamgleuf, oes opbrengs en vruggrootte ge-evalueer. Die bome se groottes is 8 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. Bome was ook ge-oes en die produksie gegradeer volgens uitvoer groottes. Die gemiddelde opbrengs per boom van al die behandelings het nie betekenisvol verskil nie. Bome met B389/1 het betekenisvol meer klein vrugte as die ander behandelings geproduseer asook die minste groot vrugte terwyl bome met GFMS 12/7 die meeste groot vrugte en die minste klein vrugte geproduseer het.

4.2.6 **PROGRESS REPORT: The effect of different CTV sources in Valencias on different rootstock combinations for the Orange River Valley**

Project 739 (2004 - 2014) by G. Cook, S.P. van Vuuren and J.H.J. Breytenbach (CRI)

Summary

Disease expression of *Citrus tristeza virus* (CTV) is influenced by host cultivar and potentially also 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, Midknight, 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 5 years after planting. Although there are significant differences in growth between treatments for each scion cultivar, it is too early to draw any conclusions with regard to the preferred pre-immunisation source as production and tree health rating data is required over a number of production seasons before final assessments can be made.

Opsomming

Siekte uitdrukking van *Citrus tristeza virus* (CTV) verskill tussen sitrusgashere en waarskynlik onder verskillende klimaatstoestande. Dit is dus nodig om verskillende CTV bronne in verskillende sitrus produserende streke te evalueer. Ponteniële CTV preïmmuniseringsbronne wat oorspronklik vanaf soetlemonbome versamel is (SM 46, SM 47, SM 48, SM 49), is gebruik om virusvrye Delta-, Midknight-, en Turkey Valencia op C35 citrange onderstam te preïmmuniseer. Hierdie bronne word met LMS 6 (die standaard preïmmuniseringsbron vir soetlemonoene) 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 5 jaar na uitplant gemeet en alhoewel daar statistiese verskille tussen die verskillende behandelings by elke cultivar was, is dit nog te vroeg om enige gevolgtrekkings te maak. Produksie en boomgesondheid data versamel oor 'n aantal jare is nodig vir volledige evaluasie.

4.2.7 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 Project 742 (2004 - 2014) by G. Cook, S.P. van Vuuren and J.H.J. Breytenbach (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 Nkwaleni 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 used 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 Letsite 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 resulted in suppressed tree growth. Although differences were observed, conclusions as to the preferred pre-immunisation source can only be made after production and tree health rating data is acquired over a number of production seasons.

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 geïnokuleer (biologiese indeksering) om te bepaal of die bome moontlik ligte rasse van *Citrus tristeza virus* (CTV) huisves wat as kruisbeskerminingsbronne 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 geïnokuleer 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 geïnokuleerde 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 viroiede, is Tabankulu 1 (versamel vanaf Star Ruby in Swaziland), New Venture 41/2 (versamel vanaf Star Ruby in die Nkwaleni Valle), ORE 8 (versamel

vanaf Marsh in die Hoedspruit gebied) 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 ontwikkeling getoon wat ook sodoende die groei belemmer het. Alhoewel daar verskille voorkom, is dit nog te vroeg om enige verdere gevolgtrekkings te maak.

4.2.8 **PROGRESS REPORT: Identification of suitable *Citrus tristeza virus* sources for pre-immunising Turkey Valencia**

Project 789 (2005 - 2015) by G. Cook, S.P. van Vuuren and J.H.J. Breytenbach (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 local citrus export portfolio 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. Tree growth and yield were measured 5 years after planting and although differences were observed, conclusions as to the preferred pre-immunisation source can only be made after production and tree health rating data is acquired over a number of production seasons.

Opsomming

Daar is gevind dat Turkey Valencia meer gevoelig vir *Citrus tristeza virus* (CTV) as ander Valencia tipes is (CRI Groep Navorsingsjaarverlag, 2003). Aangesien Turkey Valencia 'n vroeë Valencia is, is dit 'n belangrike kultivar in die bedryf en daarom is dit 'n hoë prioriteit om 'n geskikte CTV preïmmunisasie bron vir Turkey Valencia te vind. Virusvrye Turkey Valencia op Troyer citrange onderstam is in 'n glashuis voorberei en met verskeie CTV bronne, LMS 6 (standaard), SM 46, SM 47, SM 48, SM 49 (almal vanaf soetlemoene versamel), geïnkuleer om die beste ligte CTV bron vir kruisbeskermingsdoeleindes te identifiseer. Bome wat met die GFMS 12 bron geïnkuleer is en bome wat virusvry gelaat is, dien as positiewe en negatiewe kontroles onderskeidelik. Preïmmunisasie is deur middel van ELISA bevestig en die bome is gedurende Maart 2007 by Riverside in die Malelane omgewing geplant. Die boompies is 5 jaar na uitplant, vir groei en produksie ge-evalueer. Daar was verskille by boomgrootte en produksie tussen die behandelings, maar dit is nog te vroeg om enige gevolgtrekkings te maak.

4.2.9 **PROGRESS REPORT: Searching for a *Citrus tristeza virus* source suitable for cross-protecting soft citrus**

Project 968 (2004 - 2014) by G. Cook, S.P. van Vuuren and J.H.J. Breytenbach (CRI)

Summary

During re-indexing of the Citrus Foundation Block mother trees in 2003 it was found that many clementine and mandarin trees did not contain CTV despite prior pre-immunisation. This caused concern as the bud wood 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-immunized with different CTV sources; *i.e.* EM, 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. Due to frost and drainage problems one year after planting at both sites, new trees had to be prepared and the trial re-planted. New trees were prepared and the trial replanted during December 2012 in the Citrusdal area. Trees for the Groblersdal site have been prepared and will be planted in spring, 2013.

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 plantuise oorgedra word nie. Die CTV bekermingsbron is noodgedwonge na GFMS12 verander totdat 'n meer 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. EM, 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). As gevolg van dreineringsprobleme en koue-skade vestiging van hierdie bome belemmer het, is nuwe bome voorberei. Die nuwe boompies is gedurende Desember 2012 in die Citrusdal omgewing geplant. Boompies vir die Groblersdal omgewing sal gedurende die lente van 2013 geplant word.

4.2.10 FINAL REPORT: Investigation into the seasonal population fluctuation of *Trioza erytreae* and infection with the greening organism, *Candidatus Liberibacter africanus* Project 988 (2010 - 2013) by G. Cook, Zama Maqutu and S.P. van Vuuren (CRI)

Summary

Trioza erytreae (Del G.) (Hemiptera: Triozidae) is in itself a minor pest of citrus, but its significance is attributed to its ability to vector the pathogen of African Citrus Greening disease, *Candidatus Liberibacter africanus* (Laf). The population fluctuation of the citrus trioqid is correlated to the flushing rhythm of the citrus host, but seasonal fluctuation in infection of populations carrying the Greening pathogen have been difficult to monitor in the past due to limited detection methodologies. This study explored this fluctuation in infectivity in an orchard entirely infected with African greening by using PCR to test individual triozids caught on sticky traps placed weekly in an orchard. Two season's data was collected from a small sour orange orchard in the Nelspruit district. The project approach was changed from the original approach which aimed to trap triozids from natural vegetation and test transmission of the Greening pathogen to the trap plants. This change was implemented as no positive transmissions to a total of 420 trap plants was obtained in the first season by placing flushing citrus trap plants adjacent to natural vegetation some distance away from any citrus plantation. Additionally triozids were very seldom detected on the traps at these sites. The change was therefore made to test within an infected citrus orchard. An additional experiment was also conducted when a trioqid outbreak was observed in a sweet orange orchard, which showed a high incidence of African Greening, also in the Nelspruit district. Triozids collected here were used in a challenge study.

The trioqid population fluctuations monitored in the sour orange orchard correlated to previous findings of population peaks following the citrus flush cycles and population decline in response to extreme weather conditions, reflected in high saturation deficit values and lethal days. Fluctuations in the percentage infectivity of the *T. erytreae* populations were observed, with infectivity peaking with or just after the citrus flush seasons, but with peaks in infectivity differing to population peaks in that infectivity levels were maintained for longer than the population peaks and were also not dependent on population size. The transmission study again demonstrated that individual triozids are inefficient vectors in that although plants were exposed to vectors that tested positive, this did not relate to positive transmissions in every instance. Nonetheless this inefficiency is compensated for by the high population numbers in a natural environment, increasing the vectoring ability substantially. Further, it was established that positive *T.erytreae* individuals are found throughout most of the season in an infected orchard even though peaks in infectivity are found. The study underscores the importance of the current trioqid control recommendations to control the spread of African Greening by limiting population build-up during the first flush period. This remains vital due to the combination of high infectivity levels and larger populations of the vector over that period. A low population survival early in the season also limits later population build-up. This is important as triozids developing on subsequent flushes can have similarly high infection levels as in the early season and can still vector the disease despite lower vector numbers.

Opsomming

Trioza erytrae (Del G.) (Hemiptera: Triozidae), die sitrusbladvlooi, is op sigself nie 'n ernstige sitrusplaag nie, maar sy belangrikheid as sitrusplaag is weens sy vermoë om die sitrus-vergroeningsiekte patoogen, *Candidatus Liberibacter africanus*, oor te dra. Seisoenale bevolking-fluktuasies van die sitrusbladvlooi word gekorreleer met die groeistuwing van die sitrus gasheer, maar seisoenale wisseling in besmetting van die bladvlooi met die vergroeningspatoogen was moeilik bepaal in die verlede weens beperkte opsporing metodes. Hierdie fluktuasie in besmetting is bestudeer in 'n sitrus boord, tenvolte besmet met vergroeningsiekte. Bladvlooi is met geel, taai lokvalle gevang en individuele bladvlooi is met PKR getoets. Twee seisoene se data, vanuit 'n klein bitterlemoen boord in die Nelspruit-distrik, is ingesamel. Die projek benadering is verander van die oorspronklike benadering wat daarop gemik was om bladvlooi van natuurlike plantegroei te ondersoek deur oordraging van die vergroeningsiekte na lokval plante te toets. Bladvlooi was selde gevang en geen positiewe oordraging na 420 lokval plante was verkry deur die plasing van lokval plante aangrensend aan natuurlike plantegroei 'n entjie weg van enige sitrus aanplanting. Hierdie benadering was nie suksesvol nie en die verandering is dus gemaak om die ondersoek in 'n besmette sitrusboord te doen. Daarbenewens is 'n bladvlooi uitbraak in 'n soet lemoen boord in Nelspruit distrik waargeneem, wat ook 'n hoë besmetting van Vergroening getoon het. Bladvlooi is hier versamel en gebruik in 'n oordraging studie.

Bevolkings wisseling van die sitrus bladvlooi het gekorreleer met vorige bevindings van populasiepieke na aanleiding van die sitrus groeistuwing siklusse. Die bevolkings het ook gedaal in reaksie op uiterste weerstoestande weerspieël in hoë temperature en laë lugvog. Wisseling in die persentasie besmetting van *T. erytrae* bevolkings is waargeneem waar besmettingspieke gelyktydig met, of net na die sitrus groeistuwing seisoene geval het. Hierdie besmettings pieke het verskil van populasiepieke deurdat besmetting vlakke vir langer in stand gehou was en was onafhanklik van die bevolking getalle. Die oordragstudie het weereens gewys dat individuele sitrusbladvlooi, ondoeltreffende vektore van vergroeningsiekte is deurdat alhoewel plante blootgestel is aan vektore wat positief getoets het vir die patoogen, dit nie in elke geval 'n positiewe oordraging verseker het nie. Nietemin, hierdie ondoeltreffendheid is vergoed met die hoë bevolking getalle in 'n natuurlike omgewing wat die doeltreffendheid van die oordragingsvermoë aansienlik verhoog.

Vêrder is daar vasgestel dat positiewe *T.erytrae* deurgangs die jaar gevind word in 'n besmette boord, al is pieke in besmetting wel gevind. Die studie beklemtoon die belangrikheid van die huidige sitrusbladvlooi beheer aanbevelings wat die verspreiding van vergroeningsiekte beheer deur die beperking van opbou van vektor bevolkings gedurende die eerste groeistuwing tydperk. Beheer oor hierdie tydperk is noodsaaklik as gevolg van die kombinasie van hoë besmettingsvlakke asook groter bevolkings van die vektor oor hierdie tydperk. 'n Laë bevolking oorlewing vroeg in die seisoen beperk later populasiegetalle. Hierdie vroeë beperking is belangrik omdat bevolkings later in die seisoen soorgelyke vlakke van infeksie kan bereik as wat daar in die vroeë seisoen gekry word en dus kan vergroeningsiekte wel ook oor hierdie tydperk versprei word al is die vektor getalle laër.

Introduction

Studies have shown that the *Trioza erytrae* populations in citrus orchards increase with cooler, moist weather conditions and that population increase coincides with the flush cycles of citrus (van Vuuren & Moll, 1984). In this study, Greening transmission was evaluated based on symptom development on trap plants and transmission was also correlated to an increase in the trioqid population. This study spanned 3 years in which the trioqid populations were high in the first year and very low in the subsequent 2 years due to very hot, dry conditions and greening transmission was therefore very low (7). In an even earlier study, seasonal fluctuations in the infectivity of trioqid populations were seen, also based on transmission to trap plants (McClean, 1974). PCR now enables testing of individual trioquids for the greening organism, "*Candidatus Liberibacter africanus*" (Laf). In this manner populations can now be monitored for their infectivity and transmission of Laf to the citrus host can also be confirmed.

This trial aims to re-examine the population dynamics and greening transmission of *T. erytrae* in citrus orchards and adjacent natural vegetation. Continual movement of trioqid populations between citrus and natural vegetation has been demonstrated (van den Berg et al., 1991b) and trioqid populations were shown to increase more rapidly in indigenous vegetation during certain periods and in citrus in other periods (van den Berg et al., 1991b). PCR will enable us to determine whether the trioqid adults originating mainly from natural vegetation are infective all year round or more seasonally. Alternate hosts for Laf have not yet been established and this aspect is currently under investigation in project 886; (*Epidemiology of citrus greening disease-alternate host and spread*). With this project we hope to determine whether natural vegetation harbouring possible alternate hosts for Laf, is a source of infection, as well as to determine if there are

seasonal fluctuations in the infectivity of triozid populations. The information gleaned from the study will aid effective control of triozid populations by pre-empting the population and greening transmission danger periods.

Objectives

Monitor seasonal fluctuation of *Trioza erytreae* and infectivity with Laf by:

Initial approach

- trapping triozids in indigenous vegetation and the CRI premises on traps, replaced fortnightly.
- test individual triozids for Laf
- test citrus trap plants for Laf that are re-placed fortnightly

Adapted approach

- trapping triozids using yellow sticky traps in citrus orchards
- test individual triozids for Laf by PCR
- Replace traps weekly

Materials and methods

Initial approach to investigate triozid infectivity originating from natural vegetation

Two sites were initially identified for trapping. The first being a site just outside Nelspruit belonging to Crocodile Valley Citrus Co. that extends along natural vegetation, a good distance away from any citrus plantation (approximately 1 km). The site enables monitoring of triozids in a variety of ecological niches; along a river, grassland and also more bush terrain. The second site was at the Nelspruit CRI premises in a bushy area, but adjacent to where citrus plants are maintained, some being greening infected.

Twenty Chempac Yellow Sticky Traps (85mm×200mm) (Chempac (Pty) Ltd, RSA) were hung on individual poles 1m off the ground and distributed along the trial sites. They were replaced every fortnight and checked for triozids.

Citrus seedlings used as trap plants were planted in pots and placed at the trial sites on stands 1m off the ground at 15m spacing from each yellow sticky trap. Twenty citrus trap plants were placed and watered twice weekly. The trap plants were replaced every fortnight with new healthy plants with young flush.

Citrus sinensis cv. Madam Vinous seedlings were used as trap plants and each trap plant was confirmed negative by PCR for Laf before placement in the field. When the trap plants were retrieved from the field they were sprayed with a broad spectrum carbamate insecticide, methomyl, to kill all triozids (and other insects) before being transferred to the glasshouse. Plants were then maintained at 24-28°C for symptom development and after 3 months leaf samples were collected from each plant for PCR detection of Laf to establish whether they were infected after field exposure.

Additionally twenty *Vepris lanceolata*, a known host for *T.erytreae* and a potential host for Laf were also placed as trap plants at the first site to expose them to natural infestation and then subsequently also tested for possible Laf transmission to investigate the alternate host potential of this species.

Location and description of orchards used in the study

A sour orange (*Citrus aurantium* L.) orchard located on the farm, Hilltop 458JT, in the Nelspruit district in the Mpumalanga Province of South Africa was used as the trapping site. The orchard consists of 80 trees and is entirely infected with African Greening. A single lemon tree (*C. limon* L.) is found in the orchard and is also infected with African Greening. No triozid control is done at the orchard, which is a standard management practice in most other commercial orchards. This site enabled triozids to be found throughout most of the season and the proximity to the research station enabled traps to be serviced weekly.

Weather data analysis

Temperature and humidity data were obtained from the Agricultural Research Council – ITSC weather station in Nelspruit. The maximum saturation deficit (SD_{max}) was calculated using the saturation vapour pressure (e_s) and minimum daily RH values $SD_{max}=e_s(T_{max}).(1-\frac{RH_{min}}{100})$. Lethal days were identified as days with maximum saturation deficits exceeding 34.4 mbars.

Adult triozid trapping and collection

Ten yellow sticky traps were hung weekly; nine traps in sour orange trees distributed randomly through the orchard and one trap in the single lemon tree. The traps were hung in the same trees throughout the monitoring period. Traps were changed weekly and checked for triozids under a stereo microscope the same day. Triozids were removed from traps using chloroform and then preserved in 100% ethanol at -20°C until DNA was extracted for PCR analysis. Traps were placed from the beginning of March 2011 to the end of March 2013.

DNA extraction and PCR

Total DNA was extracted from plants and from individual triozids using a modified CTAB (hexadecyltrimethylammonium bromide) extraction protocol (Doyle and Doyle, 1990). For single triozids, individuals were macerated in a 1.5 ml reaction tube using a pipette tip. Two hundred and fifty microliters of CTAB buffer was added and incubated at 60°C for 30 min. An equal volume of chloroform was added, vortexed and centrifuged at 13 000 rpm. Nucleic acids in the supernatant were precipitated with ethanol. After centrifugation the pellets were suspended in 25 µl distilled water. Extraction from citrus leaves differed in that midribs of 4 leaves per plant were macerated in a maceration bag with 4 ml CTAB buffer containing 1% mercapto-ethanol. The extraction is essentially the same but with 1.2 ml of the extract used for processing and 600 µl chloroform added for further extraction and precipitation was done with equal volumes of iso-propanol increase in volumes further. Final suspension was in 100 µl distilled water.

A PCR using the A2/J5 primer set, which targets the bacterial β -operon coding for ribosomal proteins of *Liberibacter* species, was used for detection of Laf (Hocquellet et al., 1999). PCR reactions were done in 20 µl reaction volumes using GoTaq® Hot Start Green Master Mix (Promega, Madison, USA) and 2 µl of DNA extract. Thermo-cycling parameters consisted of one cycle of 94°C for 3min followed by 35 cycles of 94°C for 20 s, 57°C for 30 s, 72°C for 20 s and a final extension at 72°C for 5 min.

Transmission experiment using field collected T.erytreae

After an outbreak of *T. erytreae* was detected in a commercial sweet orange orchard in Nelspruit in September 2011, adult triozids were collected on three days, over a two week period, for a transmission study. The triozid numbers were high compared to that found in the sour orange orchard and adults could easily be found and caught in the tree canopy. Greening symptoms of blotchy mottle were visible in all the trees where triozids were collected.

Plants of Olinda Valencia (*C. sinensis*) and Ponkan Mandarin (*C. reticulata*) derived from seedlings, were exposed to adult *T. erytreae* caught during September and October 2011. Three to five adult triozids were confined in a small plastic cage on a young shoot of each plant for seven days. Triozids were recovered from the challenged plants after the feeding period and stored separately in 100% ethanol at -20°C until DNA extractions and PCR were done. Thereafter the plants were sprayed with a suitable insecticide to kill all triozid eggs and transferred to a greenhouse maintained at 18- 26°C with 18h photoperiod for three months. During this time Greening symptom development was monitored followed by PCR testing after three months to check transmission.

Results

Initial approach to investigate triozid infectivity originating from natural vegetation

Trap plants and yellow sticky traps were monitored for triozids every fortnight at the various sites. Ten traps were monitored adjacent to natural vegetation some distance away from any citrus. Nine triozids were caught at this site from May 2010 to March 2011. More traps were placed in January 2011 adjacent to a greening infected citrus block at another site of Crocodile Valley Farm. Triozid numbers here too were extremely low and only 3 triozids were caught from Jan to March 2011. The highest triozid numbers detected was in December 2010 at the CRI premises. Here 95 triozids were caught of which 7 were Laf positive.

Transmission of greening to citrus trap plants was confirmed by PCR in only 2 plants, both of which were placed at the CRI premises and none that were placed adjacent to natural vegetation. These positive plants were placed mid November 2010 and the other at the end of December 2010. These transmissions coincided with the elevated triozid numbers at the CRI site. A total of 420 plants were placed over the report period and tested before placement in the field and again 3 months after returning from the field. No positive transmissions to trap plants were obtained from plants placed adjacent to natural vegetation.

T. erytreae population dynamics in a sour orange orchard

Numbers of adult *T. erytreae* caught in the sour orange orchard were low but demonstrated seasonal fluctuations. Nonetheless, since no vector control was done in the orchard it allowed detection of *T. erytreae*

through most of the season, although in some months few individuals were trapped (Figs. 1, 3). Generally the seasonal population fluctuations followed the normal citrus flushing rhythm except that in 2011 only a single major population peak was detected in September with a lesser peak observed in December that year (Fig. 1). The expected population peak associated with the flush in November 2011 was not observed, but weather data from September of 2011 indicate a high average maximum SD value, 31% higher than the equivalent period in 2012. (Fig. 2). For similar reasons the September population peak in 2012 was lower than anticipated considering population build-up started earlier. Again, high SD values and 9 lethal days recorded for August impacted population growth. Eight of these 9 days were concentrated over a short period from 20 August- 4 Sept 2012. This spring triozyd population is normally larger than the following summer populations, but this was not the case in 2012.

The total number of triozyds trapped in the lemon tree over the total monitoring period was double that caught in the sour orange trees, when comparing average numbers trapped over the 9 traps (Table1).

Seasonal fluctuation in triozyd infectivity

Fluctuations in the percentage infectivity of the *T. erytreae* populations were observed with infectivity reaching higher levels after the citrus flush seasons, but these levels of infectivity were maintained for two to three months despite declines in triozyd populations (Fig 3). Peaks in infectivity in September following the August flush were noted in both the 2011 and 2012 season and were 68 and 51% respectively. Infectivity peaks were reached again in January of both seasons and the percentage infectivity was equal (2011) and similar (2012) to the earlier peaks in each season even though the population sizes were smaller (Fig 3). Accuracy of percentage infectivity in periods where few triozyds were trapped is uncertain and was mostly in the period March to June.

The average percentage infectivity over the total of each of the two seasons was 6% between 2011 and 2012. For the total trial period the average percentage infection was 43% with no differences obtained between triozyds trapped in the lemon tree or those trapped in the sour orange trees (Table 1).

Transmission experiment using field collected T. erytreae

Average percentage infectivity of the 3 triozyd catches was 71% (Table 2), similar to the 68-69% found in the sour orange orchard over the same Sept - Oct period in 2011 (Fig 3). Triozyd infectivity of the last catch in October was higher at 84% and the total transmission rate achieved with these catches was 34% (Table 2).

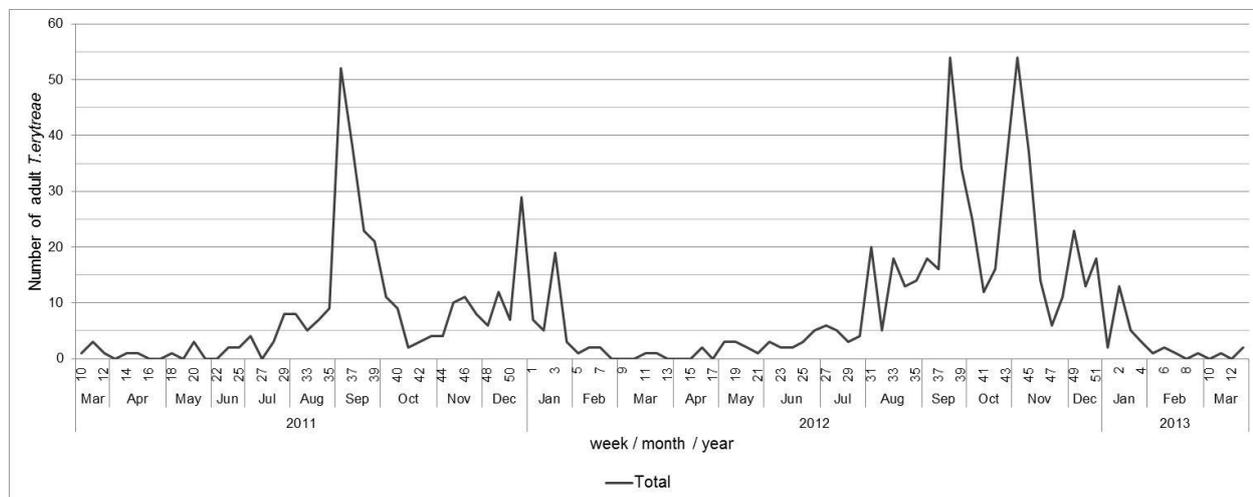


Figure 4.2.10.1. Total number of adult *T. erytreae* caught weekly on 10 sticky traps in a sour orange orchard in Nelspruit from March 2011 to March 2013.

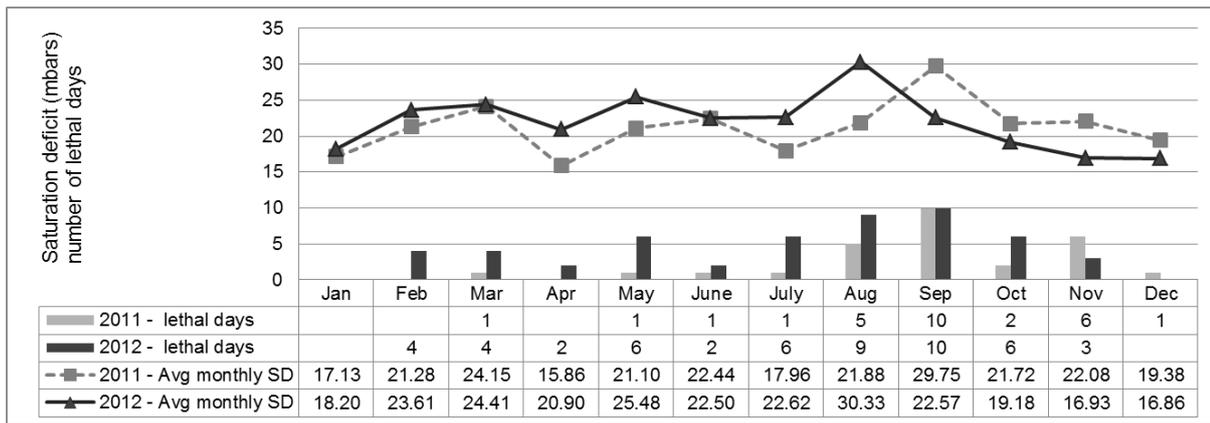


Figure 4.2.10.2. Nelspruit weather data for 2011 and 2012 expressed as average monthly saturation deficit (SD) (millibars) and number of lethal days per month (days with maximum SD exceeding 34.4 mbars)

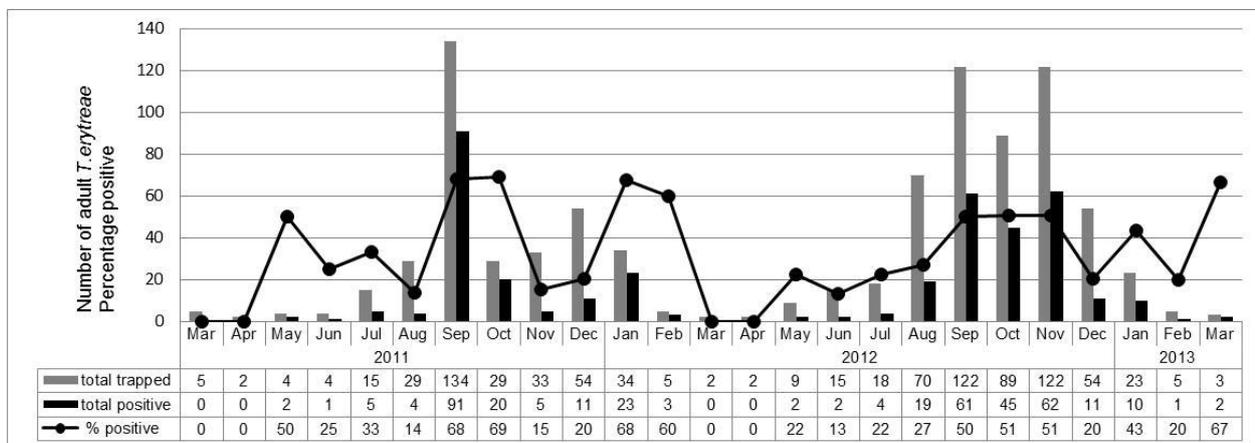


Figure 4.2.10.3. Monthly totals of adult *T. erytrae* trapped from March 2011 to March 2013 and the corresponding number of individuals testing positive for Laf as well as the percentage infectivity over the same period.

Table 4.2.10.1. Yearly totals of adult *T. erytrae* trapped from beginning March 2011 to end February 2013 and the corresponding number of individuals testing positive for Laf as well as the percentage infectivity over the same period. Trial totals for traps placed in sour orange trees and the lemon tree are recorded separately.

		Period	No. trapped	No. positive	% infected
All traps:	1 st season	2011 - 2012	348	165	47%
	2 nd season	2012 - 2013	531	217	41%
	Total for trial		879	382	43%
Sour orange traps(9):	Total for trial		700	304	43%
	AVG per tree		78	34	
Lemon trap(1):	Total for trial		179	78	44%

Table 4.2.10.2. The percentage positive triozids used for transmission and the percentage positive transmission to the citrus plants for the different batches of triozids collected on three days in September and October 2011 from a Greening infected sweet orange orchard in Nelspruit.

	Laf status of triozids used for challenge			Citrus type challenged	No. plants challenged	Laf status of plants after 3 months	
	No. Positive	Total	% infected			No. Positive	% Transmission
triozid catch 28 Sept 2011	10	15	67%	Ponkan Mandarin	3	1	33%
	33	51	65%	Olinda Valencia	13	2	15%
triozid catch 05-Oct 2011	25	36	69%	Olinda Valencia	9	4	56%
triozid catch 10-Oct 2011	27	32	84%	Olinda Valencia	7	3	43%
Total:	95	134	71%		32	11	34%

Discussion

Initially the purpose of the trial was to investigate the population dynamics and greening transmission of *T. erythrae* in both citrus orchards and adjacent natural vegetation. However, no positive Laf transmissions to a total of 420 trap plants was obtained from flushing citrus trap plants placed adjacent to natural vegetation some distance away from any citrus plantation. The project approach was then changed to only monitor triozids in citrus orchards.

A sour orange orchard in the Nelspruit district was used as the trapping site for further studies. Although no triozid control was done at the orchard triozid population numbers were low and a few factors likely contributed to this. Sour orange is a less favourable citrus host for *T. erythrae* (van den Berg et al., 1991a) and this was also observed in the relative numbers of triozids caught on the trap placed in the lemon tree compared to the average numbers trapped in the sour orange trees. Additionally, lemon trees have more growth flushes the most other citrus and therefore maintain populations over longer periods. Another factor influencing population size was the poor nutritional state of the trees due to the orchard being infected with Greening for a number of years and the orchard not being actively managed. Flush quality on a poorly nourished host is known to affect the development of the triozid. In contrast, high nitrogen content promotes ovulation and egg-laying (Catling, 1972).

Despite the low population numbers, *T. erythrae* was detected throughout most of the season in the orchard. The population fluctuations followed the citrus flushing rhythm as previously shown (Catling, 1972) except in 2011 where extreme weather conditions reflected in the high SD values likely impeded population build-up on the November flush. The spring population in 2012 was also seemingly influenced by extreme weather conditions in August, but milder conditions as seen in the average SD values from September to December in 2012 allowed for normal population fluctuations associated with the citrus flushing rhythms. The number of lethal days alone did not seem to explain the weather influence on the population. Catling (1969) did note that consecutive days of extreme weather had a more pronounced influence on survival of the young stages. The average SD values over the trial period are more clearly correlated to changes in population in this study than the number of lethal days.

The percentage infectivity of the *T. erythrae* populations in both 2011 and 2012 first peaked in September. These populations would have originated from the first citrus flush season beginning in August and were maintained for two and three months for 2011 and 2012 respectively before declining. Infectivity peaked again in January of both seasons and the percentage infectivity was equal (2011) and similar (2012) to the earlier peaks in each season.

In a study monitoring progression of plant ultra-structural changes due to infection with Las, the Asian form of the pathogen, some results indicated a strong possibility that as the bacteria translocate into the active growing sectors of the plant or flush, most of the bacterial population is viable. As the tissue ages and foliar symptoms develop, most of the bacterial population found within the older tissue is non-viable (Hashemian et al., 2009). Considering this possibility and the likelihood that the related African form, Laf, would behave in much the same manner, this can offer an explanation for the higher infectivity of triozid populations that are

associated with the flush periods as they would be acquiring viable bacteria that can multiply within the vector, thus raising the percentage infectivity of the population. Adults emerging and feeding on the older tissue, where few live bacteria are present, would acquire less bacteria and this would be reflected in the population infectivity.

Also of interest is that the infectivity of triozids trapped at the same site in the lemon tree and those trapped in the sour orange trees did not differ in their infectivity levels and that the two citrus hosts did not influence the vector infectivity implying that acquisition from both hosts was equal.

Results presented in this paper show that higher incidence of Laf within the vector population was not correlated with the population numbers and the same or similar infectivity levels were obtained in the later summer broods as is the spring broods where the population numbers were highest. This trend was similarly seen for Las in *Diaphorina citri* the natural vector of Huanglongbing (Manjunath et al., 2008)

The transmission experiment again demonstrated that individual triozids are inefficient vectors in that although plants were exposed to vectors that tested positive, this did not relate to positive transmissions in every instance. Nonetheless this inefficiency is compensated for by the high population numbers in a natural environment, increasing the vectoring ability substantially.

Further, it was established that positive *T.erytrae* individuals are found throughout most of the season in an infected orchard even though peaks in infectivity are found. The study underscores the importance of the current triozid control recommendations to control the spread of Greening by limiting the population build-up during the first flush period. This remains vital due to the combination of high infectivity levels and larger populations of the vector over that period. A low population survival early in the season also limits later population build-up. This is pertinent as triozids developing on subsequent flush periods can have similarly high infection levels as in the early season and can still vector the disease despite lower numbers.

Technology transfer

Oral Presentation

G. Cook, V. Maqutu and S.P. van Vuuren 2012. Investigation of the Seasonal Incidence of "Candidatus" *Liberibacter africanus* in *Trioza erytrae*. 7th Citrus Research Symposium, Champagne Sports Resort, Drakensberg, 20 - 22 August 2012.

Poster Presentation

G. Cook, V. Maqutu and S.P. van Vuuren 2013. Population dynamics and seasonal fluctuation in the percentage infection of *Trioza erytrae* with *Candidatus* *Liberibacter africanus*, the African citrus greening pathogen, in an orchard fully infected with African greening. Nineteenth Conference of the International Organisation of Citrus Virologists., Skakuza, Kruger Park, 28th July – 2nd Aug 2013.

Draft publication

Population dynamics and seasonal fluctuation in the percentage infection of *Trioza erytrae* with *Candidatus* *Liberibacter africanus*, the African Citrus Greening pathogen, in an orchard fully infected with African greening and transmission data from field collected triozids.

G. Cook, V. Maqutu and S.P. van Vuuren

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4.2.11 FINAL REPORT: Epidemiology of greening disease– alternate hosts and spread

Project 886 (April 2007 – April 2014) by Gerhard Pieterse, M.N.B. Phahladira and Ronel Viljoen (ARC-PPRI and 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 help 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. No evidence of spread from indigenous vegetation was obtained in a number of citrus groves monitored for Laf spread, mainly due to the low rate of spread observed. No evidence of spread of LafC from *Calodendrum capense* to citrus was observed in a well-tested grove. A new, sensitive real-time PCR was developed to detect Liberibacters in a generic manner as well as Laf and LafC specifically. Large numbers of *C. capense*, *Vepris lanceolata*, *Zanthoxylum capense* (and a few *Z. davyii*) and *Clausena anisata* have been collected, mainly from within the natural distribution of these plants, and analyzed for the presence of Laf and other 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 analyzed thus far. Amongst a total of 278 *C. capense* samples collected throughout South Africa 100 tested positive for LafC as confirmed by sequencing the amplicon derived from conventional PCR of the β -operon of the ribosomal protein gene region of the first 17 infected trees found and of a representative sample from each district. Liberibacters were obtained by real-time PCR from 33 of the 234 *Clausena anisata* specimens collected, 17 of 289 *Vepris lanceolata*-, and 10 of 231 *Zanthoxylum capense/davyii*. The Liberibacters obtained were characterized by amplifying and sequencing the *rplJ*, *16S* and *omp* gene regions of each. When aligned, the sequences of the *rplJ* and *omp* gene regions for each given tree species were unique, but with all the specimens from that species being the same. Just one exception to this was observed. Based on these two genes the Liberibacters from *C. anisata* and *V. lanceolata* each had distinct Liberibacters related to LafC from *C. capense*. One specimen of *Z. capense* contained a LafC sequence while the remaining *Z. capense* specimens contained unique Liberibacter sequences related to Laf. Based on the *16S* rDNA sequence, which has very poor resolution amongst Liberibacter species, Liberibacters from *V. lanceolata* and *C. anisata* were marginally more similar to the cognate *16S* sequences for LafC (from *Calodendrum capense*) than to those of Laf from citrus. The *16S* rRNA sequence of those from *Zanthoxylum* species, with the exception of the one containing the LafC-like Liberibacter grouped separately. To confirm the identity of the tree host species from which Liberibacter sequences were obtained, the sequence of the large subunit of the ribulose 1,5-biphosphate carboxylase-coding gene of the hosts was determined. This confirmed the

identity of specimens identified by morphological means, including that of the *Z. capense* sample containing LafC. The absence of citrus-like Laf in these Rutaceous species and the absence of these Liberibacters in citrus suggest that the current epidemiology of each is independent. However, it is possible that that citrus became infected with a Laf-like Liberibacter progenitor from one of the indigenous Rutaceous species on the African continent, from which the current citrus-infecting Laf arose. LafC has been detected at high concentrations in an indigenous psyllid, commonly observed on *C. capense*, identified as *Moraniella calodendri*. The whole genome of Laf has been sequenced, deposited with Genbank and will be released following annotation and publication of data. Significant progress towards sequencing of LafC has been made, but further analysis is required.

Opsomming

Sitrus vergroening is in Suid-Afrika tot ekonomies aanvaarbare vlakke beheer deur die streng beheer van die vektor en ook die stelselmatige verwydering van inokulum deur die verwydering van ge-infekteerde 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 inheemse lede van die sitrus-familie (*Rutaceae*) in Suid-Afrika kan dien tot meer doeltreffende siektedruk beheer. 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. Geen bewyse van verspreiding vanaf inheemse bome na sitrus is gevind in verskeie gemoniteerde sitrus boorde; hoofsaaklik as gevolg van die baie lae vlakke van verspreiding van Laf gevind. Geen bewys vir verspreiding van “*Candidatus Liberibacter africanus* sp. *capense*” (LafC) na sitrus kon gevind word nie in een volledig getoetse boord. ’n Nuwe, sensitiewe opsporingstegniek, gebaseer op “real-time PCR” is ontwikkel om Liberibacter op ’n generiese manier op te spoor sowel as LafC spesifiek. Groot getalle *Calodendrum capense*, *Vepris lanceolata*, *Zanthoxylum capense* (en klein hoeveelhede *Z. davyii*) en *Clausena anisata* eksemplare is versamel, hoofsaaklik binne die natuurlike verspreiding van die spesies, en vir die teenwoordigheid van Laf spesifiek en Liberibacters in die algemeen geanaliseer. Laf, in streng gebruik van die term, 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. Een honderd monsters van *C. capense* uit 278 wat dwarsdeur Suid-Afrika versamel is, was positief vir LafC. Hierdie is bevestig deur die nukleienvolgordebepaling van die amplikon van die beta-operon van die ribosomale proteïengeen van die eerste 17 besmette bome gevind, sowel as dit van verteenwoordigende bome uit elke streek. Liberibacters is m.b.v. “real-time PCR” in 33 uit 234 *Clausena anisata* eksemplare gevind, asook 17 uit 289 *Vepris lanceolata* en 10 uit 231 *Zanthoxylum capense/davyii*. Hierdie Liberibacters is volgens hul *rplJ*, 16S en *omp* geen areas gekarakteriseer. Na belyning van die nukleotiedvolgordes, was dit duidelik dat die *omp* en *rplJ* volgordes uniek per boom spesie was, maar dat al die eksemplare van ’n gegewe boomspesie dieselfde Liberibacter volgorde bevat. Daar was net een uitsondering hierop. Gebaseer op hierdie twee gene is die Liberibacters van *C. anisata* en *V. lanceolata* besmet met unieke variante naasverwant aan LafC van *C. capense*, een eksemplaar van *Z. capense* was besmet met ’n tipiese LafC, teryl die ander almal ’n unieke Laf-agtige variant bevat het. Die 16S geen, wat ’n baie lae resolusie met Liberibacter spesies het, het aangetoon dat die Liberibacters van *C. anisata* en *V. lanceolata* effens meer verwant is aan die soortgelyke volgorde vanaf LafC (van *C. capense*) as aan Laf van sitrus. Die nukleotiedvolgorde van die 16s rRNA van Liberibacter van *Z. capense*, met die uitsondering van die LafC bevattende een, vorm ’n unieke groepering weg van beide Laf en LafC. Om identifikasie van die bome waaruit Liberibacters gevind is te bevestig, is die nukleotiedvolgorde van groot subeenheid van die ribulose 1,5-bifosfaat carboksilase geen bepaal. Hierdie is die standaard plant “bar-coding” geen. Dit het die identifikasie van hierdie bome bevestig insluitend die *Z. capense* wat met ’n LafC besmet was. Die afwesigheid van sitrus geassosiëerde Laf in inheemse lede van die *Rutaceae*, sowel as die afwesigheid van die Liberibacters van inheemse bome in sitrus, dui daarop dat die epidemiologie van hulle onafhanklik van mekaar is. Dit is egter moontlik dat sitrus in Afrika besmet geraak het met ’n Laf-agtige voorganger uit ’n inheemse boom, waaruit Laf in sitrus geselekteer is. LafC is teen hoë titers in ’n inheemse bladspringer algemeen gevind op *C. capense*, *Moraniella calodendri* opgespoor. Die nukleotiedvolgorde van die hele genoom van Laf is bepaal en in Genbank gedeponeer, en sal vrygestel word sodra dit ge-annotateer is en die werk gepubliseer is. Betekenisvolle vordering is gemaak met die nukleotied volgorde bepaling van die genoom van LafC, maar verdere analiese word verlang.

Introduction

Citrus greening is a destructive disease of citrus and is associated in South Africa with a fastidious bacterium “*Candidatus Liberibacter africanus*” (Laf). The disease has been reduced to manageable levels through stringent vector control strategies, but remains a problem in cooler citrus production areas of South Africa. The perpetuation of the disease may be due to the presence of hosts other than citrus, which may serve as reservoirs of the disease. In this study we propose to study the possibility that other hosts of the bacteria

exist. We are starting with the indigenous Rutaceous species, firstly, by monitoring the spatial distribution of greening infected citrus in a number of citrus groves adjoined by indigenous vegetation, in order to search for disease gradients. Secondly, we intend to determine alternate hosts by a) developing and using very sensitive PCR techniques to detect the bacteria in field-collected indigenous Rutaceous plants in regions of high greening infection pressure, and b) by transmission of Laf through grafting to indigenous Rutaceous species. Thirdly, to determine whether any possible Liberibacter variants detected in indigenous plants can infect Citrus and whether *Trioza erytreae* can serve as a vector

Objectives

- 1) Monitoring the spatial distribution of greening infected citrus in a number of citrus groves adjoined by indigenous vegetation, in order to search for evidence of spread (disease gradients).
- 2) Determine the presence of Laf alternative hosts by;
 - a) developing and using very sensitive PCR techniques to detect the bacteria in field-collected indigenous Rutaceous plants in regions of high greening infection pressure
 - b) by transmission of Laf through grafting to indigenous Rutaceous species.
- 3) Determine whether any possible Liberibacter variants detected in indigenous plants can infect Citrus and whether *Trioza erytreae* can serve as a vector
- 4) Start to characterize Liberibacters detected.

Materials and methods

Spatial analysis for disease gradients from indigenous vegetation.

Three Citrus groves, one in Rustenburg, one in Nelspruit, and one in Schoemanskloof, adjoining indigenous vegetation were monitored on a tree-for-tree basis for greening symptoms annually in winter. This was done by a team of people trained in greening symptom detection and postgraduate students. Trees are assigned a row/plant position coordinate and infected and missing trees are plotted and analyzed for gradients in order to ascertain whether any external source of the disease, in the direction of indigenous vegetation exists.

A further trial was located at a farm near Sunlands, Sundays River Valley, Eastern Cape (33°30.407S, 25°38.750E, Elevation 28m), where two *C. capense* trees had earlier been found to be infected with LafC. These trees were part of a *C. capense* bordered lane of 72 *C. capense* trees of one row on either side. The lane bisected a 15-year old Citrus orchard (Midnight Valencia) with the closest citrus row being 8m from the *C. capense* lane. The planting of the *C. capense* trees predated that of the citrus by an unknown period. Leaf and petiole samples were collected from 44 *C. capense* specimens, representing all of those in the lane bisecting the first citrus orchard. Leaf and petiole samples of 273 citrus specimens representing two rows of citrus on either side of the *C. capense*-bordered lane were also collected. All Citrus and *C. capense* nucleic acid extracts were subjected to Liberibacter general real-time PCR tests. This was followed by conventional PCR (Hocquellet et al. 1999) and sequencing as described above on those with Ct values of less than 30 in real-time PCR.

Universal, sensitive detection of Liberibacters

By ICAN

In order to determine whether a published *Isothermal and Chimeric-primer initiated Amplification of Nucleic acid (ICAN)* protocol for the detection of Las (Mukai et al., 2007; Urasaki et al., 2008) was able to detect Laf, the primer sequences were compared to those of Laf, and shown to be identical. In theory therefore this technique would also be able to detect Laf. It was determined that it is capable of detecting Laf, but its sensitivity in our hands was relatively poor, and the technique was not deemed useful for continued, routine detection of Laf.

By Real-time PCR

Total DNA extracts from known Laf infected citrus or LafC-infected *C. capense* tree were utilized as a positive control in PCR. DNA extracts from tree specimens were tested for Liberibacters and for LafC specifically using a the labeled probe (HLBp) and reverse primer (HLBr) from the real-time PCR of Li et al. (2006) but as the forward primer LibUF, to a conserved region of known Liberibacters (5'-GGCAGGCCTAACACATGC-3') ("Generic Liberibacter real-time PCR") or a LafC specific primer, LafC F (5'-ATTGCGCGTATCGAATACGACG-3') and using as template 1µl of the DNA extract. The specificity of the generic Liberibacter PCR were tested against total DNA extracts containing Laf, LafC, "Ca. Liberibacter asiaticus" (Las); "Ca. Liberibacter solanacearum" (Lso) and "Ca. Liberibacter americanus" (Lam) (results not shown). Real-time PCR was performed using a Lightcycler® 1.5 (Roche, Mannheim, Germany) capillary-based thermocycler. Lightcycler® Taqman® Master kits were used along with the LibUF, LafC primers and probes and conditions as described (by Li et al. 2006). A positive/negative crossing threshold (Ct) of 30 was used after parallel tests showed that samples with Ct values of 30 in the LafC-specific and Generic

Liberibacter systems no longer yielded amplicons in conventional PCR. Samples with Ct of 30 to 35 were generally retested, as well as being tested with conventional PCR.

Collection of samples.

The location of indigenous Rutaceous trees was obtained by sending a questionnaire to professional and amateur botanists and by surveys for specimens in a number of city gardens and parks, and botanical gardens and in natural settings. Samples were collected from trees with no regard for symptoms, although these were noted when observed. Leaf and petiole samples were collected throughout South Africa. Samples consisted of approximately 20 leaves and petioles collected from various branches of an individual tree.

Extraction of DNA for PCR.

Total DNA was extracted from 0.5 g of the pooled sample of petioles or midribs from each tree using a standard CTAB (hexadecyltrimethylammonium bromide) procedure (Doyle and Doyle 1990).

*PCR amplification of *rplJ**

Liberibacter *rplJ* was amplified as described by Hocquellet et al., (1999). 0.5µl of DNA template was added to a final reaction volume of 50µl consisting of 2.5µl 2% Triton X-100, 5µl 10X NH₄ reaction buffer (Bioline, Boston, USA), 5µl 2mM deoxynucleotide triphosphate mix (dNTP), 2.5µl primer A2 (5'-TAT AAA GGT TGA CCT TTC GAG TTT-3'), 2.5µl 10µM primer J5 (5'-ACA AAA GCA GAA ATA GCA ACA A-3'), 2.0µl 0.05M MgCl₂, 5.0µl 2000µg/ml BSA, 0.5µl 2.5 units (U) Biotaq® (Bioline, Boston, USA) and 20µl molecular grade H₂O (Sigma-Aldrich, St. Louis, MO, USA). PCR cycling reaction was performed on a T100™ Thermal Cycler (Bio-Rad, CA, USA). Cycling conditions were set up as follow; initial denaturation at 92°C for 3 min, followed by 35 cycles of denaturation at 92°C for 20s, annealing at 62°C for 20s and elongation at 72°C for 45s, with a final elongation step at 72°C for 5 min. Following amplification, 8µl of PCR product was mixed with 2µl loading dye and loaded onto a 1% (w/v) agarose gel containing 4% ethidium bromide. The gel was electrophoresed at 100V for 45min and viewed under an ultraviolet trans-illuminator to confirm amplification.

*PCR of outer membrane protein (*omp*)*

The outer membrane protein (*omp*) of Liberibacters was amplified from Liberibacter universal PCR positive samples. The protocol of Bastianel et al. (2005) was used with modifications for the detection of unknown Liberibacters. PCR was as for amplification of *rplJ* but with HPlinv (5'-ATG AAT TTG TTG CCT ATT CC-3') and OMP8inv (5'-TCA CGA CGA ATC ACA GAA TC-3') primers. Amplification of template DNA was performed on a T100™ Thermal Cycler (Bio-Rad, California, USA) with cycling conditions as follows; initial denaturation at 92°C for 3 min, followed by 35 cycles of denaturation at 92°C for 30s, annealing at 55°C for 30s and elongation at 72°C for 2 min, with a final elongation step at 72°C for 10 min. Amplification products were visualized on a 1% agarose gel following electrophoreses as previously explained.

PCR of 16S ribosomal gene

The protocol described by Jagoueix et al. (1996) was followed for the amplification of Liberibacter 16S rDNA sequences. Minor modifications were made to detect unknown Liberibacters from samples. PCR was performed by the addition of 0.5µl of DNA template to 50µl reaction mix as previously described using OA1 (5'-GCG CGT ATT TTA TAC GAG CGG CA-3') and OI2c (5'-GCC TCG CGA CTT CGC AAC CCA T-3') primers. Amplification was carried out under the following conditions; initial denaturation at 92°C for 5 min, followed by 35 cycles of denaturation at 92°C for 30s, annealing at 62°C for 30s and elongation at 72°C for 90s, with a final elongation step at 72°C for 10 min. Amplification products were visualized following gel electrophoreses as previously described.

Nucleotide sequencing.

To obtain templates for sequencing, a number of samples were amplified using the A2/J5, *omp*, or 16sRNA primers. Reaction conditions were identical to those described for PCR for detection of Laf (Pietersen et al. 2010) except cycling conditions were conducted at an annealing temperature of 62°C for 20 s. Amplicons were purified by mixing 19µl of amplicon with 10U Exonuclease I from *Escherichia coli* (Fermentas, Maryland USA) and 2U FASTAP™ Thermosensitive alkaline phosphatase (Fermentas, Maryland USA) at 37°C for 15 min. The reaction was stopped by incubation at 85°C for 15min. The purified PCR products were subjected to cycle sequencing using the Big Dye® Terminator v3.1 Cycle Sequencing kit and Big Dye® Terminator v1.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA) according to the manufacturers specifications. For identification purposes sequencing was only conducted in one direction using one of the primers. The extension products were purified by ethanol/sodium-acetate precipitation, and then sequenced at the core sequencing facility of the University of Pretoria on an ABI Prism 3100/3130 sequencer (Applied Biosystems, Foster City, CA).

Sequence analysis.

Nucleotide sequence was analyzed using the DNAMAN software suite (Lynnon Biosoft, Quebec, Canada). Nucleotide similarity searches of Genbank were conducted using the BLAST algorithm of the National Centre for Biotechnology Information. Amplicon sequences were compared to cognate regions of the following Genbank accessions for Laf (LAU09675); LafC (AF248498), Lam (EF122254), Las (M94319), and a recently discovered Liberibacter from tomato, Lso (EU834131) (Liefing et al. 2009). Multiple alignments were prepared in DNAMAN and alignments were used to prepare phylogenetic trees using a maximum likelihood method (Hasegawa et al. 1985; Tamura and Nei 1993).

Confirmation of Tree host species identification

To verify the tree host species from which Liberibacters were identified, these were identified by amplification and direct sequencing of the large subunit of ribulose 1,5-biphosphate carboxylase-coding gene (*rbcl*), the standard, plant barcoding gene. Amplification was in 50µl reactions as described for Liberibacter PCRs above, but using primer *rbcl* F (5'-ATG TCA CCA CAA ACA GAG ACT AAA GC-3') and primer *rbcl* R (5'-GTA AAA TCA AGT CCA CCR CG-3') (reference?). PCR cycling reaction was performed on a T100™ Thermal Cycler (Bio-Rad, California, USA). Cycling conditions were set up as follow; Initial denaturation at 92°C for 3 min, followed by 35 cycles of denaturation at 92°C for 20s, annealing at 55°C for 20s and elongation at 72°C for 90s, with a final elongation step at 72°C for 5 min. Amplification products were then gel electrophoresed and visualized under an ultraviolet trans-illuminator. Amplicons were sequenced and subject to phylogenetic analyses as previously discussed.

Graft transmission:

Three bark patches of Laf infected citrus material (UPCRI 06-0150, 06-0195 and 06-0280) were grafted on 10 seedlings of each of the following Rutaceous species; *Agosthema capensis*, *A. ciliaris*, *Calodendrum capensis*, *Clausena anasita*, *Vepris lanceolata* and *Zanthoxylum capense* and two citrus controls in September, 2007. Symptom expression has been monitored on a regular basis since then. Samples from inoculated plants were tested for Laf or Las three months, six months, a year, and 3 years and six years post inoculation. Initially tests were conducted with the multiplex and conventional A2/J5 PCR tests. At three years post-inoculation real-time PCR using Taqman probe HLBpr and primers HLBafr/HLBbr specific for Laf (Li et al., 2006) were used to re-test all samples.

Results and discussion

Spatial analysis for disease gradients from indigenous vegetation.

The first orchard in which these studies were initiated (Union Homestead) was unfortunately removed due to road works in the Nelspruit area in 2008 and further monitoring of it could not continue. An orange grove in Rustenburg was fully analyzed until 2009. No evidence of spread of greening was obtained, with trees showing symptoms and testing positive in earlier seasons being the only infected ones in 2009. Infection gradients could not be determined due to the very small number of infected plants. As the producer had started utilizing imidachloprid to control psyllids this could no longer be analyzed. Analyses of the grove at Kingstonvale, Crocodile Valley, Nelspruit was also done up until 2009. In this grove it also appeared as though no spread of greening had occurred during the period recorded. The grove at Joubert Brothers, Schoemanskloof was monitored until 2009. However no correlation could be obtained with individual trees which had displayed symptoms the previous season, possibly due to slight variations in time in monitoring the grove, and also due to the fact that the producer had started to control greening spread by infected branch removal. The approach of trying to gain evidence of a greening disease gradient in the direction of indigenous vegetation, as a means of proving the existence of indigenous alternate hosts was unexpectedly fraught with obstacles and it was decided that it is unlikely to yield meaningful data, and was therefore abandoned since 2010, with the exception of a farm near Sunlands, Sundays River Valley, Eastern Cape (33°30.407S, 25°38.750E, Elevation 28m), where two of the *C. capense* trees found to be infected with LafC during an earlier survey were part of a roadside lane of 72 *C. capense* specimens bisecting and pre-dating four 15-year old Citrus (Midnight Valencia) orchards. Leaf and petiole samples from 44 *C. capense* specimens, representing all of those dividing the first orchard were collected. Leaf and petiole samples of 273 citrus specimens representing the four (two on either side) most proximal rows of citrus plants to the *C. capense* lane were also collected. Total nucleic extracts were obtained from all samples using the protocol of Li et al., (2008). Both Citrus and *C. capense* nucleic acid extracts were subjected to real-time PCR tests, using the universal Liberibacter primers (LibUNiv), followed by conventional PCR (Hocquellet et al., 1999) and sequencing as described above, of those with Cp values of less than 30 in real-time PCR. Samples with Ct values between 30 and 35 were retested. All samples in this trial were also tested for citrus tristeza virus (CTV) by triple antibody sandwich (TAS) ELISA using reagents (polyclonal goat anti-CTV G-604 and polyclonal rabbit anti-CTV CTV-20) and protocol supplied by Richard Lee (CREC, University of Florida, USA).

Forty-three of the 44 *C. capense* trees occurring as the lane bisecting the orange grove were found positive for Liberibacters after being tested using a Liberibacter universal detection real-time PCR assay (all had Ct values less than 35, the highest being 32.75). LafC was confirmed in 36 samples by unidirectional direct sequencing (primed with A2) of amplicons from conventional PCR, using primer pair A2/J5. Three representative sequences amongst the 36 sequences generated (which are all essentially identical) have been submitted to Genbank with the following accession numbers (JF419553, JF419554, and JF419555). None of the 273 citrus trees sampled, all within the two rows immediately adjacent to the *C. capense* lane tested clearly positive for Liberibacters (average Ct of less than 30 from two sets of test). The vast majority of trees however had Ct values between 32 and 33). Conventional A2/J5 PCR on 28 of the samples with the lowest Ct values did not yield any amplicons. All 273 Citrus trees, but none of the 44 *C. capense* trees tested positive for CTV. The ubiquitous CTV infection in citrus is expected as these trees would have been pre-immunized with CTV. No evidence of *Trioza erytreae* feeding or breeding was observed on two inspections of the citrus and *C. capense* trees, but the vector is known in this region and over the 15 years of co-existence of the citrus and the *C. capense* lane numerous outbreaks of *T. erytreae* must have occurred. Based on circumstantial evidence from this site, it would appear as though LafC on *C. capense* is not transmitted to citrus.

Development of a sensitive method for Liberibacter detection.

The ICAN protocol was assessed experimentally by extracting total DNA from the following known Laf-infected citrus samples (09-0849, 09-0850, 09-0851, and 09-0703). This DNA was then utilized in the ICAN procedure as templates. Two of the samples (09-0850 and 09-0849) tested positive while samples 09-851 and 09-0703 were negative. Conventional PCR using A2 and J5 primers were performed on the same samples and showed that the 09-0703 and 09-0851 samples no longer contained viable DNA, and therefore confirmed the specificity of the ICAN technique. In further tests, 6 of 10 further samples (all shown to be Laf positive by conventional PCR) were detected by ICAN. In order to optimize ICAN to detect all ten samples, the run conditions of ICAN have been varied and the test optimized. In a comparison of the sensitivity of this technique vs. conventional A2/J5 PCR a serial dilution series was made of DNA from samples 09-0849 and 09-0850 comprising 1:10, 1:15, 1:20, 1:50 and 1:100 dilutions of the DNA. PCR was shown to detect samples up to 1:100 dilution while ICAN could only detect up to a 1:20 dilution of the DNA showing that PCR is more sensitive than the ICAN system as used in our hands. While the test shows great promise as a means of detecting Laf without the requirement of sophisticated apparatus such as thermocyclers and electrophoresis equipment, its relative lack of sensitivity in our hands suggest that it is unlikely to detect low concentrations of Laf.

A real-time PCR using Taqman probe HLBpr and primers HLBafr/HLBBr (Li *et al.*, 2006) was modified from the published multiplex PCR to be specific for Laf and was used to detect Laf in various experiments during the course of this project. The capabilities of this real-time PCR published protocol was expanded to also detect LafC specifically and to detect all known Liberibacters (including those of the Solanaceae) by designing LafC specific forward primers, and primers to a conserved sequence in all sequenced Liberibacters to date to complement the published reverse primers and Taqman® probe. The primers designed were: HLBafC 5'-ATTGCGCGTATCGAATACGACG-3' and LibUF 5'-GGCAGGCCTAACACATGC-3'. The "universal" Liberibacter detection method was capable of detecting Laf, Las, Lam, and Lso, however the LafC specific system unexpectedly was capable of detecting both LafC and Las. This possible heterologous detection is not predicted by published sequence data available and may suggest that the Las positive control may contain some LafC-like sequences either through contamination or as part of its natural population. However, even if this result reflects a true ability to detect both LafC and Las, the absence of Las in South Africa (Pietersen *et al.*, 2010) allows us to use the technique for the specific detection of LafC.

Detection of Laf in indigenous members of the Rutaceae

Initial studies were focused on detection of Laf in *Calodendrum capense* (Cape Chestnut) as a Liberibacter (LafC) had already been recorded on it (Garnier *et al.*, 2000) and it could therefore potentially serve as an alternative host to citrus of Laf. A total of 278 *C. capense* samples were collected throughout South Africa and tested for Liberibacters using real-time PCR. While LafC was found in 100 samples, distributed from all areas where collected, no evidence of Laf infection in any sample was found. The identity of the LafC present was confirmed by sequencing the amplicon derived from conventional PCR of the β -operon of the ribosomal protein gene region of the first 17 infected trees found and of a representative sample from each district. This study is comprehensively reported on in a publication enclosed as appendix A; PHAHLADIRA, M.N.B., VILJOEN, R., AND PIETERSEN, G., 2012. Widespread occurrence of "*Candidatus* Liberibacter africanus subspecies capensis" in *Calodendrum capense* in South Africa. *Eur. J. Plant Pathol.* 134:39-47. Subsequently *Vepris*, *Clausena* and *Zanthoxylum* spp. were surveyed for Liberibacters and Laf specifically, as these host are also known hosts of *Trioza erytreae*, and hence could also potentially serve as host of Laf. A total of 234 *C. anisata*, 289 *V. lanceolata* and 231 *Zanthoxylum* spp specimens were sampled from across

South Africa, of which 33 *C. anisata*, 16 *V. lanceolata*, 9 *Z. capense* and 1 *Z. daveyii* (as identified by barcoding, Fig. 4.2.11.5) tested positive for the presence of a Liberibacter following real-time PCR (Ct<35) (Table 4.2.11.1). These samples were obtained from different areas across South Africa in both greening and greening-free areas. Most of the samples were obtained from natural vegetation.

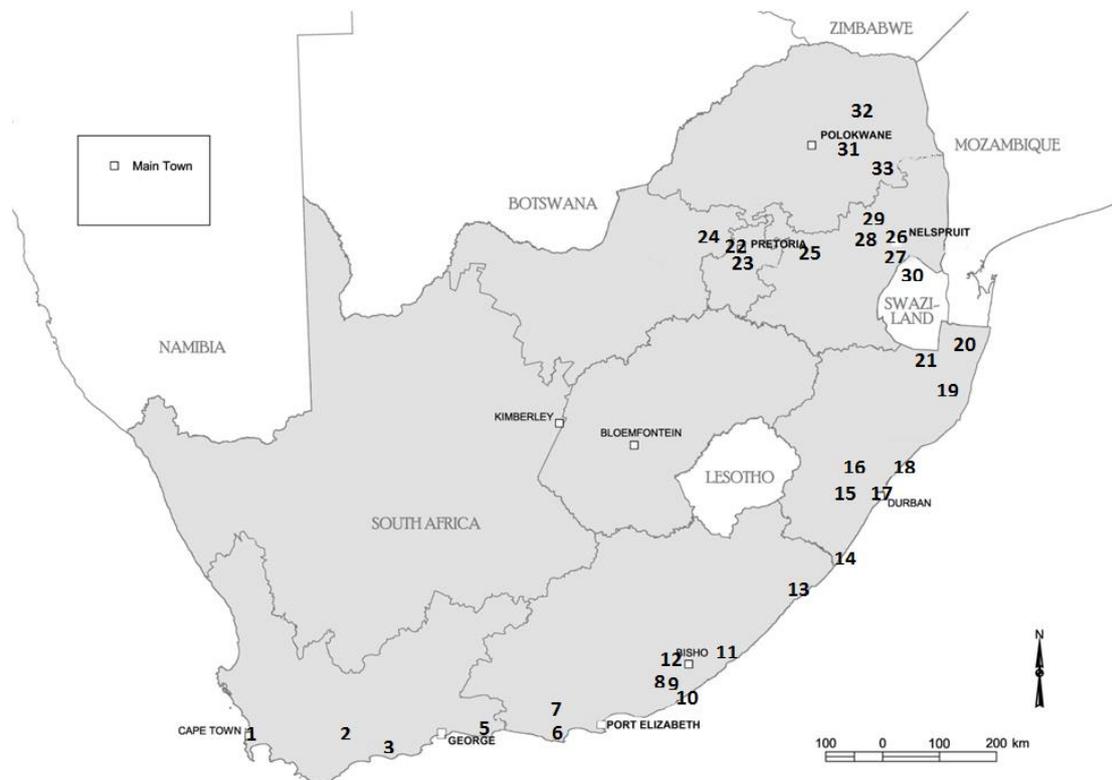


Fig. 4.2.11.1. Sampling sites of indigenous Rutaceous trees across South Africa. Site numbers (1-33) correspond to those listed in Table 4.2.11.1.

Table 4.2.11.1. Number of indigenous trees sampled per site and number of Liberibacter positive (Ct<35) samples identified per site.

Province	District	Site number (Fig)	#Samples with Ct<35/ #Sampled at site		
			<i>C. anisata</i>	<i>V. lanceolata</i>	<i>Z. capense</i>
Western Cape	Kirstenbosch	1	0	0/1	0
	Swellendam	2	0	0/3	0
	Heuningbos	3	3/13	0	0/9
	George	4	1/1	0/9	0
	Knysna	5	4/11	12/70	5/40
Eastern Cape	St. Francis Bay	6	3/7	0	1/11
	Patensie	7	0/2	0/13	0/10
	Grahamstown	8	0/14	2/23	0/10
	Bathurst	9	0	0/1	0/2
	Port Alfred	10	2/4	0/3	0/7
	East London	11	0	1/19	0/11
	Ngele	12	0/3	0	1/2
KwaZulu-Natal	Port St. John	13	0	0/2	0/0
	Port Edward	14	0/5	1/15	1/12
	Richmond	15	0/5	0/20	0/1
	Pietermaritzburg	16	4/8	0/6	0/1
	Durban	17	0/1	0/6	0/5
	Balito	18	1/1	0	2/7
	Hlulwe	19	0/4	0/29	0/9

	Kosi Bay	20	0	0/4	0/1
	Pongola	21	0	0/4	0
Gauteng	Pretoria	22	0	0/24	0/21
	Johannesburg	23	0	0	0/2
North-West	Rustenburg	24	0	0/17	0/6
Mpumalanga	Schoemanskloof	25	0/46	0/15	0/30
	Nelspruit	26	0	0	0/4
	Barberton	27	4/5	0	0/1
	Sabi	28	0/48	0/2	0/3
	Mount Sheba	29	0/11	0	0/12
Swaziland	Unknown	30	0	0	0/2
Limpopo	Magoebaskloof	31	0/11	0	0/6
	Tzaneen	32	0/16	0/1	0/6
	Lekgalameetse	33	0/18	0/2	0
Totals			33/234	16/289	10/231

The *Liberibacter* specific *rplJ* gene, *omp* gene and 16S sequences were determined for those samples testing positive with the universal *Liberibacter* PCR. None of the “no-template” and healthy control samples included per reaction yielded any amplicons. Amplification of *rplJ* and 16S genes was successful for all *Liberibacter* positive samples tested, and full length *rplJ* and 16S sequences could be obtained from different host species. However, for *omp* sequences, *Liberibacter* sequences from only 29/33 *C. anisata*, 14/16 *V. lanceolata* and 8/9 *Z. capense* samples were successfully amplified and sequenced. Various attempts were made to lower stringency of the PCR in order to amplify the remaining samples but failed. Samples from which the *omp* gene could not be amplified were 11-4039, 11-4243, 11-4275, 11-4313, 11-4561, 12-0005, 12-0016 and 12-0017.

Following amplification and sequencing, phylogenetic analyses was performed using the compiled data sets per gene sequence. None of the samples contained any typical Laf sequences. Unique phylogenetic clusters however were obtained correlated with the tree host species from which *Liberibacter* sequences were obtained, for both the *rplJ* (Fig. 4.2.11.3) and *omp* (Fig. 4.2.11.4) sequences.

For *rplJ*, the aligned sequences of *Liberibacter* ex *C. anisata* samples were 97.0% identical to that of LafC sequences from *C. capense* and only 86.4% identical to Laf sequences. Similarly, for the same region, *Liberibacter* ex *V. lanceolata* sequences shared greater nucleotide identity with LafC sequences (96.1%) when compared to Laf sequences (85.3%). However, the *Liberibacter* ex *Z. capense*, whilst forming a distinct clade, shared greater nucleotide identity across aligned sequences with Laf (89.0%) than LafC (84.0%). The distinct clades, differing from known *Liberibacter*s, were shown to correlate with the host tree species.

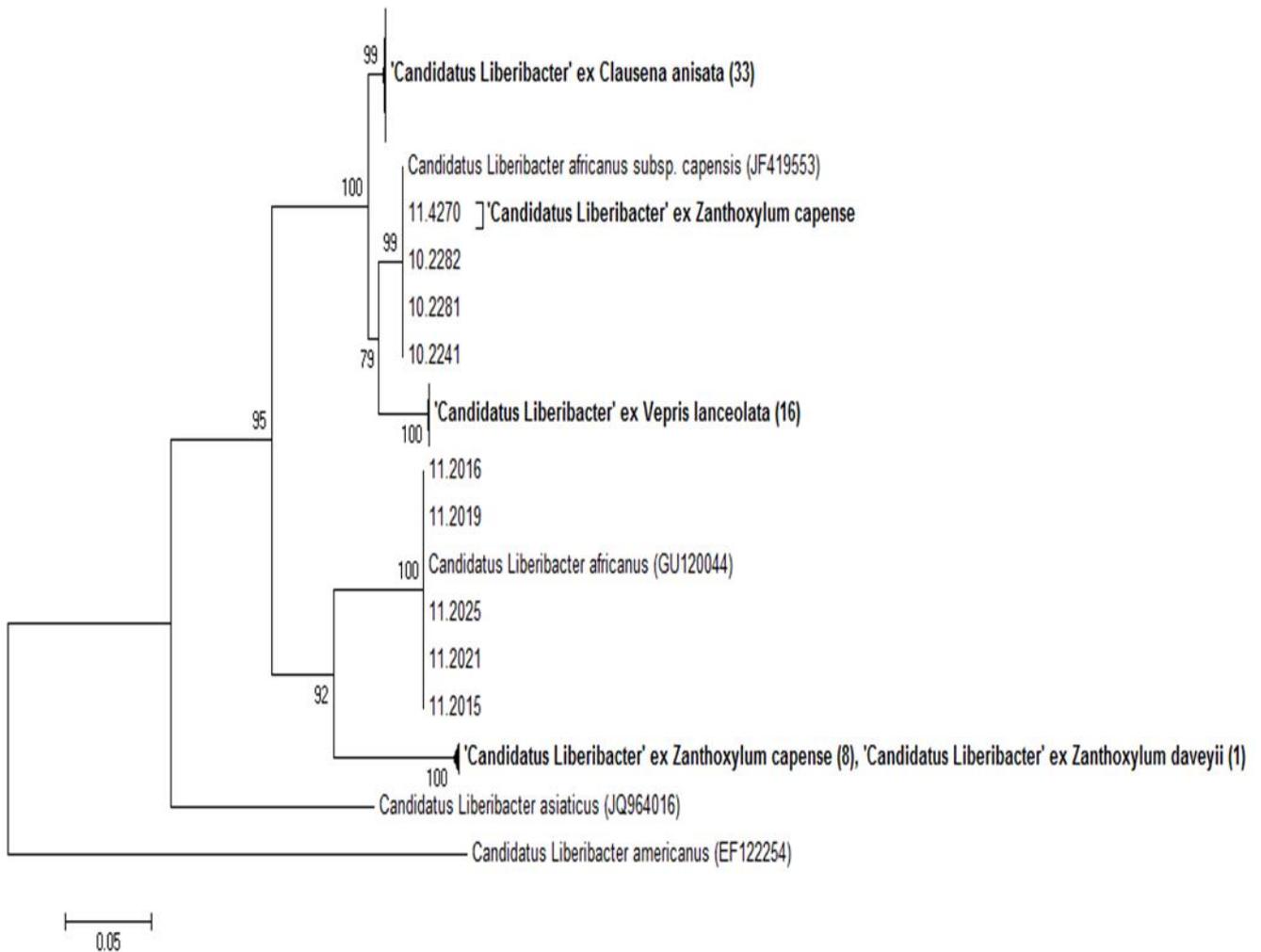


Fig. 4.2.11.2. Maximum Likelihood tree generated of aligned *rplJ* sequences obtained from Liberibacter positive *C. anisata*, *V. lanceolata* and *Z. capense* trees in Mega5 with 1000 bootstrap replicates. Las (JQ964016) and Lam (EF122254) were included as outgroups whereas Laf (GU120044) and LafC (JF419553) were included as ingroups. Bootstrap values are shown at nodes. Numbers behind Liberibacter ex “host” reflect the number of specimens with this sequence in “collapsed branches”

Aligned *omp* sequences from Liberibacter ex *C. anisata* clustered nearer to LafC (95.6% identity) than Laf (77.9% identity). This clustering was also observed for Liberibacter ex *V. lanceolata* sequences which shared 95.7% sequence identity with LafC and only 77.9% identity with Laf sequences. Liberibacter ex *Z. capense* once again clustered nearer to Laf than LafC and shared 87.0% nucleotide identity across aligned *omp* sequences with Laf and only 76.9% sequence identity with LafC. Phylogenetic clustering from both aligned *rplJ* and *omp* sequences indicates distinct Liberibacter phylogenetic clusters that correlate with the host species harboring them.

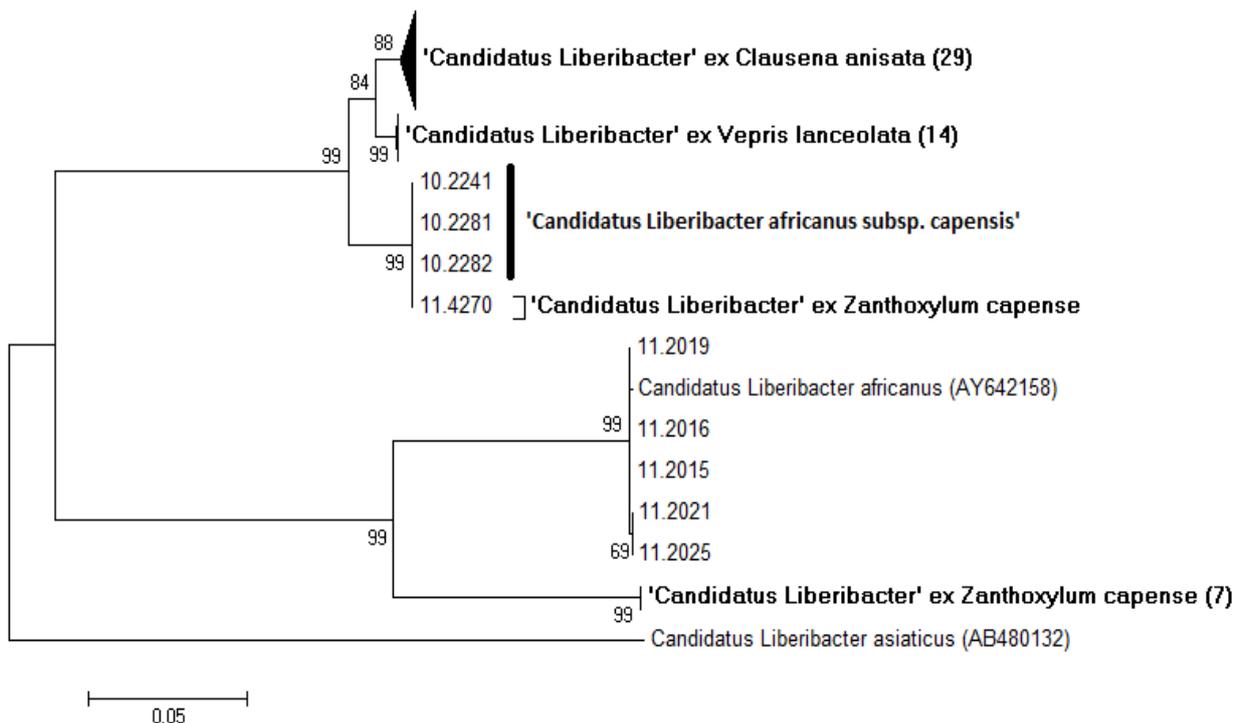


Fig. 4.2.11.3. Maximum Likelihood tree generated of aligned *omp* sequences obtained from Liberibacter positive *C. anisata*, *V. lanceolata* and *Z. capense* trees in Mega5 with 1000 bootstrap replicates. Las (AB480132) was included as an outgroup whereas Laf (AY642158) was used as an ingroup. Bootstrap values are shown at nodes. Numbers behind Liberibacter ex “host” reflect the number of specimens with this sequence in “collapsed branches” Samples 11-2019; 11-2016; 11-2015; 11-2021; and 11-2025 are Laf sequences from citrus and Samples 10-2241; 10-2281 and 10-2282 are LafC sequences from *C. capense*, from previous studies.

The clustering of Liberibacter containing samples, as determined for Liberibacter *rplJ* and *omp* gene sequences, were incongruent with those observed for the 16S rDNA sequences, which has very little resolution amongst Liberibacter species. The 16S rDNA sequence of Liberibacter ex *C. anisata* and Liberibacter ex *V. lanceolata* shared 100% sequence identity with LafC sequences, and 99.0% sequence homology with Liberibacter ex *Z. capense* sequences. Liberibacter ex *Z. capense* 16S rDNA sequences also shared 99.7% sequence homology with Laf sequences and were the only Liberibacter sequences to form a unique phylogenetic cluster separate from Laf and LafC sequences for this gene region (Fig. 4.2.11.3).

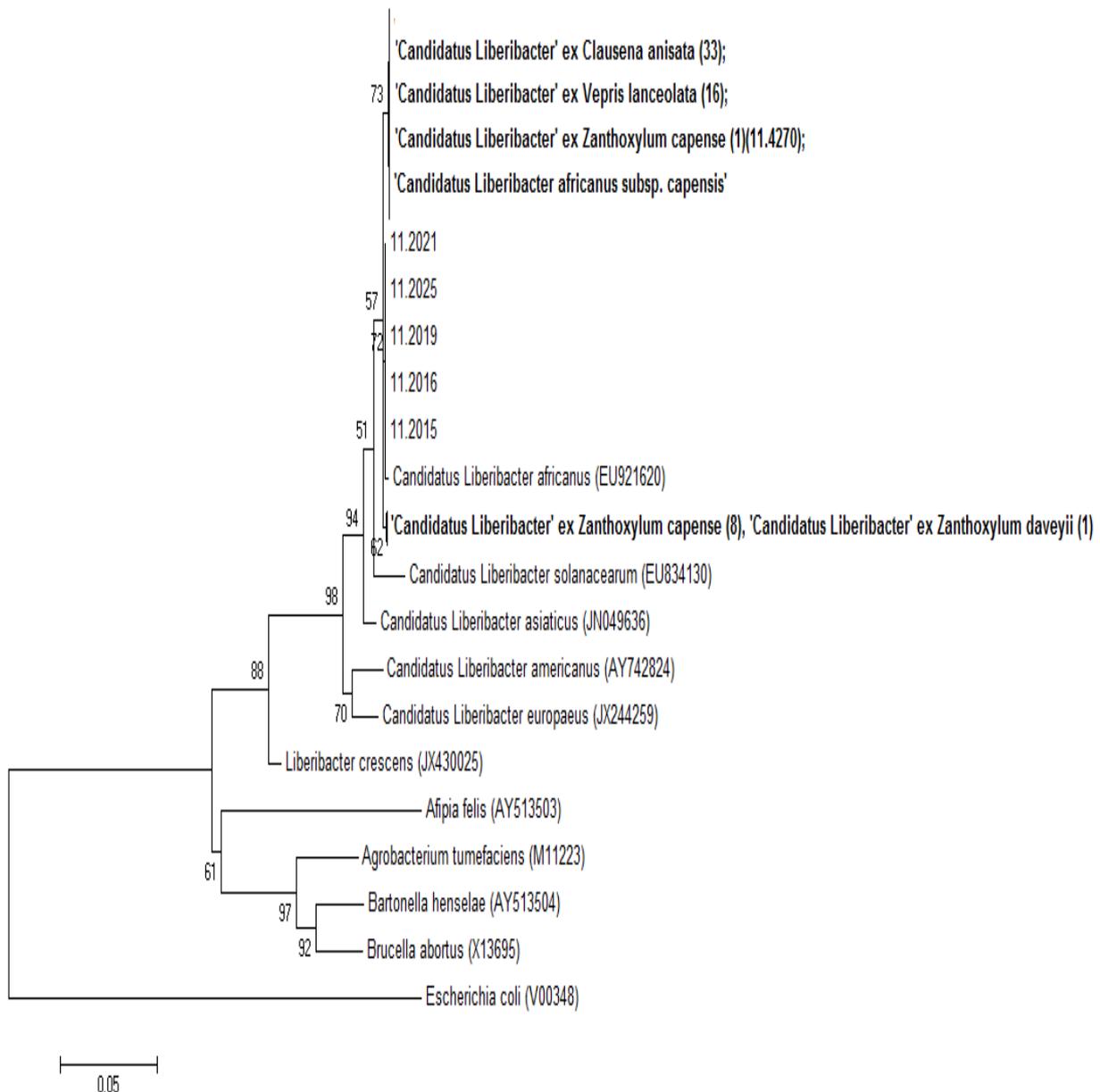


Fig. 4.2.11.4. Maximum Likelihood tree generated from aligned 16S sequences obtained from Universal Liberibacter PCR positive *C. anisata*, *V. lanceolata*, *Z. capense*, all known Liberibacter species, and other proteobacteria in Mega5 using Jukes-cantor model with 1000 bootstrap replicates. *E. coli* (V0038) was included as an outgroup. Bootstrap values are shown on nodes. Numbers behind Liberibacter ex “host” reflect the number of specimens with this sequence in “collapsed branches” Samples 11-2019; 11-2016; 11-2015; 11-2021; and 11-2025 are Laf sequences from citrus from a previous study.

Sequences from sample 11-4270 (Liberibacter ex *Z. capense*) containing LafC was an exception to the general trend of Liberibacters from *Zanthoxylum* sp. clustering with Laf. As observed in Figs. 4.2.11.3-5 it had a 100% identity to LafC sequences in all genes (*rpIJ*, *omp*, *16S*) tested. This is the first report of a Rutaceous host in South Africa with two different Liberibacters.

To assess the correlation of phylogenetic clustering observed for Liberibacter *rpIJ* and *omp* gene sequences and specific tree host species, the accepted barcoding gene of plants, the *rbcL* gene sequence, of the host of Liberibacter positive samples were determined (Fig. 4.2.11.6). This confirmed that tree host species have some clearly associated specific Liberibacter within them (Fig. 4.2.11.3 and 4.2.11.4). It also confirmed that sample 11-4270, clustering with Liberibacters from *C. capense* was obtained from a *Z. capense*. Furthermore analysis of the host *rbcL* gene of sample 11-4561 which was obtained from a putative *Z. davey* specimen rather than *Z. capense* was in fact distinct from other *Z. capense* *rbcL* sequences.

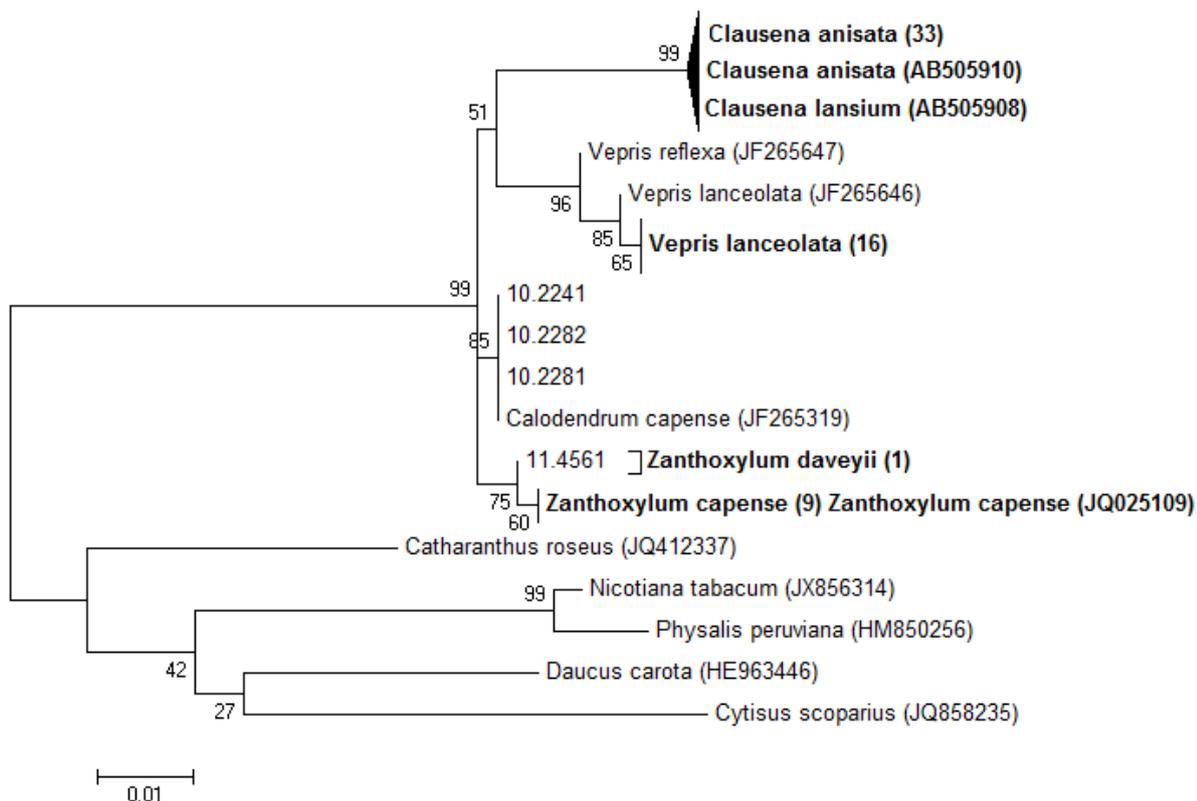


Fig. 4.2.11.5. Maximum likelihood tree generated from aligned *rbcL* barcoding gene for tree host species. Alternative experimental hosts of Las were included as outgroups. Bootstrap values are shown at nodes. Numbers after the tested tree species represent the number of specimens “collapsed” into a branch. The survey of Liberibacters in *Vepris*, *Clausena* and *Zanthoxylum* is being prepared in a manuscript for publication.

Conduct experimental transmission to host other than citrus.

None of the *C. capense*, *Clausena anasita*, *Vepris lanceolata* and *Z. capense* samples inoculated with Laf showed symptoms associated with greening over the entire monitoring period since September, 2007 when the graft transmission was done, however a number of samples died during this period, it is assumed primarily due to some root rot problems. In retests of grafted plants at 36 months PI with real-time PCR one *Calodendrum capensis* plant, inoculated with Laf, yielded low Ct values, indicative of a Laf infection. While it was planned to amplify the bacterial DNA template from this plant using the *rpl* primer-based PCR and sequencing the amplicon in order to confirm the identity of the bacteria, the plant died before this could be achieved. Tests have not been conducted on DNA extracted from this plant previously. A number of the other hosts had high Ct values and may represent Laf infection at very low levels, however only one of the three control citrus plants grafted with the bark patches appeared to be infected with Laf, albeit with a high Ct value, and it is possible that graft transmission of the bacteria was very inefficient under the conditions utilized or that the at the time the tests were conducted that Laf concentrations were generally low in the samples. Tests done five year PI suggest that none of the plants were infected with Laf. Due to the very high temperature this tunnel experiences we have very little faith in the results of this transmission and would like to repeat these transmissions once the tunnel has shadecloth and a more effective cooling system during power-outs.

Conduct transmission studies to assess whether Liberibacters obtained are able to infect Citrus sp. and are transmissible by T. erytraea.

This aspect of the project was severely disrupted when Me. Phahladira changed employment in 2010 as it could not be incorporated immediately in the MSc. of her successor Me. R. Viljoen who started her MSc in 2011 (takes too long for a standard MSc in Microbiology). Some progress in especially establishing the infrastructure do this has been achieved. This aspect will be addressed in a successive project and will form part of a PhD study of Me. Viljoen. The following aspects however were conducted towards this objective:

a) *Establish and maintain Trioza erytreae colony on its preferred citrus host*

Following various early failed attempts to establish a colony of *T. erytreae* on citrus, we determined late in 2010 that *V. lanceolata* appears to be a better host for this psylla than citrus and hence managed to sustain colonies on two occasions that year for a short period of time. The one colony was terminated when it was overrun with mealybugs and the second when the colony numbers dramatically fell due to the presence of *T. erytreae* parasitoids. Subsequently a few, healthy appearing *T. erytreae* adults (to avoid parasitoids) were collected from a citrus grove in Pretoria and placed on healthy *V. lanceolata* seedlings grown from seed under insect-free conditions in greenhouses. These were maintained in insect-proof cages at 23°C in a room with natural day-length and light from proximal windows supplemented with cool-white fluorescent light from the centre of the room for 10 hours a day. Several successful passages of plants and generations of *T. erytreae* were obtained passing through full cycles from oviposition to adulthood. The colony was maintained throughout 2011 and until February, 2012, when it unfortunately also was overrun by mealybugs, probably introduced when fresh *V. lanceolata* saplings were added to the colony to maintain it. Subsequently various attempts to maintain a colony has met with limited success as on a further two occasions colonies were overrun with mites or mealybugs. In the latest attempt, 2012/13, healthy, Vepris plants, untreated with insecticides were placed in cages with *Cryptoleamus* ladybirds which appeared to rid the plant of any mealybugs, unfortunately no *Trioza*'s were obtained subsequently.

b) *Obtain plants needed for transmission trials*

Experiments planned and scheduled for vector and graft transmission studies amongst Rutaceous species are still planned within the successive project. Collection of sufficient Liberibacter free Citrus, *C. capense*, *V. lanceolata*, *Z. capense* and *C. anisata* trees to perform both grafting and vector transmission studies were acquired and their health status tested and can be used in transmission studies once a new *T. erytreae* colony is established.

Total DNA was extracted from these plants which was tested for the presence of Liberibacters using real-time PCR. All trees were negative and can therefore along with all the other plants testing negative can be used in transmission studies.

Vector transmission of Laf and Indigenous Rutaceous host Liberibacters

During a field trip to Knysna in 2011, psyllids unlike *Trioza erytreae* were observed on some trees and collected into ethanol or maintained on branchlets in plastic bags. A *Trioza*-like species (not *T. erytreae*) was tentatively identified by Ian Millar, ARC-PPRI from *V. lanceolata*, while a single individual of a *Diaphorina* sp. was identified from *Z. capense*. Contact was made with Dr. Daniel Burckhardt in Naturhistorisches Museum, Switzerland, the current world authority on psyllid taxonomy, to have these specimens identified to species level. Unfortunately Dr. Burckhardt requires many individuals of a specimen and will be unable to identify the *Diaphorina* species from a single individual specimen. During a subsequent trip to Knysna, no additional psyllids were observed and those tentatively ascribed to *Trioza* by Dr Millar were submitted to Dr. Burckhardt who has identified them as *Parapsylla capensis* (Petty, 1933), a previously described Southern African species. Its role in transmission of Liberibacters is undetermined. Psyllid-like insects were also collected from Schoemanskloof in February, 2012 (Accession 12-0116), these were identified as belonging to the Psyllidae by Mr. Ian Millar at ARC-PPRI, but could not be identified to genus or species level.

LafC infected *C. capense* trees infested during 2011 with the *Empoasca* leafhopper were monitored on three occasions in the 2011 report period with no individuals being observed. In February, 2012 some individuals were observed and collected from a LafC infected *C. capense* tree in Springbok park. DNA was extracted from two sets of five leafhoppers after which a Generic Liberibacter real-time PCR was performed. One of the samples tested negative for the presence of Liberibacters whereas the other sample had a Ct value of 37.25 (ie. Possibly a low concentration positive). While this is not evidence that the Leafhoppers are vectors of the Liberibacter but just contain the Liberibacter at least in their gut, it does suggest that a colony of leafhoppers should be established. Subsequently however no leafhoppers were observed on the *C. capense* tree. . Psyllids collected from *C. capense* in Springbokpark, Pretoria, were identified as *Moraniella calodendri* (Moran) by Dr. Burckhardt. These were shown to contain LafC at high concentration based on real-time PCR reactions, conventional PCR and sequencing. A number of individuals of these were selected and submitted for next generation sequencing.

Whole genome sequencing of Laf.

This aspect was done in collaboration with the Dr. Hong Lin of the USDA-ARS Crop Diseases, Pests and Genetics Research Unit, Puller, California, and have resulted in one publication thus far [DODDAPANENI, H., LIAO, H., LIN, H., BAI, X., ZHAO, X., CIVEROLO, E.L., IREY, M., COLETTA-FILHO, H., AND PIETERSEN, G., 2008. Comparative phylogenomics and multi-gene cluster analyses of the Citrus Huanglongbing (HLB)-associated bacterium Candidatus Liberibacter" BMC Research Notes 1(75)]. Leaf

samples from 27 *Citrus sinensis* cv. Kara Kara plants growing on the Experimental Station of the University of Pretoria were collected and subjected to total DNA extraction using a CTAB protocol. 25 of these plants tested positive for the presence of Laf using real-time PCR. Individual *Trioza erytreae* insects were collected from the trees that tested positive. In order to test for the presence of Laf in these insects, individuals were subjected to total DNA extraction, using a modified CTAB protocol. All *Trioza erytreae* DNA extracts that were tested using real-time PCR were positive for the presence of Laf. A few hundred individuals with high concentrations of Laf were sent to Dr H. Lin at the USDA Crop Diseases, Pests and Genetics Research Unit, where the Laf template was prepared for whole genome sequencing of the bacterium using an Illumina next generation sequencing platform. A sequencing run was performed and data assembled using Las and *Liberibacter solanacearum* (Lso) as reference conducted. David Read spent two months in this laboratory (Feb., March, 2012). Contigs were generated from the Illumina reads and gaps were closed using conventional Sanger sequencing. The genome is now essentially fully sequenced, and has been checked through the NCBI Prokaryotic Genome Annotation Pipeline. Full analysis of the data is currently underway and a manuscript is in preparation. The genome has been deposited with Genbank and a release date of December 2013 has been imposed on it, in order to finalize publication of the results.

Three representative LafC sequences from the ribosomal protein gene rplA to rplJ regions of samples 10-2271, 10-2273 and 10-2269 have been submitted to Genbank with accession numbers; JF419553, JF419554, and JF419555.

DNA from a LafC infected *C. capense* sample (10-2279) was subjected to two Illumina runs locally. Sequence data of a completed run was obtained at the end of this report period and is currently being analyzed. While coverage along the genome is relatively low, it may be possible to get most of the genome sequenced. Further LafC-containing psyllids and *C. capense* samples have been prepared, the LafC titer determined and submitted for sequencing.

Conclusions to date

Laf, in the strict sense has not been found in any alternate Rutaceous hosts thus far, and based on current information no other host, other than citrus sp., appears to play a role in the epidemiology of Citrus greening. However Laf-like *Liberibacter*s appear to occur in many of the indigenous Rutaceae. These *Liberibacter*s appear to be associated with specific genera of the Rutaceae, with the one most closely related to Laf thus far, being found in *Zanthoxylum capensis*.

Technology transfer

Peer Reviewed scientific article:

DODDAPANENI, H., LIAO, H., LIN, H., BAI, X., ZHAO, X., CIVEROLO, E.L., IREY, M., COLETTA-FILHO, H., AND PIETERSEN, G., 2008. Comparative phylogenomics and multi-gene cluster analyses of the Citrus Huanglongbing (HLB)-associated bacterium *Candidatus Liberibacter*" BMC Research Notes 1(75).

VAN VUUREN, S. P., COOK G., AND PIETERSEN, G. 2011 Lack of evidence for seed transmission of "*Candidatus Liberibacter africanus*" associated with Greening (Huanglongbing) in citrus in South Africa. Plant Disease 95(8):1026.

PHAHLADIRA, M.N.B., VILJOEN, R., and PIETERSEN, G., 2012 Widespread occurrence of "*Candidatus Liberibacter africanus* subspecies *capensis*" in *Calodendrum capense* in South Africa. European Journal of Plant Pathology 134:39-47.

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PHAHLADIRA, M.N.B. AND PIETERSEN G., 2010. Detection of “*Candidatus Liberibacter africanus*” subspecies *capense* widely occurring in *Calodendrum capense* in South Africa. 6th Citrus Research Symposium, Champagne Sports Resort, Drakensberg, 15 - 18 August 2010

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PIETERSEN, G. Background, current situation and management of HLB and its vector in South Africa. 2nd International Workshop on Citrus Huanglongbing and The Asian Citrus Psyllid Mérida, Yucatán, México. July 19-23, 2010.

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4.2.12 **PROGRESS REPORT: Differential cultivar selection or suppression of *Citrus tristeza virus* (CTV) genotypes**

Project 1056 (2012/13 – 2013-14) by G. Cook, V. Maqutu, J.H.J. Breytenbach and S.P. van Vuuren (CRI)

Summary

Citrus tristeza virus (CTV) is a complex of variants. This insight and the subsequent development of diagnostics for strain determination enable the analysis of mixed populations. We have expanded on a published CTV strain detection system and determined the strain composition of most of the CTV sources maintained at CRI using this methodology, including the sources used within this trial. In addition we tested various maintenance source plants of GFMS12 and GFMS35 pre-immunisation sources at 3 different institutions including all grapefruit mother trees maintained at the Citrus Foundation Block. This investigation indicated that segregation of strains within these primary sources has occurred and that various maintenance sources lack different strains that are detected as components of the original sources. These findings could potentially explain the cross-protection break down experienced in the field. Four different, single strain CTV sources were used to inoculate various citrus cultivars and transmission, translocation and symptom expression of each strain is being studied. ELISA tests to detect CTV within each trial plant were done at 7, 13 and 24 weeks after inoculation by sampling at 15cm, 30cm and at the shoot tip respectively. Various susceptibilities are noted with the different cultivars and citrus types and are detailed within the body of the report. Plant growth measurements were also done after six months. Thus far significant reduction in plant growth was only observed in one commercial cultivar, Nules Clementine, and the susceptible indicator host, Mexican Lime, with specific strains. All plants were cut back and plants will be allowed a second regrowth cycle to allow sufficient time for symptom expression. Final analysis will again include plant growth measurements after 6 months, stem pitting ratings will be done for the first time, 12 months after initial inoculation.

Opsomming

Sitrus tristeza virus (CTV) bestaan uit 'n kompleks van rasse. Hierdie insig gekoppel met die ontwikkeling van diagnostiese toetse verskaf die vermoë om gemengde CTV bevolkings te ontleed. Ons het uitgebrei op 'n gepubliseerde opsporingstelsel vir CTV rasse en het die rasprofiel opgestel van meeste van die CTV bronne wat by CRI instand gehou word, insluitend die bronne wat gebruik is in die huidige proef. Daarbenewens het ons verskeie instandhoudingsbronne van GFMS12 en GFMS35 pre-

immuniseringsbronne getoets by 3 verskillende instellings, asook alle pomelo moederbome by die Sitrus Grondvesblok wat met GFMS 35 gepre-immuniseer is. Hierdie ondersoek het aangedui dat afskeiding van rasse plaasgevind het en dat verskeie instandhoudingsbronne nie meer die volle komponente van rasse besit wat in die ouer bronne teenwoordig is nie. Hierdie bevindinge is 'n moontlike verduideliking vir die kruis-beskerming-afbraak wat in boorde ervaar word.

Vier verskillende, enkelras CTV bronne is gebruik om verskeie sitrus kultivars te inokuleer en transmissie, translokasie en simptoom uitdrukking van elke ras te bestudeer. CTV ELISA toetse is op elke proefplant gedoen. Monsterneming is 7, 13 en 24 weke na inenting gedoen en is onderskeidelik op 15cm, 30cm en by die groeipunt geneem. Die verskillende kultivars en sitrus tipes het verskil in vatbaarheid. Gedetailleerde inligting word in die verslag verskaf. Plantgroeimetings is ook gedoen ná 6 maande. Vermindering in groei as gevolg van spesifieke ras besmettings is slegs waargeneem in een kommersiële kultivar nl. Nules Clementine, asook by die gevoelige biologiese indikator gasheer, Meksikaanse lemmetjie. Alle plante is terug gesny en 'n tweede hergroeitogelaat om voldoende tyd te laat vir simptoom uitdrukking. Finale proefanalise sal hergroeimetings na 'n verdere 6 maande insluit asook stamgleuf evaluasies wat op 12 maande na die aanvanklike inenting sal plaasvind.

4.3 **PROGRAMME: FRUIT AND FOLIAR DISEASES**

Programme coordinator: G.C. Schutte (CRI)

4.3.1 **Programme summary**

Results from field trials where new systemic and contact fungicides alone or in combination with registered fungicides were tested on 'nova' mandarins for the control of *Alternaria* brown spot, showed that eight applications of copper oxychloride performed the best but also resulted in serious copper stippling. Combinations and altering of different fungicides with different modes of action showed promise and should be investigated on a commercial scale (4.3.2).

From various spray trials using different machines at 1000 to 24000 L/ha, it was clear that excessively high spray volumes (>10 000 L/ha) did not result in better spray deposition on leaves. Improved spray deposition quantity and uniformity on leaves at better spray efficiency could be obtained at lower spray volumes through optimal use of equipment or through the use of more efficient sprayers. Preliminary data showed promising deposition quantity, uniformity and efficiency results at low volumes (2000 to 4000 L/ha), lower spray pressures (15 bar) and adequate, efficient spraying speeds (1.9 to 2.9 km/h). Penetration of dense canopies was shown to be a problem. Adjuvants may affect canopy penetration in dense canopies. There is a need for the development of a tree-row-volume sprayer calibration system (4.3.3).

The saprophyte, *Botrytis cinerea* Pers. the causal pathogen of blossom blight on lemons can survive on organic matter with a low pH. The pH, Brix and mycelium growth rates of *Botrytis* on a petal- and stamen-extract media of different citrus cultivars were compared over two seasons from different production areas. No pattern could be detected as results were inconsistent. Field trials showed that benomyl and iprodione performed the best in controlling the disease, while two new fungicides tested in 2012, fenhexamid and pyrimethanil, also performed well. A commercial application of benomyl in Addo showed that blossoms in the balloon stage were better protected than open blossoms (4.3.4).

Programopsomming

Resultate van 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 agt bespuitings met koperoksichloried die beste gevaar het. Kombinasies en afwisseling van verskillende swamdoders met verskillende meganismes van werking toon groot belofte en moet verder op kommersiële skaal ondersoek word (4.3.2).

Verskeie spuitproewe met verskillende masjiene by spuitvolumes vanaf 1 000 tot 24 000 L/ha wys dat uitermate hoë spuitvolumes (> 10 000 L/ha) nie beter spuit neerslag op blare realiseer het nie. Verbeterde spuitdeponering, eenvormigheid en beter spuitdoeltreffendheidsvlakke kan d.m.v laer volume toedienings tesame met optimale gebruik van toerusting of deur die gebruik van meer doeltreffende spuit-tegnologie verkry word (4.3.3). Resultate van voorlopige studies van lae-energie spuitmasjiene dui op belowende deponeringskwantiteit, uniformiteit en spuit-effektiwiteit teen lae spuitvolumes (2000 tot 4000 L/ha), laer spuit-druk (15 bar) en spuit-spoed (1.9 tot 2.9 km/h). Penetrasie van digte blaredakke is as probleem uitgewys. Benatters kan penetrasie in digte bome benadeel. Daarom moet 'n boom-ry-volume kalibrasiesisteen ontwikkel word (4.3.3).

Die saprofitiese *Botrytis cinerea* Pers., wat bloeiserversenging 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 van verskillende produksiegebiede bepaal. Geen patroon kon bepaal word nie aangesien resultate wisselvallig was. In veldproewe het benomyl en iprodione asook twee nuwe swamdoders, fenhexamied en pyrimethanil, goeie beheer van die siekte tot gevolg gehad het. 'n Kommersiële bespuiting van benomyl in Addo het getoon dat blomme wat toe is (ballonstadium) langer as oop blomme beskerm word (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**

Project 750 (September 2004 – June 2012) by G.C. Schutte and C. Kotze (CRI)

Opsomming

Nuwe sistemiese en kontak swamdoders is op hul eie asook in kombinasies met geregistreerde swamdoders vir die beheer van *Alternaria* bruinvlek op 'Nova' mandaryne getoets. Resultate toon dat sewe tot agt maandelikse koperoksichloried bespuitings die beste gevaar het, maar gepaardgaande met erge stippelvorming. Kombinasies en afwisseling van verskillende swamdoders met verskillende meganismes van werking toon groot belofte en moet verder op kommersiële skaal ondersoek word.

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. Results showed that copper oxychloride applied as seven to eight monthly applications performed the best, but accompanied with serious copper stippling. Combinations and altering fungicides with different modes of action showed promise and should be investigated on a commercial scale.

4.3.3 **FINAL REPORT: Optimisation of fungicide spray applications in citrus orchards**

Project PPL 891 (April 2007 - March 2013) by Paul Fourie (CRI at SU)

Summary

In South Africa, fungicide spray application at medium to high 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 run-off and drift, which results in considerable environmental pollution of soils and air and reduced spray efficiency. This study uses a spray deposition assessment protocol and previously developed deposition benchmarks indicative of the biological effectiveness of depositions to improve spray application and possibly through cost-saving lower volume foliar application. From various spray trials using various machines at spray volumes from 1000 to 24000 L/ha, it was clear that excessively high spray volumes ($>10\ 000$ L/ha) did not result in better spray deposition. 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 on leaves. In the past season, the potential of low-energy sprayers were evaluated on leaves. Preliminary data showed promising deposition quantity, uniformity and efficiency results at low volumes (2000 to 4000 L/ha), lower spray pressures (15 bar) and adequate, efficient spraying speeds (1.9 to 2.9 km/h). The negative effects on deposition parameters as influenced by density of canopy were also shown in this trial. The denser a canopy, the harder it is to penetrate it with the spray mixture and the more uneconomical it is to spray. The effect of canopy density was also seen with field evaluation of adjuvants Break-Thru S240 and Break-Thru Union, showing that super spreader adjuvants may negatively affect canopy penetration in dense canopies. From field spray trials it is also evident for the need of the development for a tree-row-volume sprayer calibration system, since canopy geometry and density clearly influences spray application dramatically.

Opsomming

In Suid-Afrika word swamdoders teen medium dek-bespuitings ($\pm 9\ 000$ L/ha) aanbeveel om vrug- 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 gebruik 'n unieke spuit neerslag-assesseringsprotokol vir beter interpretasie van spuit deponeringsresultate asook drempelwaardes vir die aanduiding van biologiese doeltreffendheid van spesifieke deponeringshoeveelhede om verbeterde of kostebesparende spuit-toediening op blare te bestudeer. 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 L/ha), nie beter spuit neerslag op blare realiseer het nie. Soortgelyke en selfs beter spuitdeponering, eenvormigheid en beter spuitdoeltreffendheidsvlakke kan d.m.v laer volume toedienings tesame met optimale gebruik van toerusting of deur die gebruik van meer doeltreffende spuit-tegnologie op blare verkry word. Tydens die afgelope seisoen is die potensiaal van lae-energie spuitmasjiene op blare bestudeer. Voorlopige resultate dui op belowende deponeringskwantiteit, uniformiteit en spuit-effektiwiteit teen laer volumes (2000 tot 4000 L/ha), laer spuit-druk (15 bar) en spuit-spoed (1.9 tot 2.9 km/h). Die negatiewe effekte van boomdigtheid (blaredak) is duidelik gewys, siende dat digter bome moeiliker penetreer word, en dus duurder is om effektief te spuit. Proewe met Break-Thru S240 en Break-Thru Union wys dat benatters (“super-spreaders”) penetrasie in digte bome kan benadeel. Hierdie proewe wys duidelik dat ’n boom-ry-volume kalibrasiesisteem ontwikkel moet word siende dat boom-vorm en -digtheid spuit-toediening dramaties beïnvloed.

Introduction

Several economically important fungal diseases (such as citrus black spot and *Alternaria* brown spot) and insect pests (such as false codling moth, mealybug, red scale and citrus thrips) are primarily controlled by means of regular fungicide or insecticide sprays. At present, full cover fungicide/insecticide spray application to citrus trees in South Africa involves applications of 10 000 to 16 000 L/ha (Grout, 1997). However, mature citrus trees are reported to hold sprays to a maximum of 2 300 L/ha only, depending on the canopy geometry and foliage density (Cunningham and Harden, 1998, 1999). As much as 85% of the excessive spray volume is therefore lost to endo- and exodrift, which results not only in considerable environmental pollution of soils and air, but also increased run-off, reduced spray cover and therewith reduced spray efficacy (Furness et al., 2006a; Landers and Farooq, 2004). Moreover, excessively high spray volumes are not time and cost effective. Scope for improvement of the current spray application in southern Africa certainly exist as growers of citrus for processing in Florida (USA) apply 1 500 L/ha to mature trees (Pete Timmer, pers. comm.), while the use of novel spray applicators allowed a reduction in spray volumes to below 6 000 L/ha in Australia (Furness et al., 2006b). Citrus trees differ in geometry and density depending on type, cultivar and growing region. Yet, in South Africa, set spray volumes exist for application types (Grout, 1997). Furness et al (1998) developed a sprayer calibration system based on canopy size, length and row which proved to be effective for the use in citrus. Reviewing current spray volume/application strategies and the development of a tree row volume (TRV) calibration system, specific for South Africa is needed.

In order to study the optimisation of spray application on grape vineyards, researchers at Stellenbosch University’s Plant Pathology department (USPP) have developed a spray assessment protocol using fluorometry, photomicrography and digital image analyses (Brink *et al.*, 2004, 2006). Following the determination of benchmark levels for biologically effective spray deposits, they clearly demonstrated that the current best-practice spray applications in table and wine grape vineyards did not result in biologically effective spray deposits. One method of improving the *status quo* was to use spray applicators within specific optimal volume output ranges. USPP’s research has shown that optimal use for an air shear machine (Cima™) in table or wine grape vineyards was between 250 and 500 L/ha, compared with the standard 1,000-1,500 L/ha. Biologically effective spray deposits on leaves and bunches were effected by increasing the fungicide concentration relative to the decrease in volume (2- or 4-fold).

A similar study is herewith proposed for the citrus industry, with ultimate aims to optimise spray application in citrus orchards and to improve cost and time effectiveness, without compromising biological efficacy.

The following objectives are proposed for this study:

1. Conduct a thorough survey of current spray application materials and methods in all major citrus growing areas.
2. Determine benchmarks for biological efficacy of copper oxychloride against *Alternaria* brown spot.
3. Characterisation of spray deposition with current spray application methods.
 - a. Evaluate methods for optimisation of spray application with commonly-used applicators.
 - b. Evaluate methods for optimisation of spray application with novel applicators.
4. Development and validation of a user-friendly calibration system.
5. Develop a guideline for optimal use of commonly-used and novel spray applicators in citrus orchards.
6. Use of adjuvants for improved spray deposition on citrus leaves and fruit

Current objectives as part of Gideon van Zyl’s PhD(Agric) study

7. Physical and chemical effects of adjuvants on spray deposition and control of *Alternaria* brown spot following copper oxychloride sprays on mandarin leaves
8. Modelling of the influence of quantity and quality of copper oxychloride sprays on control of *Alternaria* brown spot on mandarin leaves

Materials and methods

1. Conduct a thorough survey of current spray application materials and methods in all major citrus growing areas

A comprehensive questionnaire comprising all aspects of spray application in citrus orchards was compiled. This questionnaire was handed out after grower study group meetings in various citrus growing areas. The questionnaire was also circulated on CRInet. The data will be summarised to accurately reflect the current status of spray application in the citrus industry, which is essential for conceptualisation of following experimentation. The information will furthermore prove invaluable when future changes to the *status quo* are negotiated with growers, the agrichemical industry and the Registrar for Agricultural Remedies.

2. Determine benchmarks for biological efficacy of copper oxychloride against *Alternaria* brown spot

Earlier work on this aspect has been reported on in the 2007/8 report. A scientific article was published: PH Fourie, M du Preez, JC Brink and GC Schutte, 2008. The effect of run-off on spray deposition and control of *Alternaria* brown spot of mandarins. Australasian Plant Pathology 38: 173-182. A novel spray deposition assessment protocol has been described, the suitability of a fluorescent pigment as a tracer for copper fungicides was confirmed and a robust benchmark was developed for control of *Alternaria* brown spot, allowing for interpretation of biological relevance of spray deposition and optimisation of spray application in citrus. The research has been completed and a scientific article has been published: JG van Zyl, PH Fourie, GC Schutte, 2013. Spray deposition assessment and benchmarks for control of *Alternaria* brown spot leaves with copper oxychloride. Crop Protection 46: 80-87.

3. Characterisation of spray deposition with current spray application methods

In the 2011 annual report, all earlier spray trial work was comprehensively reported.

In 2012/13, one spray trial has been conducted with two spray machines at different spray volumes at Citrusdal (Western Cape) in February 2013. Using less spray machines makes throughput of trials more efficient and allows for evaluation of machines at more variables. The trial has been completed and reported (Addendum A). A repeat of this trial is being planned for later 2013.

4. Development and validation of a user-friendly calibration system.

This objective will be postponed, as it is not a critical control point in excessively high-volume spray application. As more knowledge is gained, it will be addressed through extension and re-analysis of spray trial data.

5. Develop a guideline for optimal use of commonly-used and novel spray applicators in citrus orchards.

This objective will be addressed through extension, and firstly by updating the relevant sections in the Production Guidelines.

6. Use of adjuvants for improved spray deposition on citrus leaves and fruit

Final report was included in the 2009/10 annual report. However, the work is on-going as part of the PhD(Agric) project of Gideon van Zyl.

Laboratory spray trials. Several (18) biotests were conducted (using similar methodologies as described for the bench-mark objective; detail methodology described in 2011/12 report, Addendum B) to determine the effect of adjuvants on the bio-efficacy of copper oxychloride. Deposition quantity and quality data from these trials will also be used to improve the benchmark model, by including the deposition quality parameter.

Field evaluation. Four field spray trials were conducted with the selected adjuvants, BreakThru®, NuFilm17®, Exit®, Villa51®, Wetcit® en Herbiplus®, as well as Citrole100®, a mineral oil adjuvant, as well as Entrée®, an adjuvant similar to Exit®, but used at lower dosages. A scientific paper is in preparation (included as Addendum C in 2011/12 report).

Field evaluation of adjuvants Break-Thru S240 and Union at different spray volumes in South African citrus orchards. Two field spray trials were conducted with Break-Thru S240 and Union at different spray volumes

in the Western- and Eastern Cape The research has been completed and a scientific article is being prepared for publication (Addendum B).

7. Physical and chemical effects of adjuvants on spray deposition and control of *Alternaria* brown spot following copper oxychloride sprays on mandarin leaves

Effect of adjuvants and deposition quality on ABS control. Selected adjuvants and yellow fluorescent pigment will be sprayed at industry recommended concentrations in laboratory spray trials on young flush mandarin leaves with and without copper oxychloride contact fungicide. These applications will be done at a predetermined 'pre run-off' volume to minimise the influence spray run-off has on the quantity parameter. To determine the effect of "post run-off" sprays on deposition quality, sprays will also be done at post run-off volumes as was done in Chapter 3. Subsequently, sprayed leaves will be inoculated with a virulent strain of *Alternaria alternata* pv. *citri*. Deposition parameters and disease incidence will be evaluated for pre- and post-run-off adjuvant alone and adjuvant+copper oxychloride sprays. Previous methodology described in Objective 2 will be used for spray application, spray inoculation and deposition and ABS analysis. Each sprayed leaf batch will be stored, batched and Cu residue levels determined. Collected data will be used to relate quality deposition data with disease control achieved. ABS control data of adjuvant and adjuvant + copper oxychloride sprays will also be compared to determine synergistic effects of different spray adjuvants with copper oxychloride fungicide. All relevant data, especially deposition quality data, will be used to improve the FPC deposition benchmark model through statistical modelling.

Histopathology study. Adjuvant alone and adjuvant + copper oxychloride sprays will be evaluated for fungicidal activity. Through an *in vitro* study, adjuvant influence on pathogen development will be evaluated. PDA growth medium will be amended with adjuvants at recommended concentrations with and without copper oxychloride after which the amended plates will be inoculated with *Alternaria alternata* pv. *citri*. Fungicidal activity of adjuvants will be evaluated by comparing spore germination and fungal growth measurements on amended and non-amended plates. The pH of each spray mixture will be measured and correlated with Cu residue level.

Physical and chemical effect of adjuvants influencing quality of copper oxychloride deposition on the plant surface, and through it, pathogen development, will be consequently evaluated through microscopically investigating laboratory sprayed and inoculated leaves following similar methodology as described by van Zyl *et al.* (2010). Young flush mandarin leaves will be sprayed, inoculated and incubated as described previously (Objective 2). At the beginning of visual symptom development, leaves will be appropriately prepared by cutting out a small piece for experimental purposes. Cut sections will be stained with appropriate dyes for epi-fluorescence microscopy to make visualisation of fungal structures possible. Germination, germ tube lengths and mortality of fungal structures will be examined (van Zyl *et al.*, 2011) to determine the various treatments' effect on pathogen development.

Scanning Electron Microscopy. Physical modification of the cuticle layer as influenced by the addition of selected adjuvants will be studied through scanning electron microscopy (SEM). This will be done on inoculated and non-inoculated leaves of untreated leaves, adjuvant alone and adjuvant + copper oxychloride sprayed leaves. SEM images will be compared to untreated surfaces in terms of surface differences, pathogen behaviour on disrupted surfaces and evaluation of copper oxychloride deposition on the plant surface. Selected adjuvants will be sprayed at industry recommended concentrations in laboratory spray trials on young flush mandarin leaves with and without copper oxychloride contact fungicide and subsequently spray inoculated with a virulent strain of *Alternaria alternata* pv. *citri* using methodology previously described (Objective 2). Sprayed- and inoculated leaves will be prepared for SEM visualisation by cutting a 1 cm² piece from a treated leaf. The piece will be adhered to a specimen holder. Cut edges will be sealed with colloidal silver adhesive. SEM visualisation will commence without further preparation. Visualisations will be done on "fresh" samples not older than 10 min. Another viable method for preparing samples is Cryo-SEM. Cryo-SEM methodology allows for gold coating of samples and to be viewed for longer periods. It involves the immediate freezing of samples in liquid propane. However, the effect that this process will have on the leaf cuticle is unknown and has to be researched further. SEM images will be taken of treated surfaces and compared to untreated surfaces in terms of pathogen interaction and surface differences.

8. Modelling of the influence of quantity and quality of copper oxychloride sprays on control of *Alternaria* brown spot on mandarin leaves

The current developed deposition benchmark model (Objective 2) did not enable accurately predict sprays including adjuvants. Datasets obtained from the current Objectives 2 and 6, as well as the new dataset

obtained from the proposed laboratory bio-efficacy trials will be used to improve the model, most probably through the inclusion of deposition quality as an additional parameter.

Results and discussion

Objective / Milestone	Achievement
1. Conduct a thorough survey of current spray application materials and methods in all major citrus growing areas	In total, 61 questionnaires were received, which is used by the investigators as indication of the industry's spraying practices. This objective was terminated but needs to be reviewed for the development of an effective and user friendly spray application calibration system.
2. Determine benchmarks for biological efficacy of copper oxychloride against <i>Alternaria</i> brown spot	A novel spray deposition assessment protocol has been described, the suitability of a fluorescent pigment as a tracer for copper fungicides was confirmed and a robust benchmark was developed for control of <i>Alternaria</i> brown spot, allowing for interpretation of biological relevance of spray deposition and optimisation of spray application in citrus. The research has been completed and a scientific article has been published (van Zyl <i>et al.</i> , 2013).
3. Characterisation of spray deposition with current spray application methods	To date, 6 orchard spray trial were conducted. Two spray applicators, the Nieuwoudt tower spray and low volume Martignani has been evaluated successfully at various spray volumes and is reported in addendum B. This trial shall be repeated. Results from all field trials shall be used for preparation of a scientific paper
4. Development and validation of a user-friendly calibration system	Lots of information and experience gained, but to date this aspect has not been addressed as it is not a critical control point in excessively high-volume spray application. Will be submitted as new proposal.
5. Develop a guideline for optimal use of commonly-used and novel spray applicators in citrus orchards	These guidelines are being developed following continued experimentation; nonetheless, technology transfer ensures that the latest recommendations and research findings are communicated to the growers.
6. Use of adjuvants for improved spray deposition on citrus leaves and fruit	Two-year contract research project completed and final report submitted to funders and CRI in 2010. Research ongoing as part of PhD-study: additional orchard and laboratory trials completed. One article is in preparation, pending improvement of method to determine quality of deposition. Two orchards spray trials were conducted under contract for Evonik-Degussa to evaluate BreakThru® and BreakThru-Advance® at various spray volumes. A scientific article is being prepared.
7. Physical and chemical effects of adjuvants on spray deposition and control of <i>Alternaria</i> brown spot following copper oxychloride sprays on mandarin leaves	To be conducted in 2013/14
8. Modelling of the influence of quantity and quality of copper oxychloride sprays on control of <i>Alternaria</i> brown spot on mandarin leaves	To be conducted in 2013/14

1. Conduct a thorough survey of current spray application materials and methods in all major citrus growing areas

In total, 61 questionnaires were received, which are used by the investigators as an indication of the industry's spraying practices. Gideon van Zyl was appointed as researcher on contract to focus on spray application research in January 2012. As part of his spray application extension responsibilities, he will use various extension forums for progress with this objective.

2. Determine benchmarks for biological efficacy of copper oxychloride against *Alternaria* brown spot

A scientific article was published: JG van Zyl, PH Fourie, GC Schutte, 2013. Spray deposition assessment and benchmarks for control of *Alternaria* brown spot leaves with copper oxychloride. *Crop Protection* 46: 80-87.

3. Characterisation of spray deposition with current spray application methods

Previous spray trials were reported on in depth for 2011 CRI Annual Report. See addendum A for report of research in progress.

The outcomes of this trial again confirmed the potential of two low-energy sprayers. In a previous Citrusdal spray trial, the Nieuwoudt sprayer proved to be one of the better high volume sprayers evaluated. Those results were supported by the recent trial. However, it was clear that an increase in spray pressure and therewith volume, reduced spray efficiency with no meaningful deposition quantity and uniformity benefit. The lower volume Nieuwoudt application yielded relatively poor results. The Martignani demonstrated its potential as a low-energy low-volume sprayer, despite a too fast tractor speed in a previous trial resulted in poor canopy penetration. In the current trial, it was tested at 1.9 and 2.9 km h⁻¹, which resulted in deposition uniformity and penetration comparable with the high-volume sprays. In fact, based on spray efficiency, the Martignani at 2000 l ha⁻¹ at 2.88 km h⁻¹ proved to be the best application. Interestingly, this application proved to be better than the 4000 l ha⁻¹ application, most probably due to the air shear and electrostatic technology being superior at lower volumes and smaller droplet size. *Please note that these findings should be viewed as preliminary; this trial will be repeated in another orchard (different canopy structure) before final conclusions are drawn.*

4. Development and validation of a user-friendly calibration system

Not specifically researched. Will be submitted as new proposal.

5. Develop a guideline for optimal use of commonly-used and novel spray applicators in citrus orchards

These guidelines are being developed following continued experimentation; nonetheless, technology transfer ensures that the latest recommendations and research findings are communicated to the growers.

6. Use of adjuvants for improved spray deposition on citrus leaves and fruit

Laboratory spray trials. A scientific paper is being prepared (included as Addendum B in 2011/12 report); however, significant changes were made to the deposition quality analysis and following completion and validation of the new analysis, the photos from these trials will have to be re-analysed and results included in the paper. This objective should be concluded in 2013.

Field evaluation. See Addendum D of 2011/12 report. As with lab-trial, we need to re-analyse the photos for with the improved deposition quality analysis. This objective should be concluded in 2013.

Field evaluation of adjuvants Break-Thru S240 and Union at different spray volumes in South African citrus orchards. Objective concluded; see Addendum B. Abstract of the prepared paper:

Citrus producers in South Africa use very high spray volumes (8000 to 12000 l ha⁻¹) to control certain pests and diseases. In order to study the benefit of spray adjuvants on reducing spray volumes, trials were conducted with the organo tri-siloxane surfactants Break-Thru S240 (L6764) and Break-Thru Union (Registration pending). Two separate spray trials were conducted in the Western and Eastern Cape provinces of South Africa in uniform navel orange orchards. Break-Thru S240 and Break-Thru Union, at recommended dosages per hectare (300 ml ha⁻¹, respectively), were sprayed separately at three different spray volumes together with SARDI Yellow Fluorescent Pigment (1 ml l⁻¹) at high (20 l tree⁻¹), medium (14 l

tree⁻¹) and low (8 l tree⁻¹) spray volume. Sprays consisting of the pigment only were used as control treatments. Trees were sprayed from both sides with a commercial multi-fan tower sprayer (BSF-Multiwing) at a constant tractor speed (2.4 km h⁻¹) and pressure (15 bar). Spray volume was calibrated by using different spray nozzles (TeeJet Disc-Core type; full and hollow cone nozzles D3-DC56/46, D4-DC56/46, D5-DC56/46). Leaves were sampled from six canopy positions (inner and outer canopy position at bottom, middle and top of tree). Deposition quantity and quality of fluorescent pigment were determined on upper and lower leaf surfaces using fluorometry, digital photomacrography and image analyses. Spray uniformity in the canopy and efficiency were also compared between treatments. Deposition quantity generally increased with increasing spray volume, but normalised values showed better spray efficiency at lower volumes. In “spray friendly” canopies, the value adding effect of adjuvants, especially Break-Thru Union, was observed in terms of deposition quantity, efficiency and uniformity, especially at low volume applications (8 l tree⁻¹) on the inside and outside of the canopy. As these effects were not as evident in very dense canopies, the importance of canopy management must be stressed.

Conclusion to date

The benchmarks for biological efficacy were satisfactorily determined and support a more comprehensive interpretation of the results. 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. FPC benchmarks were developed and are used to evaluate spray technology research, specifically for control of ABS and similar citrus fruit and foliar diseases.

From the orchard spray trial results obtained to date, it was clear that the highest quantitative deposition per leaf values at the lowest variation between leaves was generally obtained with higher spray volumes. However, it was obvious that the quality of pigment deposition on individual leaves declined with increasing spray volumes due to more run-off, which might also have a detrimental effect on biological efficacy (Fourie *et al.*, 2008). It should furthermore be stressed that the fluorescent pigment dosage of 1x was used when comparing all the different sprayers and calibration settings, even though spray volumes differed. Hence, the dosage per hectare differed substantially between treatments. In relative terms, spray efficiency (expressed as quantitative deposition per leaf per 1000 L of spray volume) in combination with spray uniformity (expressed by the variation in deposition quantity between leaves) are therefore the parameters that should be used when comparing sprayers and calibration settings.

From various spray trials using spray volumes from 1000 to 24000 L/ha, it was clear that excessively high spray volumes (>10 000 L/ha) did not result in better spray deposition. Similar and even improved spray deposition quantity and uniformity at better spray efficiency can thus be obtained at lower spray volumes through optimal use of equipment or through the use of more efficient sprayers, especially if dosage per hectare is equated between treatments.

In terms of tractor speed, it seems that c. 3 km/h should be the upper limit for medium cover spray application; defined as adequate cover of outer and inner canopy leaves and fruit, without emphasis on film-wetting of the trunk and branches as would be required for full cover application. Faster tractor speed reduced spray penetration and spray uniformity, especially in denser canopies.

From the findings to date, it seems clear that at least medium cover sprays can be adequately delivered with 2-sided sprayers. This holds a big advantage over 1-sided sprayers as work rates are improved, costs reduced and orchard traffic and concomitant soil compaction are also halved.

The research is still on-going, but in future evaluation will focus on power-efficient and low-volume sprayers and low-drift nozzles to address the priorities of reducing carbon footprint, reducing production cost and minimising environmental impact of spray application.

In terms of spray pressure, we observed that higher spray pressures (>30 bar) do improve deposition quantity and uniformity, but not statistically more than that of lower spray pressures (15 bar). The use of lower spray pressures is thus adequate for effective application. Lower spray pressures are also more economical (Addendum A).

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. Very little applicable literature is available to elucidate this phenomenon, which necessitates additional fundamental research. Importantly, this research needs to be conducted to give relevance to the results from 6 orchard spray evaluation trials of selected adjuvants, which has already been conducted. The additional research will include biological efficacy studies following laboratory sprays with and without selected adjuvants using the developed methodology, as well as histopathology trials using epi-fluorescence and scanning electron microscopy. The generated data will also be used to improve the benchmark model, possibly through the inclusion of a deposition quality parameter in addition to the quantity parameter currently used.

From field trials with adjuvants, we saw significant differences between adjuvants, and recent work indicates that certain adjuvants (spreaders) can negatively affect deposition and penetration of spray deposition in very dense (4 to 4.5 on a 5 point scale) canopies due to increased run-off on outer canopy leaves and reduced penetration of thick leaf wall (Addendum B).

Development of the deposition quality parameter is proving to be challenging, since a sound statistical method still have to be found that would indicate differences in deposition quality of pigment deposition on leaf surfaces.

Technology transfer

- JG van Zyl and PH Fourie. "General spray application guidelines". Invited lecture at USPP Short Course day 2012.
- JG van Zyl, PH Fourie and GC Schutte. Evaluation of adjuvants to improve fungicide spray deposition and control of Alternaria brown spot in South African citrus orchards. Oral presentation at SASPP Western Cape PhD research day 2012.
- JG van Zyl, PH Fourie and GC Schutte. Spray deposition benchmarks for control of Alternaria brown spot and evaluation of adjuvants to improve fungicide spray deposition in citrus orchards. Oral presentation at 7th CRI Symposium 2012.
- JG van Zyl, D Viljoen, E Sieverding and PH Fourie. Evaluation of Break-Thru S240 and Break-Thru Union at different application volumes in South African citrus orchards. Poster presentation at 7th CRI Symposium 2012.
- JG van Zyl, PH Fourie and GC Schutte. General spray application guidelines for citrus production in South Africa. Oral presentation at CRI Road show 2012 South Africa
- JG van Zyl, PH Fourie and GC Schutte. Spray deposition benchmarks for control of Alternaria brown spot and evaluation of adjuvants to improve fungicide deposition in citrus orchards. Oral presentation at International Citrus Congress (ICC) 2012 Spain.
- JG van Zyl, PH Fourie and GC Schutte. Improvement of spray deposition and control Alternaria brown spot on mandarin leaves following sprays with copper oxychloride and selected adjuvants. Poster presentation at 48th SASPP congress 2013.
- JG van Zyl, PH Fourie and GC Schutte. General spray application guidelines for citrus production in South Africa. Oral presentation at Citrusdal producer technical study group, February 2013.
- Gideon van Zyl – Successful proposal to upgrade his MSc to PhD study
- Van Zyl JG, Fourie PH, Schutte GC. 2013. Spray deposition assessment and benchmarks for control of Alternaria brown spot on Mandarin leaves with copper oxychloride. Research article published in Crop Protection 46: 80-87.

Further research

1. Conduct a thorough survey of current spray application materials and methods in all major citrus growing areas
2. Determine benchmarks for biological efficacy of copper oxychloride against Alternaria brown spot
 - a. Improve FPC/Control model by including a deposition quality parameter
3. Evaluation and optimisation of spray deposition with current and novel spray application methods.
 - a. Optimised use of commonly used sprayers
 - b. Evaluation of low-drift nozzles
 - c. Evaluation of energy-efficient and low-volume sprayers
 - d. Season-long evaluation of ultra-low volume sprayer (subject to contract funding)
 - i. *Contract funding proposal was not successful*
4. Development and validation of a user-friendly calibration system
 - a. To be submitted as new proposal

- b. Update production guidelines
 - 5. Develop a guideline for optimal use of commonly-used and novel spray applicators in citrus orchards
 - a. Update production guidelines
 - 6. Use of adjuvants for improved spray deposition on citrus leaves and fruit
 - a. Complete as part of PhD study
 - b. Evaluate adjuvants for use in lower volume spray application (subject to contract funding)
 - i. *Contract funding was successful*
 - 7. Physical and chemical effects of adjuvants on spray deposition and control of *Alternaria* brown spot following copper oxychloride sprays on mandarin leaves
- Quarterly milestones for Apr-Jun, Jul-Sep, Oct-Dec 2014 and Jan-Mar 2015

None; project terminates end-March 2014. New proposals will be submitted for 2014/15.

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Addendum A

Evaluation of the Nieuwoudt and Martignani spray machines and different spray volumes

J. G. Van Zyl and P. H. Fourie

1. Materials and methods

1.1 Spray application

1.1.1 Field evaluation

The trial was conducted in uniform sections of a Washington navel [3.6x3.4 m trees (HxW) with density of 4.5 on a 5-point scale; 4x6 m row spacing] orchard on the farm Boontjiesrivier (Citrusdal, Western Cape, South Africa) in February 2013. For each treatment combination, a row-section with 10 trees was marked with hazard tape and sprayed from both sides. Sprays consisted of eight separate treatments consisting of water and yellow fluorescent pigment 40% EC (SARDI, Loxton, South Australia); 1 ml l⁻¹. Treatment one to four were sprayed with a Nieuwoudt sprayer (www.Nieuwoudt.co.za) at different spray volumes, with volume

being manipulated by either changing nozzles or spray pressure: high volume – 1.8 mm @ 1500 KPa = 12458 l ha⁻¹; high volume – 1.8 mm @ 3000 KPa = 17694 l ha⁻¹; medium volume – 1.5 mm @ 1500 KPa = 8125 l ha⁻¹; low volume – 1.2 mm @ 1500 KPa = 5417 l ha⁻¹. Tractor speed was kept constant at 2.88 km h⁻¹ for each treatment. Treatment five to eight were sprayed with a Martignani Whirlwind sprayer (www.Martignani.com) at different spray volumes at a constant spray pressure and tractor speed of 150 KPa and 2.88 km h⁻¹, respectively, except for treatment five, where tractor speed was reduced to 1.78 km h⁻¹: high volume @ 1.78 km h⁻¹ = 4000 l ha⁻¹; high volume @ 2.88 km h⁻¹ = 4000 l ha⁻¹; medium volume @ 2.88 km h⁻¹ = 2000 l ha⁻¹; low volume @ 2.88 km h⁻¹ = 1000 l ha⁻¹. Two buffer rows were left unsprayed between treatments. The spray tank, spray nozzles, filter and pipes of the spray machine was thoroughly washed and flushed after each treatment.

2.1.2 Sampling of field evaluations

As replications, three uniform trees were selected from each sprayed section (treatment) from which leaves were sampled for spray deposition analysis. Twelve randomly selected intact leaves were carefully sampled from each of the various positions in the tree canopy; inner (>30 cm into the tree) and outer canopy (leaves on the outside of the tree) at the top, middle and bottom parts of each of the selected trees (72 leaves per replication). Leaves picked from these six various positions were collected and stored separately in marked sandwich bags. Stored leaves were transported back under cool, dry conditions to the Department of Plant Pathology at the University of Stellenbosch where it was stored at 4°C until further analysis.

1.2 Spray deposition analysis

For deposition analysis, petioles were removed from leaves with a scissor by cutting it just in front of the start of the leaf blade. A single leaf was positioned in the middle of a back-illuminated red Perspex box (300×210×110 mm) to reduce any shadowing and to enhance edging of leaves in captured images during analysis. The leaf was covered with a glass pane (200×200×2 mm) and illuminated using an ultra-violet light source (UV-A; ≈ 365 nm; Labino Mid-Light; www.labino.com). Digital photos were taken in Canon RAW file format (.CR2 ≈ 10 MB) of the upper and lower leaf surfaces using a Canon EOS 40D camera equipped with a 60 mm macro lens. The camera was attached to a tripod in a fixed position directly above the leaf. RAW image files were converted to 8-bit Exif-TIFF (.TIF ≈ 30 MB) with Digital Photo Professional version 3.1.0.0 (CANON INC.; www.canon.com) files for digital image analysis to determine the deposition parameters.

Spray deposition assessment involved digital image analysis with Image Pro Plus software version 7.0 (Media Cybernetics, www.mediacy.com) to determine the deposition quantity and quality per leaf. Similar to the methodology used in van Zyl *et al.* (2013), deposition quantity was measured as percent total leaf area covered by pigment particles (percentage fluorescent particle coverage; %FPC) (van Zyl *et al.*, 2013). Deposition uniformity was calculated as the CV% in pigment deposition in a 12 leaf batch and deposition efficiency was expressed as deposition quantity normalised to %FPC per 1000 l ha⁻¹.

2.3 Benchmarking

Deposition data was subjected to the FPC benchmark model developed by van Zyl *et al.* (2013) to evaluate the effectiveness of deposition in relation to theoretical disease control that can be achieved. The FPC₅₀ (2.07 %FPC) and FPC₇₅ (4.14% FPC) benchmarks indicate 50% and 75% control of *Alternaria* Brown Spot on mandarin leaves, respectively.

2.4 Statistical analysis

Deposition quantity (%FPC), efficiency (FPC% normalised to 1000 l ha⁻¹), and uniformity data were subjected to appropriate analysis of variance (ANOVA). Fisher's LSD was calculated to identify significant differences between treatments at a confidence interval of 95%. Data from upper and lower leaf surfaces was analysed separately, but were combined when describing the results. Data was also subjected to regression analysis and Pearson's correlation to demonstrate the relation between deposition quantity, quality and uniformity measurements. SAS version 8.2 statistical software (SAS institute Inc., 1999) was used for analysis.

2. Results and discussion

2.1. Deposition quantity

Analysis of variance of deposition quantity (%FPC) indicated a significant interaction for treatment x horizontal canopy position x vertical canopy position ($P < 0.0001$). The significance of the interaction can be ascribed to the significantly higher deposition quantity realised by the Nieuwoudt spray at 17694 l ha⁻¹ on middle inside canopy leaves (7.37%FPC) as well as the significantly lower deposition quantity realised by the Martignani spray at 1000 l ha⁻¹ on bottom inside leaves (0.273%FPC) (results not shown). Due to the complexity of this 3-factor interaction, interactions between treatment x horizontal canopy position ($P < 0.0149$) and treatment x vertical canopy position ($P < 0.1366$) were discussed separately.

Overall, as expected in very dense canopies, deposition quantity was higher on outer canopy leaves than on inner canopy leaves. On the outer canopy leaves, the highest deposition was realised by Nieuwoudt sprays at 12458 I ha⁻¹ (5.67%FPC). At 17694 I ha⁻¹; Nieuwoudt sprays realised a lower deposition quantity (4.94%FPC), but still statistically similar. The lower deposition quantity at the higher spray volume can be ascribed to excessive run-off. The 17694 I ha⁻¹ spray was realised by increasing spray pressure from 1500 KPa to 3000 KPa. This is often the industry norm for spray pressure. However, the increase in spray pressure did not improve deposition quantity on the outer canopy, but rather worsened it, due to increased shielding of leaves, and therefore increased run-off. Nieuwoudt sprays at 8125 I ha⁻¹, 5417 I ha⁻¹ at 2.88 km h⁻¹ and Martignani sprays at 4000 I ha⁻¹ at 1.78 km h⁻¹ on outer canopy leaves deposited statistically lower deposition quantities and did not differ statistically from each other (4.01, 3.65 and 3.76%FPC, respectively). The slower tractor speed with the Martignani spray in this case improved deposition quantity at the lower spray volume. However, 4000 I ha⁻¹ Martignani sprays at 2.88 km h⁻¹ deposited significantly lower quantities (2.76%FPC), which did not differ significantly from 2000 I ha⁻¹ at this tractor speed (2.71%FPC); 1000 I ha⁻¹ at 2.88 km h⁻¹ realised the lowest deposition on the outside canopy leaves (0.99%FPC) (Table 1) (Figure 1).

On the inner canopy leaves, the highest deposition quantity was realised by Nieuwoudt sprays at 17694 I ha⁻¹ (5.38%FPC), statistically higher than all other treatments. The improved penetration can be ascribed to the increase in spray pressure from 1500 KPa to 3000 KPa. The 12458 I ha⁻¹ and 8125 I ha⁻¹ Nieuwoudt spray deposition quantities (4.16 and 3.50%FPC, respectively) did not differ significantly. Martignani sprays at 4000 I ha⁻¹ @ 1.78 km h⁻¹ (2.74%FPC) realised deposition quantity significantly higher than that of Nieuwoudt sprays at 5417 I ha⁻¹ (1.26%FPC). The improved deposition quantity can be ascribed to the slower tractor speed, allowing more time for spray penetration into the canopy. Nieuwoudt sprays at 5417 I ha⁻¹ did not differ significantly from the Martignani sprays at 4000 I ha⁻¹ and 2000 I ha⁻¹ (1.58 and 1.51%FPC, respectively). At 1000 I ha⁻¹ the Martignani realised the lowest deposition quantity of all the treatments on the inner canopy leaves (0.56%FPC) (Table 1) (Figure 1).

Evaluation of the non-significant interaction treatment x vertical canopy position ($P < 0.1366$) indicated that the Nieuwoudt spray at 17694 I ha⁻¹ landed a significantly higher quantity of pigment on middle canopy leaves (6.24%FPC) than all other treatments at all canopy positions. This was also the case at 12458 I ha⁻¹ on top of the tree leaves (5.93%FPC). All other treatments at various canopy positions did not differ in deposition quantity per treatment. However, the Martignani spray at 2000 I ha⁻¹ did realise a statistically lower deposition quantity on bottom canopy leaves (0.80%FPC), in relation to middle and top canopy leaves (results not shown).

2.2. Deposition uniformity

Analysis of variance of deposition uniformity between leaves (in a 12 leaf batch) indicated a significant interaction for treatment x horizontal canopy position ($P < 0.0480$). Deposition uniformity was generally better on the outside than on the inside of the canopy. This can be ascribed to the density of the canopy, not allowing spray to freely enter the canopy. On the outside of the tree, the lowest variation in deposition quantity (*i.e.* best uniformity) was realised by the Nieuwoudt spray at 17694 I ha⁻¹ (50.06%), significantly lower than that of the Martignani sprays at 4000 I ha⁻¹ at 2.88 km h⁻¹, 2000 and 1000 I ha⁻¹ (72.31 to 87.14%). Improved uniformity at 17694 I ha⁻¹ is due to the higher spray pressure, forcing the spray mixture through the dense outer canopy. The rest of the treatments did not differ significantly in deposition uniformity (Table 1) (Figure 2).

On the inside of the canopy, the Nieuwoudt spray at 5417 I ha⁻¹ realised the poorest deposition uniformity (100.88%), significantly higher than the deposition uniformity realised by Nieuwoudt sprays at 12458 I ha⁻¹ (72.65%) and 17694 I ha⁻¹ (58.28%). Interestingly, uniformity realised by the Martignani spray at 4000 I ha⁻¹ at 1.78 km h⁻¹ (72.03%) and 2000 I ha⁻¹ at 2.88 km h⁻¹ (73.22%) realised similar deposition uniformity; these did not differ significantly from the other Martignani treatments (81.29 to 89.09%) (Table 1) (Figure 2).

2.3. Deposition efficiency

When deposition quantity analyses is normalised and expressed as %FPC per 1000 I ha⁻¹ spray volume, lower volume sprays indicated better results. Analysis of deposition efficiency (normalised deposition %FPC to a spray volume of 1000 I ha⁻¹) indicated significant interactions for treatment x horizontal canopy position ($P < 0.0001$) and treatment x vertical canopy position ($P = 0.0005$).

On the outside canopy leaves, the highest deposition efficiency was realised by the Martignani spray at 2000 I ha⁻¹ (1.35%FPC), statistically higher than the other treatments. Martignani sprays at 1000 (0.99%FPC), 4000 @ 1.78 km h⁻¹ (0.94%FPC) and Nieuwoudt spray at 5417 I ha⁻¹ (0.88%FPC) realised lower but statistically similar deposition efficiency. Nieuwoudt sprays at higher spray volumes generally resulted in poorer spray efficiency (0.49 to 0.32%FPC) (Table 1) (Figure 3).

On inner canopy leaves, a similar trend was observed, with Martignani sprays realising similar deposition efficiency (0.56 to 0.75%), whereas the Nieuwoudt sprays and Martignani at 4000 @ 2.88 km h⁻¹ resulted in lower deposition efficiency (0.24 to 0.43%) (Table 1) (Figure 3).

For the interaction treatment vertical canopy position, the most efficient deposition was realised by the Martignani spray at 2000 l ha⁻¹ on middle canopy leaves (1.64%), significantly better than all other sprays. The least efficient spray was realised by the Nieuwoudt at 12458 l ha⁻¹ on the bottom canopy leaves (0.24%), statistically similar to all other sprays on bottom canopy leaves (0.34 to 0.46%) (results not shown).

2.4. Benchmarking

Deposition quantity results for the treatment x horizontal canopy position interaction were compared to the FPC benchmarks. On outer canopy leaves, all sprays realised deposition quantities above the FPC₅₀ benchmark, with only the Martignani spray at 1000 l ha⁻¹ depositing quantities below the line. Only Nieuwoudt sprays at 17694 and 12458 l ha⁻¹ realised deposition above the FPC75 benchmark on outer and inner canopy leaves. On inner canopy leaves, application with the Nieuwoudt at 8125 l ha⁻¹ and Martignani at 4000 l ha⁻¹ and 1.78 km h⁻¹ showed deposition above the FPC50 line with the rest of the treatment combinations depositing below the benchmark (Figure 1). From these benchmarks, it appears that active ingredient concentrations should be increased for the lower spray volumes to ensure adequate control. For example, sprays using the best low volume application, Martignani at 2000 l ha⁻¹ at 2.88 km h⁻¹, should concentrate active ingredients by 3x in order to meet or exceed FPC75 benchmarks. Based on our results, this should realise better deposition quantities than the high volume Nieuwoudt sprays (8125 to 12458 l ha⁻¹), but at a 26 to 52% saving in chemical and water cost.

3. Conclusion (preliminary)

These results again confirmed the potential of two low-energy sprayers. In a previous Citrusdal spray trial, the Nieuwoudt sprayer proved to be one of the better high volume sprayers evaluated. Those results were supported by the recent trial. However, it was clear that an increase in spray pressure and therewith volume, reduced spray efficiency with no meaningful deposition quantity and uniformity benefit. The lower volume Nieuwoudt application yielded relatively poor results. The Martignani demonstrated its potential as a low-energy low-volume sprayer, despite a too fast tractor speed in that trial resulted in poor canopy penetration. In the current trial, it was tested at 1.9 and 2.9 km h⁻¹, which resulted in deposition uniformity and penetration comparable with the high-volume sprays. In fact, based on spray efficiency, the Martignani at 2000 l ha⁻¹ at 2.88 km h⁻¹ proved to be the best application. Interestingly, this application proved to be better than the 4000 l ha⁻¹ application, most probably due to the air shear and electrostatic technology being superior at lower volumes and smaller droplet size. *Please note that these findings should be viewed as preliminary; this trial will be repeated in another orchard (different canopy structure) before final conclusions are drawn.*

It is clear from these results, previous work and literature that different spray applicators at varying spray volumes in specific orchards that differ in planting distance and row spacing, tree size and canopy geometry/density will result in different deposition quantity, uniformity and efficiency. Excessive spray volumes result in over-application, run-off and increased spray drift whilst too low volumes result in inadequate coverage on inner and outer canopy leaves. The behaviour of sprays is dramatically influenced by all these factors. Yet still there is a reliance on simple calculation methods to determine spray volume, such as spray volume per hectare, that only takes a few parameters in account, and ignore canopy effects blatantly. Canopy geometry/density has a major effect on the amount of spray volume that can penetrate and be retained by the canopy (Cunningham and Harden, 1998; Furness et al., 1998). It is therefore suggested that a tree row volume system (TRV) be developed for the calculation of spray volumes in Southern African citrus orchards. There is an abundance of literature on the matter and together with data so far collected and additional research; it is possible to develop an efficient TRV system. Current methodology is acceptable for control but is not efficient. With the development of a TRV system will have a positive effect on reducing run-off, improving deposition parameters, reduction of application, agrochemical, water costs and environmental pollution.

Table 4.3.3.1. Mean deposition quantity, uniformity and efficiency realised following sprays with water and pigment by Nieuwoudt and Martignani applicators at various high, medium and low spray volumes on inner and outer canopy leaves.

Treatment ^a	Deposition quantity (%FPC) ^b		Deposition uniformity (%CV between leaves) ^b		Deposition efficiency (%FPC / 1000 l ha ⁻¹) ^b	
	Outer canopy	Inner canopy	Outer canopy	Inner canopy	Outer canopy	Inner canopy
Nw-12458	5.67 a	4.16 bc	53.95 ef	72.646 b-e	0.32 hi	0.24 i
Nw-17694	4.94 ab	5.38 a	50.06 f	58.28 def	0.40 ghi	0.43 ghi
Nw-8125	4.01 bc	3.50 cde	61.04 def	86.223 ab	0.49 fgh	0.43 ghi
Nw-5417	3.65 cde	1.26 fg	58.91 def	100.877 a	0.88 bcd	0.30 hi
Mg-4000 @ 1.78 km/h	3.76 cd	2.74 e	63.88 cdef	72.033 b-e	0.94 b	0.69 def
Mg-4000	2.76 e	1.58 f	72.31 bcde	81.291 abc	0.69 def	0.40 ghi
Mg-2000	2.71 e	1.51 fg	76.48 bcd	73.221 b-e	1.35 a	0.75 cde
Mg-1000	0.99 fg	0.56 g	87.14 ab	89.085 ab	0.99 b	0.56 efg

^a Treatment layout consist of spray applicator used followed by spesific spray volume

^b For each parameter separately, values in each column followed by the same letter do not differ significantly ($P > 0.05$) according to Fisher's least significant difference test

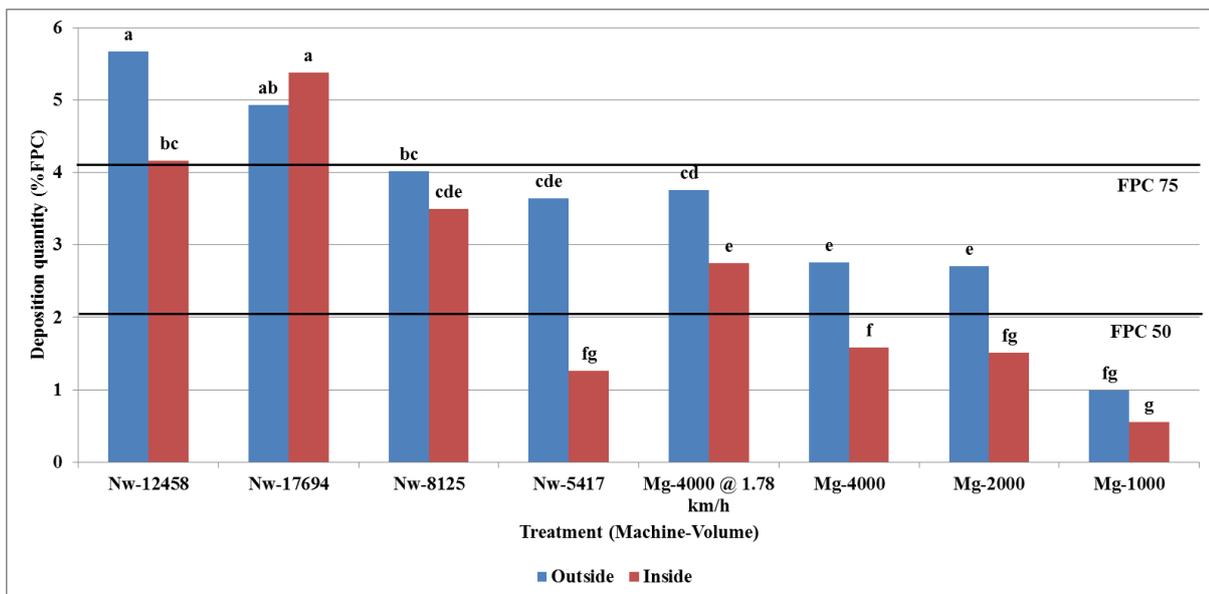


Figure 4.3.3.1. Mean deposition quantity realised following sprays with water and pigment by Nieuwoudt and Martignani applicators at various high, medium and low spray volumes on inner and outer canopy leaves.

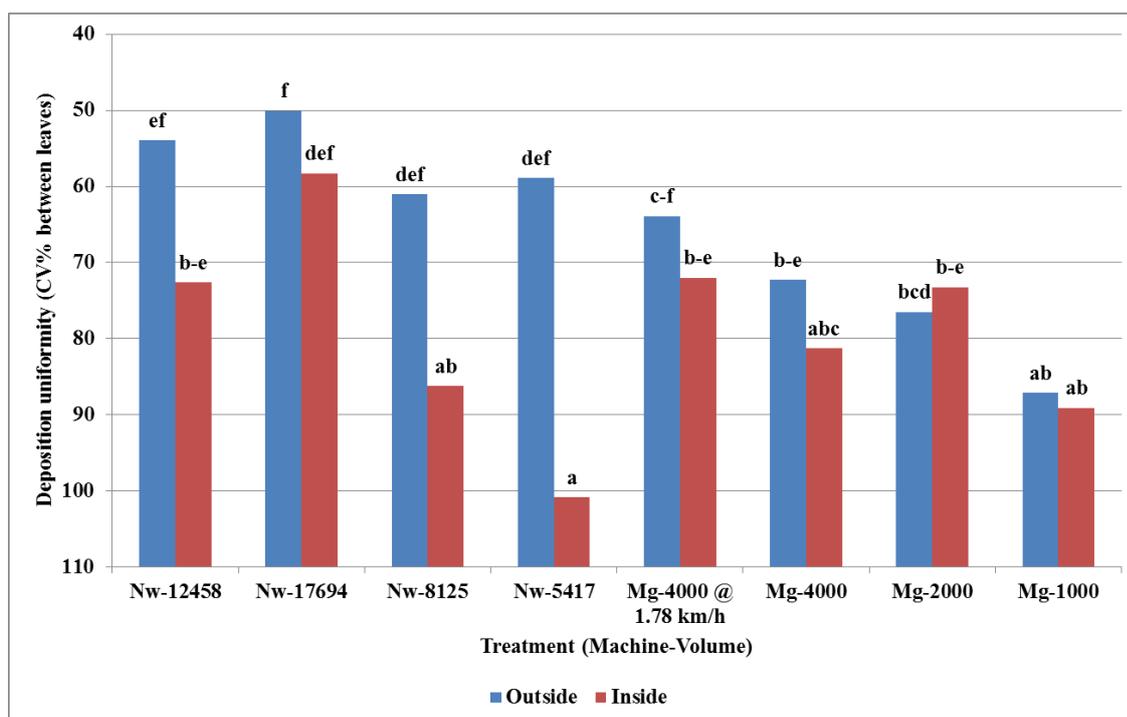


Figure 4.3.3.2. Deposition uniformity between leaves realised following sprays with water and pigment by Nieuwoudt and Martignani applicators at various high, medium and low spray volumes on inner and outer canopy leaves.

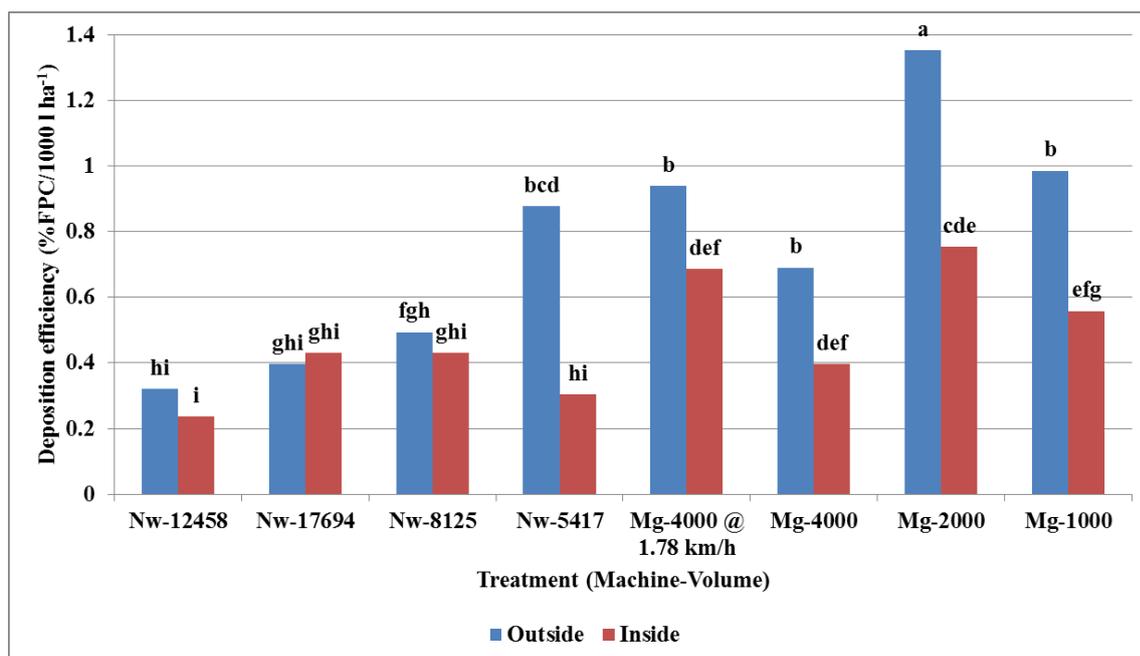


Figure 4.3.3.3. Deposition efficiency between leaves realised following sprays with water and pigment by Nieuwoudt and Martignani applicators at various high, medium and low spray volumes on inner and outer canopy leaves.

Addendum B

Evaluation of adjuvants Break-Thru S240 and Break-Thru Union at different spray volumes in South African citrus orchards

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Spray deposition

Fluorometry

Photomacrography

Canopy management

Digital image analysis

Abstract

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Citrus producers in South Africa use very high spray volumes (8000 to 12000 l ha⁻¹) to control certain pests and diseases. In order to study the benefit of spray adjuvants on reducing spray volumes, trials were conducted with the organo tri-siloxane surfactants Break-Thru S240 (L6764) and Break-Thru Union (Registration pending). Two separate spray trials were conducted in the Western and Eastern Cape provinces of South Africa in uniform navel orange orchards. Break-Thru S240 and Break-Thru Union, at recommended dosages per hectare (300 ml ha⁻¹, respectively), were sprayed separately at three different spray volumes together with SARDI Yellow Fluorescent Pigment (1 ml l⁻¹) at high (20 l tree⁻¹), medium (14 l tree⁻¹) and low (8 l tree⁻¹) spray volume. Sprays consisting of the pigment only were used as control treatments. Trees were sprayed from both sides with a commercial multi-fan tower sprayer (BSF-Multiwing) at a constant tractor speed (2.4 km h⁻¹) and pressure (15 bar). Spray volume was calibrated by using different spray nozzles (TeeJet Disc-Core type; full and hollow cone nozzles D3-DC56/46, D4-DC56/46, D5-DC56/46). Leaves were sampled from six canopy positions (inner and outer canopy position at bottom, middle and top of tree). Deposition quantity and quality of fluorescent pigment were determined on upper and lower leaf surfaces using fluorometry, digital photomacrography and image analyses. Spray uniformity in the canopy and efficiency were also compared between treatments. Deposition quantity generally increased with increasing spray volume, but normalised values showed better spray efficiency at lower volumes. In "spray friendly" canopies, the value adding effect of adjuvants, especially Break-Thru Union, was observed in terms of deposition quantity, efficiency and uniformity, especially at low volume applications (*l tree⁻¹) on the inside and outside of the canopy. As these effects were not as evident in very dense canopies, the importance of canopy management must be stressed.

1. Introduction

South African citrus farmers rely heavily on medium to high volume fungicidal spray applications to secure market access and protect, and in the latter cure, citrus fruit from challenging diseases such as Citrus black spot and *Alternaria* brown spot. Citrus trees in South Africa are large and dense, depending in which climatic region it is grown. Tree size and density complicates adequate coverage of fungicidal sprays on susceptible leaves and fruit, hence medium to high volume fungicidal spray applications ranging from 9000 to 16,000 l ha⁻¹, which is almost double or triple the volumes used in other citrus producing counties such as Spain. These application volumes do provide an acceptable balance between efficacy and efficiency based on existing economic considerations but run the risk of loss of efficacy/effectiveness due to spray run-off and exo- and endo-drift. Thus off-target application of fungicides is increased, which in turn is an economical loss and a potential environmental problem. Reduced volume sprays have the potential to reduce the economic and environmental impact/cost of fungal disease control and to be more effective. The use of adjuvants is one form of tool that citrus producers can use to lower spray volumes and improve deposition parameters. Adjuvants are a potential tool to be used with the reduction of spray application volumes to optimise spray application in terms of improved deposition of the active ingredient on the target surface. In South Africa, adjuvants are regularly used with fungicidal and pesticidal application, yet little to no literature exists on the effect of adjuvants in citrus.

2. Materials and methods

2.1 Spray application

2.1.1 Field evaluation

The first trial was conducted in uniform sections of a Bahianina Araras navel (*Citrus x aurantium* L., pro sp. [Sweet Orange Group] (sensu Mabberley 1997, Bayer et al., 2009); *Citrus sinensis* (L.) Osbeck (sensu Swingle and Reece 1967; sensu Tanaka sensu Cottin 2002) [3.7×3.4 m trees (H×W) with density of 4.5 on a 5-point scale; 3×5.5 m row spacing] orchard on the farm Die Vlei (Clanwilliam, Western Cape, South Africa) in February 2012. For each treatment combination, a row-section with 10 trees was marked with hazard tape and sprayed from both sides using a BSF-Multiwing sprayer (BSF, Hoedspruit, South Africa). Sprays consisted of three separate treatments, each at a high (20 l tree⁻¹), medium (14 l tree⁻¹) and low (8 l tree⁻¹) spray volume. The three separate treatments was yellow fluorescent pigment 40% EC (SARDI, Loxton, South Australia); 1 ml l⁻¹] alone (no adjuvant added control treatment), yellow fluorescent pigment (1 ml l⁻¹) together with adjuvant Break-Thru S240 (300ml ha⁻¹) and yellow fluorescent pigment (1 ml l⁻¹) together with Break-Thru Union (300 ml ha⁻¹). Tractor speed and spray pressure was kept constant at 2.4 km h⁻¹ and 1500 KPa respectively with spray volume being manipulated by using different spray nozzles (TeeJet Disc-Core type full and hollow cone nozzles: Low - D3-DC56/46, medium - D4-DC56/46, High - D5-DC56/46). Two buffer rows were left unsprayed between treatments. The spray tank, spray nozzles, filter and pipes of the spray machine was thoroughly washed and flushed after each treatment.

The second trial was sprayed in uniform sections of Palmer navel (*Citrus x aurantium* L., pro sp. [Grapefruit Group] (sensu Mabberley 1997, Bayer et al., 2009); *Citrus paradisi* Macfad. (sensu Swingle and Reece 1967; sensu Tanaka sec. Cottin 2002) orchard [3.65×3.2 m trees (H×W) with density of 2.5 on a 5-point scale; 4×7 m row spacing] orchard on the farm Sun Orange Farms (Addo, Eastern Cape, South Africa) in April 2012. Apart from the tree canopies being less dense, the methodology used was exactly the same as the first trial.

2.1.2 Sampling of field evaluations

As replications, three uniform trees were selected from each sprayed section (treatment) from which leaves was sampled for spray deposition analysis. Twelve randomly selected intact leaves were carefully sampled from each of the various positions in the tree canopy; inner (>30 cm into the tree) and outer canopy (leaves on the outside of the tree) at the top, middle and bottom parts of each of the selected trees (72 leaves per replication). Leaves picked from these six various positions were collected and stored separately in marked sandwich bags. Stored leaves were transported back under cool, dry conditions to the Department of Plant Pathology at the University of Stellenbosch where it was stored at 4°C until further analysis.

2.2 Spray deposition analysis

For deposition analysis, petioles were removed from leaves with a scissor by cutting it just in front of the start of the leaf blade. A single leaf was positioned in the middle of a back-illuminated red Perspex box (300×210×110 mm) to reduce any shadowing and to enhance edging of leaves in captured images during analysis. The leaf was covered with a glass pane (200×200×2 mm) and illuminated using a ultra-violet light source (UV-A; ≈ 365 nm; Labino Mid-Light; www.labino.com). Digital photos were taken in Canon RAW file format (.CR2 ≈ 10 MB) of the upper and lower leaf surfaces using a Canon EOS 40D camera equipped with a 60 mm macro lens. The camera was attached to a tripod in a fixed position directly above the leaf. RAW image files were converted to 8-bit Exif-TIFF (.TIF ≈ 30 MB) with Digital Photo Professional version 3.1.0.0 (CANON INC.; www.canon.com) files for digital image analysis to determine the deposition parameters.

Spray deposition assessment involved digital image analysis with Image Pro Plus software version 7.0 (Media Cybernetics, www.mediacy.com) to determine the deposition quantity and quality per leaf. Similar to the methodology used in van Zyl et al. (2013), deposition quantity was measured as percent total leaf area covered by pigment particles (percentage fluorescent particle coverage; %FPC) (van Zyl et al., 2013). For the deposition quality assessment, the leaf area was divided into equally-sized squares with a size of 150 × 150 pixels (22500 pixels) (van Zyl et al., 2013). Depending on the leaf size, this amounted to as few as 10 to more than 110 individual squares per leaf, of which the percent area covered by fluorescent pigment particle was determined for each square. The coefficient of variation of the mean value of all the blocks analysed per leaf (CV% = Standard Deviation × 100/ mean) was used as a measure of deposition quality per leaf, *i.e.* uniformity of deposition on leaf surface. Deposition uniformity was calculated as the CV% in pigment deposition in a 12 leaf batch and deposition efficiency was expressed as deposition quantity normalised to %FPC per l tree⁻¹.

2.3 Benchmarking

Deposition data was subjected to the FPC benchmark model developed by van Zyl et al. (2013) to evaluate the effectiveness of deposition in relation to theoretical disease control that can be achieved. The FPC₅₀ (2.07 %FPC) and FPC₇₅ (4.14% FPC) benchmarks indicate 50% and 75% control of Alternaria Brown Spot on mandarin leaves, respectively.

2.4 Statistical analysis

Deposition quantity (%FPC), quality (CV%), and uniformity data were subjected to appropriate analysis of variance (ANOVA). Fisher's LSD was calculated to identify significant differences between treatments at a confidence interval of 95%. Data from upper and lower leaf surfaces was analysed separately, but were combined when describing the results. Data was also subjected to regression analysis and Pearson's correlation to demonstrate the relation between deposition quantity, quality and uniformity measurements. SAS version 8.2 statistical software (SAS institute Inc., 1999) was used for analysis.

3. Results

Unless significant higher order interactions were observed from analysis of variance, results, the 2-factor treatment × volume and treatment × horizontal canopy position interactions were discussed to simplify interpretation of subtle treatment effects.

3.1 Trial one – Die Vlei (Clanwilliam, Western Cape) (Dense canopies)

3.1.1 Deposition quantity

Analysis of variance of deposition quantity (%FPC) indicated no significant interactions ($P > 0.05$), but some significant main effects: vertical canopy position ($P < 0.0001$) and for horizontal canopy position ($P = 0.0015$). A meaningful effect was also observed for treatment effects ($P = 0.0756$) and a significant effect for spray volume ($P = 0.0078$).

For vertical canopy position, the highest deposition was realised at the top of the tree (6.2 %FPC) which differed significantly from that of the bottom (4.79 %FPC) and the middle of the tree (4.57 %FPC), which did not differ significantly from each other (results not shown).

For horizontal canopy position, treatments generally deposited higher deposition quantities on the outside of the tree (5.65 %FPC) in relation to the inside of the canopy (4.72 %FPC) over all treatments. There was no significant interaction for treatment × horizontal canopy position ($P = 0.532$). For water and Break-Thru S240, deposition quantities on outer and inner canopy leaves were not significantly different, but inner canopy deposition was significantly lower for Break-Thru Union (Table 2).

Evaluation of the non-significant interaction treatment × volume ($P = 0.531$), indicated the control treatment of water only (7.28 %FPC) and Break-Thru S240 (7.49 %FPC) retained the highest amount of pigment on leaves at 20 l tree⁻¹, statistically more than that retained by all other treatments. Pigment retained by Break-Thru Union at 20 l tree⁻¹ (5.85 %FPC) and 14 l tree⁻¹ (5.05 %FPC) was statistically lower than that of above mentioned treatments and did not differ significantly from the amount retained on leaves by water only at 1 l tree⁻¹ (5.82 %FPC). The lowest amount of pigment was retained by Break-Thru Union at 8 l tree⁻¹ (3.46 %FPC). Adjuvant treatments did not realise deposition quantities higher than that of the control treatment, except for Break-Thru S240 at 20 l tree⁻¹ (7.49 %FPC) (Table 1).

3.1.2 Deposition uniformity

Analysis of variance of deposition uniformity between leaves (in a 12 leaf batch) indicated a significant interaction for treatment × vertical canopy position ($P = 0.0251$) and significant effects for horizontal canopy position ($P = 0.0501$) and a meaningful effect for spray volume ($P = 0.143$).

The treatment × volume interaction, although not significant ($P = 0.676$) indicated that adjuvant treatments Break-Thru Union (58.47%) at 8 l tree⁻¹ improved deposition uniformity in relation to the control treatment water only (68.07%), although not statistically. At 1 l tree⁻¹, Break-Thru Union (52.39%) improved deposition uniformity in relation to water only (57.33%), but also not significantly (Table 1).

For the significant interaction treatment × vertical canopy position, the least variation in deposition uniformity was realised by water only treatment (52.63%) in the bottom of the canopy whilst the highest variation in deposition between leaves were realised by water only in the top of the canopy (67.39%), differing statistically from that of the lowest variation. Deposition uniformity realised by adjuvant treatments

did not improve uniformity at different canopy positions in relation to the control treatment (results not shown).

Adjuvant treatments did not improve deposition uniformity statistically on the inside or outside of the canopy in relation to the control treatment (Table 2), although Union did realise marginally better uniformity than the other treatments on inner and outer canopies.

3.1.3 Deposition efficiency

Analysis of variance of deposition efficiency [normalised deposition %FPC to a spray volume of 1 l tree⁻¹] indicated no significant interactions ($P > 0.05$), but some significant main effects: vertical canopy position ($P < 0.0001$) and for horizontal canopy position ($P < 0.0001$). A meaningful effect was also observed for treatment effects ($P = 0.0582$).

Although not significant, the interaction treatment \times volume ($P = 0.237$) and treatment \times horizontal canopy position ($P = 0.267$) was evaluated for deposition efficiency. At 8 l tree⁻¹, the water only treatment (0.51%) realised the best deposition efficiency, but Break-Thru S240 (0.45%) and Union (0.43%) realised statistically similar deposition efficiency at the same volume. At 14 l tree⁻¹, Break-Thru S240 (0.37%) and water only (0.36%) realised the best deposition efficiency, however not differing statistically from the markedly lower deposition efficiency realised by Break-Thru Union (0.29%). At 20 l tree⁻¹, deposition efficiency following water only (0.42%) sprays was significantly better than that of Break-Thru S240 (0.29%), but similar to Break-Thru Union (0.36%). Thus adjuvant treatments generally did not improve deposition efficiency over that of the control treatment at the sprayed volumes. On outer canopy leaves, adjuvant treatments realised less, although statistically similar deposition efficiency than the water only control treatment on the outer canopy. However, on inner canopy leaves, water only (0.40%) realised statistically better deposition efficiency than that of the adjuvant treatments (S240 – 0.33% and Union – 0.29%).

3.1.4 Benchmarking

Deposition quantity results for the treatment \times volume \times horizontal canopy position interaction were compared to the FPC benchmarks. All treatments at 8, 14 and 20 l tree⁻¹ on the outside of the canopy was sufficiently above the FPC₅₀ and ₇₅ benchmarks, indicating sufficient deposition quantity for control above 75%. However, on the inside of the canopy, all treatments at 8 l tree⁻¹ realised deposition quantities only theoretically sufficient for 50% control. At 14 l/tree, the water only treatment and Break-Thru Union deposited quantities sufficient for >75% control. At 20 l tree⁻¹, deposition realised by the control treatment and adjuvants were sufficiently above the FPC₇₅ benchmark (Figure 1) It is important to note that the spray machine used was calibrated with care correctly, and that tractor speed and spray pressure to our knowledge, were ideal for set spraying conditions. Results obtained may vary dramatically if spray factors and technique are neglected. In previous spray trails, it was found that water only sprays with the same type of spray machine but at different nozzle selection and tractor speeds that results obtained differed. At 14 l tree⁻¹ (circa 6000 l ha⁻¹), at 1.7 km h⁻¹ spray speed, that less deposition was observed on the inside and outside of the canopy than that was observed in the current trial. The slow tractor speed in combination with the intermediate spray volume could have led to more run-off and therefore less deposition. In previous trials, we also observed that at too fast tractor speeds for example 3.3 km h⁻¹ at low volume applications 8 l tree⁻¹ (circa 3000 l ha⁻¹) also resulted in less deposition than that was observed in the current trial, since the sprayer moved too fast to assure adequate coverage (results not shown).

3.2 Trial two – Sun Orange Farms (Addo, Eastern Cape) (less dense canopies)

3.2.1 Deposition quantity

Analysis of variance of deposition quantity (%FPC) indicated a significant interaction for treatment \times volume ($P = 0.0047$) and a meaningful interaction for treatment \times horizontal canopy position ($P = 0.218$).

At 8 l tree⁻¹, Break-Thru Union (4.66 %FPC) realised markedly, but not significantly, higher deposition quantity than the water only (3.59 %FPC) and Break-Thru S240 (3.73 %FPC) treatments. At 14 l tree⁻¹, the adjuvants resulted in similar deposition quantities (5.84-5.85 %FPC), significantly higher than the water only control treatment (3.42 %FPC). At 20 l tree⁻¹, Break-Thru Union (7.17 %FPC) realised significantly higher deposition quantity than water only (5.45 %FPC) and Break-Thru S240 (4.54 %FPC) (Table 3). These results did not concur to that of trial one, but can probably be ascribed to the fact that the canopy was less dense, being more 'spray friendly', since targets was more easily reachable.

For the treatment \times horizontal canopy position interaction, Break-Thru Union realised significantly higher deposition quantity on outer canopy leaves (6.39 %FPC) than Break-Thru S240 (4.78 %FPC) and water only (4.63 %). On inner canopy leaves, adjuvant treatments Break-Thru Union (5.40 %FPC) and S240 (4.54

%FPC) realised statistically higher deposition quantities than water only treatment; Union significantly better than S240 (Table 4). Penetration was better in the less dense canopy, since spray deposition could readily penetrate the inside of the canopy.

3.2.2 Deposition uniformity

Analysis of variance of deposition uniformity between leaves (in a 12 leaf batch) indicated a significant interaction for treatment \times volume ($P = 0.0359$) and a meaningful interaction for treatment \times horizontal canopy position ($P = 0.217$).

At 8 l tree⁻¹, Break-Thru Union realised significantly better deposition uniformity (61.31%) than the control treatment (72.80%), but similar to S240 (65.18%). At 14 l tree⁻¹ treatment Break-Thru S240 (54.22%) realised the best deposition uniformity, statistically more than that of the control treatment (71.42%) and Break-Thru Union (64.13%). Deposition uniformity realised by adjuvant treatments did not differ significantly from that of the control treatment at 20 l tree⁻¹ (54.91-64.13%).

For the treatment \times horizontal canopy position interaction, treatment Break-Thru S240 and Union improved deposition uniformity on outer canopy leaves in relation to the water only treatment, with Union improving it significantly. On inner canopy leaves, both adjuvants improved deposition uniformity over that of the control treatment, with Break-Thru S240 doing so significantly.

3.2.3 Deposition efficiency

Analysis of variance of deposition efficiency [normalised deposition %FPC to a spray volume of 1 l tree⁻¹] indicated significant interactions for treatment \times horizontal canopy position \times vertical canopy position ($P = 0.0281$) and for treatment \times volume ($P = 0.0147$). A meaningful interaction was observed for the interaction treatment \times horizontal canopy position ($P = 0.240$).

For the interaction treatment \times horizontal canopy position \times vertical canopy position, best deposition efficiency was observed to be realised by Break-Thru Union (0.54%) on outer canopy leaves in the tops trees; statistically better than what was realised by the control treatment (0.35%) and Break-Thru S240 (0.40%) at this position. On outer canopy leaves in the middle trees, Break-Thru Union and the water only control treatment realised statistically similar deposition efficiency (0.44%), statistically better than Break-Thru S240 (0.40%). On outer canopy leaves in the bottom of trees, Break-Thru Union and S240 realised the best deposition efficiency (0.39% to 0.50%), statistically better than that of the control treatment (0.30%). On inner canopy leaves in tops of trees, Break-Thru Union (0.38%) realised the best deposition efficiency, statistically better than the control treatment (0.23%) whilst Break-Thru S240 (0.30%) did not differ statistically from the control treatment and Union. Similar trends were observed in the middle and bottom of trees on inner canopy leaves, where Break-Thru Union realised the best deposition efficiency, statistically better than the control treatment, and with Break-Thru S240 being statistically similar to that of the control treatment and Union (results not shown).

For the interaction treatment \times volume, At 8 l tree⁻¹, Break-Thru Union (0.58% FPC) improved deposition efficiency significantly in relation to the deposition efficiency achieved by the control treatment (0.47% FPC). Break-Thru S240 (0.45% FPC) did not differ significantly from that of the control treatment. At 14 l tree⁻¹, adjuvant treatments did not differ statistically from each other (0.42% FPC) and significantly improved deposition efficiency in relation to the control treatment water only (0.24% FPC). At 20 l tree⁻¹, Break-Thru Union again realised the best deposition efficacy; significantly better than the control treatment (0.27% FPC) and S240 (0.22% FPC), which did not differ statistically from each other.

Break-Thru Union realised significantly better deposition efficiency on outer canopy leaves (0.49% FPC) than the control treatment (0.36% FPC) and Break-Thru S240 (0.37% FPC). On inner canopy leaves, both Break-Thru Union (0.41% FPC) and S240 (0.35% FPC) improved deposition efficiency significantly compared to the control treatment water only (0.29% FPC).

3.2.4 Deposition quality

Analysis of variance indicated no significant interactions ($P > 0.05$) but did indicate a meaningful interaction for treatment \times volume ($P = 0.0965$). At 8 l tree⁻¹, no significant difference in deposition quality realised was observed between treatments (98.59% to 102.97%). At 14 l tree⁻¹, none of the adjuvant treatments improved deposition quality compared to the control treatment (94.54%). Break-Thru Union realised a deposition quality of 109.48%, statistically higher variation in deposition quantity per leaf than the control treatment. There was no significant difference between adjuvant treatments and the control treatment in terms of

deposition quality at 20 l tree⁻¹, although both adjuvants treatments had markedly higher CV% values than the control treatment.

3.2.5 Benchmarking

Deposition quantity results for the treatment × volume × horizontal canopy position interaction were compared to the FPC benchmarks. On outer canopy leaves, all treatments realised deposition quantities above the FPC₇₅ benchmark, except for Break-Thru S240 at 8 l tree⁻¹ and the control treatment at 14 l tree⁻¹, only realising deposition quantities above the FPC₅₀ benchmark. On inner canopy leaves, Break-Thru Union realised deposition quantities above the FPC₇₅ benchmark at all spray volumes. At 8 l tree⁻¹, the control treatment and Break-Thru S240 only realised deposition quantities above the FPC₅₀ benchmark. At 14 l tree⁻¹, the control treatment deposited deposition quantities above the FPC₅₀ benchmark, but below the FPC₇₅ benchmark (Figure 2). As in trial one, it is important to note that the spray machine used was calibrated with care correctly, and that tractor speed and spray pressure to our knowledge, were ideal for set spraying conditions. Results obtained may vary dramatically if spray factors and technique are neglected.

4. Discussion

This study evaluated the influence of Break-Thru S240 and Break-Thru Union on deposition parameters at different spray volumes throughout the tree canopy. Markedly different results were obtained between the two trials sprayed. Spray machines used in both trials were calibrated correctly whilst tractor speed and spray pressure used was to our knowledge, the most ideal for spray application. Tractor speed was kept constant throughout treatments and both trials to limit the variable effect application speed have on deposition parameters (Salyani and Whitney, 1990). These factors are very important, since improper calibration, speed and pressure selection along with wrongful spraying techniques, are usually the reason for most treatment failures (Grout, 1997, 2003; Fourie et al., 2009; Salyani, 1994; Stover et al., 2002b).

As observed in the trial and stated in literature, canopy density was an important factor influencing deposition parameters. In trial one, the canopy was dense, thus not as “spray friendly” as the less dense canopies sprayed in trial two, which had pruned “spray windows” and less foliage. In terms of deposition quantity, highest deposition quantity was generally realised by the water only control treatment in trial one; however, not differing statistically from adjuvant treatments, especially Break-Thru S240. In trial two, highest deposition quantity was realised by Break-Thru Union in the more “spray friendly” canopies. Deposition quantity generally increased with increase in spray volume for all treatments in all trials, with 20 L/tree realising the highest deposition quantities. Deposition quantities observed in the two trials was in a similar range, especially at 20 L/tree (4.5 to 7.2%FPC). At 8- and 14 L/tree, higher deposition quantities were observed in trial two in relation to trial one, especially with the addition of an adjuvant, indicating the value adding effect of the addition of an adjuvant at lower volume sprays for an increase in deposition quantity. However, the density of the canopy played a significant role, highlighting the importance of canopy management. More aerated/window pruned canopies were essential for improved deposition, since spray mixture could readily deposited and penetrate the canopy which allowed for free air-movement. With more dense canopies, penetration of the “leaf wall” over all volumes was more difficult, thus causing excessive run-off on the outer canopy before spray mixture could readily deposit and penetrate the canopy. Run-off could have been exacerbated by the addition of an adjuvant in these dense canopies.

Deposition quantity was sufficient in both trails at 8-, 14- and 20 L/tree for deposition above the FPC₅₀ benchmark on the inside and outside of the canopy. On the inside and outside of the canopy, only Break-Thru Union in trial two was sufficient to realise deposition quantities above the FPC₇₅ benchmark over all spray volumes, especially at the low volume application of 8 L/tree, indicating the potential the product has to improve deposition quantity at such low volumes in “spray friendly orchards”.

With the addition of Break-Thru S240 and Union, penetration of the spray mixture were certainly improved into the less dense canopy of trial two in relation to the control treatment, whereas in trial one, this was not the case. In terms of deposition efficiency, Break-Thru Union had a definite improving effect over all spray volumes in trial two, especially at lower volume applications (8 L/tree), as also observed with deposition quantity. In trial one, the addition of the adjuvants did not improve deposition efficiency.

In trial two, deposition uniformity was improved with the addition of the two adjuvants at different spray volumes on the outside of the canopy, and most importantly on the inner canopy leaves. For trial one, this was only the case for Break-Thru Union, improving deposition uniformity at all spray volumes and also on the inner and outer canopy. In both trial one and two, the addition of an adjuvant did not improve deposition quality over the different spray volumes or on the inside and outside of the canopy in relation to that of the control treatment.

From results obtained it is clear that canopy management is of cardinal importance to improve spray deposition parameters, especially with low volume applications. If a canopy is not “spray friendly”, for example, does not have pruned windows to the inside of the canopy and is too dense, spray deposition will be negatively affected which will result in loss of effectiveness of spray application and through it, reduced disease control. Furthermore, the value-adding effects of an adjuvant were especially evident in “spray-friendly canopies”. A definite value adding effect was observed with the adjuvants, especially Break-Thru Union, at low volume applications (8 L/tree), indicating the potential the product has to improve deposition quantity, efficiency and uniformity on the inner and outer canopy leaves, provided that the canopy is “spray friendly”.

Table 4.3.3.2. Mean deposition quantity, uniformity, efficiency and quality realised by the water only control treatment (Water) and adjuvant treatments Break-Thru S240 (S240) and Break-Thru Union (Union) on leaves following sprays at 8, 14 and 20 l tree⁻¹ to dense canopies (trial 1).

Treatment	Deposition quantity (%FPC) ^a			Deposition efficiency (%FPC normalised l tree ⁻¹) ^a		
	8 l tree ⁻¹	14 l tree ⁻¹	20 l tree ⁻¹	8 l tree ⁻¹	14 l tree ⁻¹	20 l tree ⁻¹
Water	4.06 cd	5.82 b	7.28 a	0.51 a	0.36 bc	0.42 ab
S240	3.61 d	4.09 cd	7.49 a	0.45 ab	0.37 bc	0.29 c
Union	3.46 d	5.05 bc	5.85 b	0.43 ab	0.29 c	0.36bc
Treatment	Deposition Uniformity (%CV between leaves) ^a			Deposition Quality (CV%) ^a		
	8 l tree ⁻¹	14 l tree ⁻¹	20 l tree ⁻¹	8 l tree ⁻¹	14 l tree ⁻¹	20 l tree ⁻¹
Water	68.07 a	55.09 ab	57.33 ab	85.56 abc	83.75 bc	86.20 abc
S240	67.54 a	60.47 ab	57.86 ab	94.69 ab	92.98 abc	82.01 c
Union	58.47 ab	59.49 ab	52.39 b	89.99 abc	96.57 a	91.65 abc

^a For each parameter separately, values in each column followed by the same letter do not differ significantly ($P > 0.05$) according to Fisher's least significant difference test.

Table 4.3.3.3. Mean deposition quantity, uniformity, efficiency and quality realised by the water only control treatment (Water) and adjuvant treatments Break-Thru S240 (S240) and Break-Thru Union (Union) on leaves on the inside and outside of the dense tree canopies (trial 1).

Treatment	Deposition quantity (%FPC) ^a		Deposition efficiency (%FPC normalised l tree ⁻¹) ^a	
	Outer canopy	Inner canopy	Outer canopy	Inner canopy
Water	6.10 a	5.35 ab	0.46 a	0.40 ab
S240	5.39 ab	4.74 bc	0.41 a	0.33 c
Union	5.48 ab	4.07 c	0.43 a	0.29 c
Treatment	Deposition uniformity (%CV between leaves) ^a		Deposition quality (%CV) ^a	
	Outer canopy	Inner canopy	Outer canopy	Inner canopy
Water	57.93 ab	62.39 ab	81.09 b	89.25 a
S240	58.53ab	65.38 a	90.67 a	89.12 a
Union	55.31 b	58.36 ab	91.69 a	93.83 a

^a For each parameter separately, values in each column followed by the same letter do not differ significantly ($P > 0.05$) according to Fisher's least significant difference test.

Table 4.3.3.4. Mean deposition quantity, uniformity, efficiency and quality realised by the control treatment (Water) and adjuvant treatments Break-Thru S240 (S240) and Break-Thru Union (Union) on leaves at different spray volumes 8-, 14- and 20 L/tree (trial 2).

Treatment	Deposition quantity (%FPC) ^a			Deposition efficiency (%FPC normalised l tree ⁻¹) ^a		
	8 l tree ⁻¹	14 l tree ⁻¹	20 l tree ⁻¹	8 l tree ⁻¹	14 l tree ⁻¹	20 l tree ⁻¹
Water	3.73 de	3.42 e	5.45 bc	0.47 b	0.24 d	0.27 d
S240	3.59 de	5.84 b	4.54 cde	0.45 b	0.42 bc	0.23 d
Union	4.66 cd	5.85 b	7.17 a	0.58 a	0.42 bc	0.36 c
Treatment	Deposition uniformity (%CV between leaves) ^a			Deposition quality (CV%) ^a		
	8 l tree ⁻¹	14 l tree ⁻¹	20 l tree ⁻¹	8 l tree ⁻¹	14 l tree ⁻¹	20 l tree ⁻¹
Water	72.80 a	71.42 a	60.39 bcd	102.97 a-d	94.54 d	100.80 a-d
S240	65.18 ab	54.22 d	64.13 bcd	98.59 bcd	97.08 cd	108.36 ab
Union	61.31 bcd	64.13 abc	54.91 cd	100.89 a-d	109.48 a	106.23 abc

^a For each parameter separately, values in each column followed by the same letter do not differ significantly ($P > 0.05$) according to Fisher's least significant difference test.

Table 4.3.3.5. Mean deposition quantity, uniformity, efficiency and quality realised by the control treatment (water) and adjuvant treatments Break-Thru S240 (S240) and Break-Thru Union (Union) on leaves on the inside and outside of the canopy (trial 2).

Treatment	Deposition quantity (%FPC) ^a		Deposition efficiency (%FPC normalised l tree ⁻¹) ^a	
	Outer canopy	Inner canopy	Outer canopy	Inner canopy
Water	4.63 c	3.77 d	0.36 c	0.29 d
S240	4.78 bc	4.54 c	0.38 bc	0.35 c
Union	6.39 a	5.40 b	0.49 a	0.41 b
Treatment	Deposition uniformity (%CV between leaves) ^a		Deposition quality (%CV) ^a	
	Outer canopy	Inner canopy	Outer canopy	Inner canopy
Water	66.07 ab	70.35 a	108.05 a	90.83 b
S240	61.66 bc	60.69 bc	108.82 a	93.87 b
Union	55.85 c	64.38 ab	115.07 a	95.90 b

^a For each parameter separately, values in each column followed by the same letter do not differ significantly ($P > 0.05$) according to Fisher's least significant difference test.

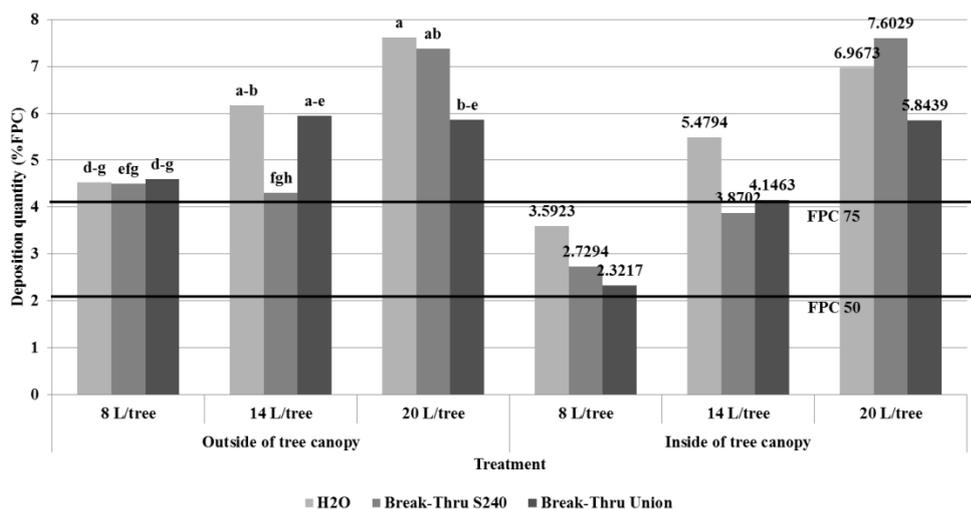


Figure 4.3.3.4. Mean deposition quantity realised by the water only control treatment (Water), Break-Thru S240 (S240) and Break Thru Union (Union) on leaves following sprays at 8, 14 and 20 L/tree on the inside and outside of citrus tree canopies when compared to FPC₅₀ and FPC₇₅ benchmarks at 2.07% and 4.14%, respectively (Trial one).

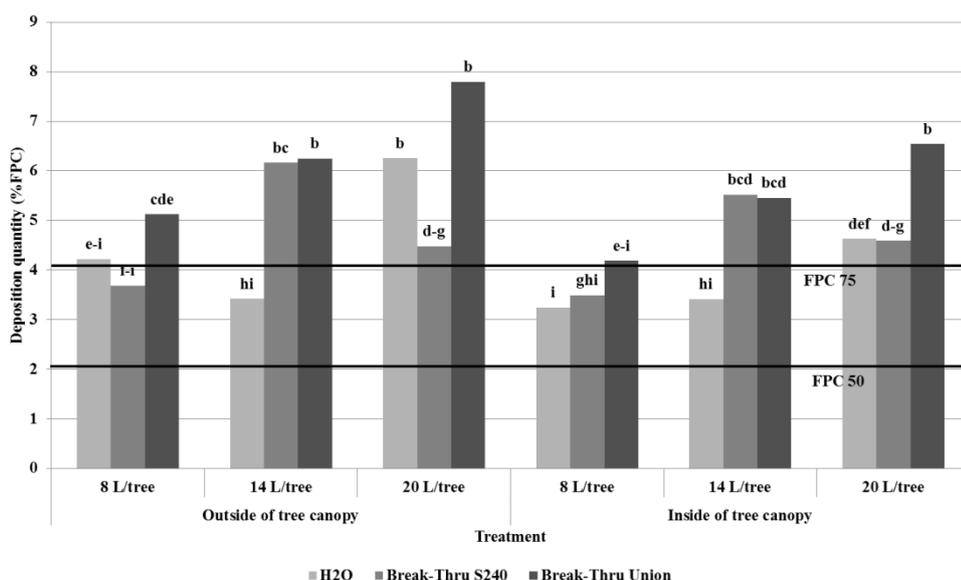


Figure 4.3.3.5. Mean deposition quantity realised by the water only control treatment (Water), Break-Thru S240 (S240) and Break Thru Union (Union) on leaves following sprays at 8, 14 and 20 L/tree on the inside and outside of citrus trees canopies when compared to FPC₅₀ and FPC₇₅ benchmarks at 2.07% and 4.14%, respectively (Trial two).

4.3.4 PROGRESS REPORT: Control of *Botrytis cinerea* Pers. on lemons

Project 1015 (April 2011 - March 2014) by G.C. Schutte, Charl Kotze and P.H. 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 over two seasons in White River. A further analysis of the same cultivars was performed on flowers from Addo. No pattern could be detected as results

were inconsistent. 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. 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 2011. The trees only started blossoming in October 2012 and the weather data showed that the conditions were unsuitable for infection. 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 after they were inoculated on 16 October 2012 with Botrytis conidia. Results over two seasons showed that benomyl and iprodione performed the best in controlling the disease, while two new fungicides tested in 2012, fenhexamid and pyramethanil, also performed well. A commercial application of Benlate in Addo showed that blossoms in the balloon stage were better protected than open blossoms.

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 oor twee seisoene in Witrivier gemeet. 'n Verdere analise is ook van dieselfde kultivars se blomme in Addo gemaak. Geen patroon kon bepaal word nie aangesien resultate wisselvallig was. 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 sensief was teen benomyl, gevolg deur iprodion. '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 2011 was. Blomme is op 1 Oktober 2011 met Botrytis spore geïnokuleer alvorens die swamdoders toegedien is om te verseker dat daar voldoende inokulum teenwoordig is. In 2012 was die blomperiode eers in Oktober en die weerdata wat in hierdie periode gemonitor was, was ook ongunstig vir die siekte. Alhoewel daar geen blominfeksies op die onbehandelde kontrole waargeneem is nie, is blomme geoes en op 'n selektiewe medium uitgeplaat nadat hulle op 16 Oktober 2012 ook met Botrytis spore geïnokuleer is, alvorens die swamdoders toegedien is om te verseker dat daar voldoende inokulum teenwoordig is. Resultate van beide seisoene toon dat benomyl en iprodione goeie beheer van die siekte tot gevolg gehad het, terwyl twee nuwe swamdoders, fenhexamid en pyrimethanil, in 2012 ook goeie beheer van die siekte tot gevolg gehad het. 'n Kommersiële bespuiting van Benlate in Addo het getoon dat blomme wat toe is (ballonstadium) langer beskerm word as oop blomme.

4.4 PROGRAMME: SOILBORNE DISEASES

Programme coordinator: M.C. Pretorius (CRI)

4.4.1 Programme summary

Using a multi-parameter approach, the effect of edaphic factors and their interactions on tree decline were studied with the purpose of identifying the early problems of citrus decline. From the initial results, utilising the ADE4 multi-variate statistical model, it could be concluded that the decline syndrome is the result of several interacting factors. The aim therefore is to influence the interactive balance in such a way, to prevent the establishment of the detrimental factors responsible for tree decline. Research in future will be part of a PhD study and will consist of: Determine whether specific factors are progressively changing over the course of two seasons; Determine whether citrus decline is caused by biological soil factor/s; Identify the specific group/s of biological factors associated with decline and determine the pathogenicity of the organism/s associated with citrus decline (4.4.2). The evaluation of alternative cost effective nematode control options in replant situations consisted of pre-plant fumigation and post plant treatments, conducted at Crocodile Valley Citrus Co. The results showed that female nematode counts started to increase in all the fumigated treatments, with only the Midas treatments being the exception having the lowest counts. Furthermore, the post plant and Metam sodium treatments had little to no effect on female nematode counts compared to the untreated control. Consistent with the nematode counts of the fumigant treatments, tree height and trunk diameter was significantly better when compared to post-plant and untreated controls. Phytophthora was present in all of the treatments, although Midas, Telone and Methyl bromide significantly less than the rest and untreated control. The results showed that the fumigants are effective but the long-term efficacy needs to be determined (4.4.3). The recent banning of aldicarb (Temik) emphasised the significance of alternative

treatments for nematode control. Therefore 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. Results indicated that several products showed initial promise, results can however only be concluded once the final set of data has been analysed. The evaluation of a new organic nematicide from Bayer suggested that it had promise, but the trial needs to be replicated to confirm the results. Selected alternative products were evaluated for the control of Phytophthora in a tunnel trial. Initial screening indicated that several products including the Bayer products controlled Phytophthora by up to 100%. The promising products will be replicated in a follow-up trial in the forthcoming season (4.4.4). The most cost effective measure of Phytophthora control remains the use of resistant rootstocks. Therefore a wide range of rootstocks were evaluated in terms of their tolerance/resistance towards Phytophthora root rot as well as the interpretation of the biochemical mechanisms involved. Tolerant rootstock samples were identified and analysed by means of thin layer chromatography and high performance liquid chromatography – mass spectroscopy (HPLC-MS) to determine the phytochemical profiles of each. Results indicate that, when Phytophthora inoculated and un-inoculated control samples of susceptible (Cairn RL) and tolerant rootstock (Swingle citrumelo) were compared to one another, several compounds were identified to be significantly higher or lower in one or the other. These identified compounds are being tested in bioassays to determine whether they are inhibiting *P. nicotianae* (4.4.6). Contract research evaluating two non-toxic nematicides was done for two international chemical 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).

Programopsomming

Die effek van edafiese faktore en hul interaksies wat lei tot boom agteruitgang is ge-evalueer en ontleed deur gebruik te maak van 'n spesifieke multi-parameter benadering. Hieruit kan moontlike aannames gemaak word vir die vroeë identifisering van probleme wat kan bydra tot sitrus-agteruitgang. Die gevolgtrekkings wat uit die aanvanklike data gemaak kan word, deur van die ADE4 multi-variate statistiese model gebruik te maak, is dat die agteruitgang sindroom die gevolg van verskeie faktore en hul interaksies met mekaar is. Daar moet dus gepoog word om die interaktiewe balans, wat 'n rol speel in die skep van 'n gunstige omgewing wat boom agteruitgang bevorder, te voorkom of te beïnvloed. Toekomstige navorsing sal deel uitmaak van 'n PhD-studie en sal die volgende insluit: Om te bepaal wat die rol van spesifieke faktore is en of daar 'n geleidelike verandering oor tyd sal wees; Bepaal of sitrusagteruitgang veroorsaak word deur biologiese grond faktore; identifiseer die spesifieke biologiese faktore en bepaal die patogenisiteit van organismes wat verband hou met sitrusagteruitgang (4.4.2). Die doeltreffendheid en die ekonomiese impak van verskeie voor-plant beroking en na-plant behandelings is by Crocodile Valley Citrus Co evalueer met die doel om alternatiewe herplantbeheer opsies te ondersoek. Ten spyte daarvan dat die resultate steeds lae aalwurmfyfie getalle vir alle geëvalueerde berokings behandelings toon, het slegs die Midas toedienings na wense presteer met die laagste getalle, terwyl Metam-natrium en die na-plant behandelings swak presteer het. Die boomhoogte en stamdeursnee is aansienlik beter in die berokingsbehandelings indien vergelyk word met die na-plant behandelings en onbehandelde kontrole. Alhoewel Phytophthora in al die behandelings teenwoordig was, was daar egter statisties minder in die Midas, Telone en Metiel bromied behandelings. Die resultate toon duidelik dat die lang termyn effek van die benadering eers deeglik bestudeer moet word voordat finale aanbevelings moontlik sal wees (4.4.3). Die verlies van (aldicarb - Temik) het die noodsaaklikheid vir alternatiewe behandelings vir aalwurm beheer weereens beklemtoon. Daarom is die volgende produkte in 'n veldproef geëvalueer: Nontox-Silica (silika), Biolan (ZZ2, produk), CRI aalwurmeier stimulant, 'n kombinasie van 'n aalwurmeier stimulant + 'n aalwurmdoder, standaard aalwurmdoder Rugby (cadusaphos), Foodprint en Diatomied. Voorlopige resultate dui aan dat meeste van die produkte wel 'n mate van beheer teen die sitrusaalwurm kon toon maar die finale stel resultate is nodig om sinvolle afleidings te kan maak. Evaluasie van 'n nuwe Bayer produk, 'n organiese aalwurmbheer middel, lyk uiters belowend, maar moet herhaal word om hierdie resultate te bevestig. Verskeie nuwe en alternatiewe produkte is in 'n tunnel eksperiment ge-evalueer met die doel om Phytophthora infeksie te beheer. Aanvanklike resultate toon dat sekere van die produkte, sowel as 'n nuwe Bayer produk het Phytophthora met 100% beheer. Die effektiwste produkte sal in die komende seisoen weer ge-evalueer moet word met die doel om die resultate te bevestig (4.4.4). Bestande onderstamme bly die mees koste-effektiewe wyse om Phytophthora wortelvrot in sitrus te beheer. Met dit in gedagte, is 'n wye reeks onderstamme nie net geëvalueer ten opsigte van hul weerstandbiedendheid nie, maar ook om die biochemiese meganismes te identifiseer, en met dunlaag-chromatografie en HPLC-MS ontledings die fitochemiese profile in die onderskeie wortelmonsters te bepaal. Resultate het getoon dat 'n aantal verbindings vanaf 'n vatbare onderstam (Cairn growweskil suurlemoen) en 'n weerstandbiedende onderstam (Swingle citrumelo) in betekenisvol hoër of laer konsentrasies voorgekom het wanneer dit in geïnokuleerde en ongeïnokuleerde plante vergelyk is. Hierdie verbindings word verder getoets in bioassaerings om te

bepaal of hulle *P. nicotianae* inhibeer (4.4.6). Kontrak navorsing: Evaluering van twee nie-giftige aalwurmdoders is gedoen vir twee internasionale maatskappye in België en Israel (4.4.7, 4.4.8) sowel as 'n tonnel eksperiment op Sitrusvergroening vir 'n maatskappy in Duitsland (4.4.9).

4.4.2 FINAL REPORT: Investigation into edaphic factors and their interactions on citrus tree decline

Project 910 (2008 – 2012) by M.C. 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 aim of the project was 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. The following parameters analysed were: 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 zink accumulation in xylem). The following conclusions can be made from the initial results obtained by utilising the ADE4 multi-variate statistical model: the technique is suitable to identify interactions; preliminary results indicated that the decline syndrome is the result of several factors interacting with one another; the aim is to influence the interactive balance to prevent the establishment of the detrimental environment which leads to the tree decline. Up until now we were analyzing data vertically using parameters individually, eg; nematodes and *Phytophthora* or leaf and soil analysis. Another advantage is the projection of the interactions on field trial maps to determine possible areas which are more subjective to trigger decline. Future research which will be part of a PhD study will be included in a new project proposal.

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, asook boom vrektes. Die doel van hierdie projek was om grond faktore en hul interaksies te identifiseer wat moontlik tot sitrusagteruitgang kan lei, asook om 'n "multi-parameter" model te gebruik om voortydig sitrusagteruitgang simptome te identifiseer. Die parameters wat ondersoek en waarvan data versamel en met die ADE4 program ontleed is, 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 zink akkumulاسie in xileem). Na ontleding van die data met die "multi-variate" statistiese program kan die volgende afleidings gemaak word: die analiese tegniek is geskik om interaksies te kan bepaal; volgens die voorlopige resultate kan wel bevestig word dat sitrusagteruitgang die gevolg is van die interaksie van verskeie faktore; opvolgende doelwitte is om die interaktiewe balans te beïnvloed en om sodoende boom agteruitgang te stuit. Tot op hede is data individueel ontleed maar met dié metode is dit moontlik om al die parameters gelyktydig te ontleed. Van die interaksies kan geprojekteer word op die veldplan wat areas kan uit wys waar sitrusagteruitgang 'n moontlikheid is. Die projek sal voortgesit word in 'n nuwe projekaansoek wat ook deel uitmaak van hierdie PhD studie.

Introduction

Soil, tree and root health is the new buzz word in most of the agricultural industries and research institutes worldwide in order to develop and maintain good management practices ensuring well managed sustainable farming units able to provide good quality products to the markets.

The symptoms associated with Citrus Decline are distinguished by sparse foliage, dead wood and reduced growth. The trees do not die from the disease, but remain in a declined state following the initial onset of symptoms. The declined trees will have a reduced yield and fruit size. These trees will always wilt sooner during a dry period than the adjoining healthy trees. All trees within a decline area show the same general symptoms, with a distinct margin present at the border between the decline and healthy trees. The disease will gradually spread from the declined trees to the adjacent apparently healthy trees. Suit et al. (1947 & 1949) reported that, based on tree behaviour, this spreading decline appeared to be the result of feeder root rot infections.

In order to sustain a healthy citrus tree, specific growth conditions are necessary. Many health problems occur in citrus trees as a result of improper growth conditions. Poor soils, lack of nutrient content or inappropriate water can contribute to health problems in citrus trees. These improper growing conditions will stress trees, increasing the risk of secondary problems. Certain insects attack citrus and cause disease-related problems, viz. aphids and citrus psylla, with vectors Tristeza virus and Citrus greening disease, respectively. Diseases associated with tree health include *Phytophthora* spp. responsible for root-collar rot and stem canker. Nematodes cause slow decline in citrus. A typical declined tree will have yellowing, discolouration, distorted leaves, loss of leaves and branch die-back (Schalau, 2010).

The complexity of decline on citrus is important due to the lack of knowledge regarding the original causes and also the consequence thereof. In many instances decline is only emphasised when the symptoms become visible and in this instance it is normally too late to make any corrections in order to reverse the situation. It is a very cost effective exercise with no guaranteed results. Numerous factors can play a role in causing citrus decline and in many instances it is not only one factor but a combination of different factors complicating the matter even further. Currently there are no early-diagnosis tools that one can utilise to address the problem. An early-diagnosis tool would assist researchers, consultants and citrus growers to attend to the decline problem proactively.

Objectives

To identify edaphic factors and their interactions that lead to tree decline by using a specific multi-parameter approach, therewith enabling the early identification of citrus decline problems.

Materials and methods

Two typical root disease-related, declined orchards were identified. The one orchard was identified at Crocodile Valley's, Valley View farm at Karino (Trial 1), this being a 20-year-old Delta Valencia orchard on Rough Lemon with visual root disease decline symptoms. Four decline category trees were identified representing the following criteria: Category 1 – Visually healthy tree; Category 2 – Visually healthy tree with slight indication of decline symptoms; Category 3 – Typical root disease declined tree and Category 4 – severely declined tree. The trial consisted of 15 single trees per category. The second trial was laid out at Friedenheim Estate on 15-year-old Midnight Valencia on rough lemon rootstock trees (Trial 2). Only three categories of decline were identified: Category 1 – Visually healthy tree; Category 2 – Visually healthy tree with slight indication of decline symptoms; Category 3 – Typical root disease decline trees. The orchard is visually in a much better condition and no Category 4 decline trees could therefore be found. The total number of trees for the two trial sites where data was collected was 105 trees. The following data was collected over a period of two seasons:

1. Nematode and *Phytophthora* samples: Collect 250 g soil and 500 g of feeder roots per tree. Samples analysed by the Diagnostic Centre in Nelspruit to determine the second stage larvae in the soil according to the method of Whitehead and Hemming (1965) and the female populations in the roots according to the method of Van der Vegte (1973). *Phytophthora* in the soil was determined by means of the leaf baiting technique.
2. Chlorophyll content: 30 leaves per tree were monitored by means of a mobile SPAD meter.
3. Leaf size: 30 leaves per tree were collected, leaf size was determined by the University of Pretoria.
4. Soil depth: determined by means of a penetrometer, readings were collected on the northern side of each tree.
5. Soil and leaf samples for chemical fertiliser analysis: the soil samples were collected using a spade, at a depth of 10 cm, before May of each year, on the eastern side of each tree. Fifteen leaves per tree are needed for nutritional analysis. Samples were analysed by Nvirotek Laboratories, Brits, South Africa.
6. Starch content: Roots were collected (500 g) from each tree – samples were grinded, refrigerated and sent to University of Pretoria for analyses.
7. Blight tests: Water test - drill one hole per tree. A 100 ml syringe was filled with 10 ml of water. The time of water up-take by the tree determined the disease status (< 10 sec. – healthy; > 20 sec. Blight symptoms); Root test (Zink content in roots), collect 100g of feeder roots, grind to fine powder, freeze, samples analysed by LabServ laboratory, Nelspruit, South Africa.
8. Yield and fruit size data: fruit was hand-picked and a mobile fruit counter used to collect the data.

Data analysis on the physical, chemical and biological factors with regard to soil, plant (roots and leaves) and root diseases was analysed by means of the Multi-Variate Statistical programme (ADE- 4) for a period of two

seasons. Certain of the parameters were analysed with the assistance of Dr. S Berry (Becker Underwood) from data collected during the first year. However Dr. P Cadet (France) assisted more directly with training and the analyses of the data with the Multi-Variate statistical programme. Analysis were done on all data (all parameters analysed together) for the two sites. The following analyses were done for both trials: Soil vs. Leaf vs. Disease vs. Yield. Parameters were analysed to indicate possible, if any, correlations or relationships between factors and on their own.

Results and discussion

Milestones	Achievement
Data collection second year – determine nematode and <i>Phytophthora</i> presence, soil and leaf analysis (soil-chemical/physical), climatic conditions (temp, rainfall and soil temperature on monthly basis), yield data (yield and fruit size and quality).	The second year's data were collected during 2011/12 – The outstanding data was received during November 2012 and was entered in Excel spreadsheets – All data of all the parameters monitored for two seasons is ready to be analysed.
Data collected from field trials will be incorporated and analysed with the ADE-4 multivariate statistical package in collaboration with Dr. P Cadet (France) and Dr. A McLeod.	The available data were analysed with the assistance and guidance of Dr. P Cadet as part of a training session over a period of two weeks which included Dr. A McLeod.
Glasshouse trial - to study certain tendencies that could be manipulated in a pot trial and confirm the field data by means of the Multivariate Statistical programme - (ADE4)	The trial did not commence; however, with Dr. A McLeod as promoter, a new approach will be followed – Two planning discussions with Dr. H le Roux and Prof. N Labushagne were held in 2013.
Future research and the way forward will form part of a PhD study.	The progress of the PhD study was discussed. Dr. A McLeod as promoter, Dr. H le Roux and Prof N Labushagne are involved. The possible chapters were determined as well as future research options – the research will be presented as a new proposal during the 2013 proposal process.

All data collected over a period of two seasons were utilised in the training sessions which took place during October 2012, presented by Dr. P Cadet. Dr. A McLeod also attended the training sessions. The data collected included chemical, physical, biological and pathological data associated with soil health in citrus declining orchards. A basic PCA analysis was completed on the first season's data early on in 2012 and was reported on during last year's (2012), progress report. The PCA analysis offers a number of different options to visually present analysed data. Large numbers of recorded data can be studied more meaningfully with this method. Not all the data could be analysed for the two seasons because some of the second season's data was not available during the training sessions. The initial analysed data was represented at the Soilborne Disease Interest group meeting in Stellenbosch during September 2012 and at the International Citrus Congress in Spain in November 2012.

The training consisted of a theoretical and applied introduction to the ADE 4 (PCA) data analysis model. The following steps and questions were identified and form part of the training outline:

Step 1: 1. Are the two sites different or similar to each other?

2. If they are different – data will have to be analyzed separately.

Conclusions: The sites are different and will therefore have to be analyzed separately (results presented in 2012 report)

Step 2: 1. Are the identified parameter categories, namely yield, soil, leaf and symptom category changing according to increasing classes of visual decline?

2. What are the most important factors for each of the five groups of parameters?

3. Are factors grouped together in the different categories relevant in the analyzing process?

Results: PCA analysis performed on all the collected data at site 1 and 2 for year 1 was re-analysed (Same results as in 2012 progress report) and the summary of the most important factors for each site was identified (Table 4.4.2.1). The parameters were originally grouped into five groups, namely Leaf = only leaf chemical; Soil physical = texture and density; Soil chemical = nutritional, CEC and pH; Disease = organisms + blight + starch + leaf size; and Yield = total fruit, big fruit and small fruit. As a result of the first set of results the question was raised whether these groupings are justifiable and therefore different groupings were

identified with the exception of the Yield grouping that were kept the same. The parameters were re-grouped and again analysed. The new groups were identified as: Plant = all leaf nutrients + blight + starch + leaf size; Organism = nematode roots, nematode soil, free-living and Phytophthora; All soil = chemical and physical; Soil minus texture = All soil minus texture (sand, clay and silt); yield = total fruit, big fruit and small fruit. All the data was analysed and presented as factorial plans and correlation circles. Some of the results, Plant data site 1 and 2, All soil site 2 and soil minus texture site 2 are presented in Figs 4.4.2.1 – 4.4.2.6.

The results indicated insubstantial differences between the previous groupings and the latest analysis of the re-grouped parameters.

Where the plant data were analysed and compared between the different categories, it clearly shows that in site 1 the categories are distributed along the 2nd diagonal. Mainly because B, % Na, Mn and % Ca or % P increased and Mo decreased when moving from category 1 & 2 to 4 (Fig 4.4.2.1). These results correlate with last year's data (2012 progress report) with no difference when the other plant related factors were included in the analyses as part of the re-classification of the parameters. The new parameters included in the analyses indicated an increase in the Blight value for the category 3 and 4 trees whereas the leaf size increased toward the category 1 and 2 trees.

The leaf analysis in site 2 also correlates with last year's progress report data and the re-grouped parameter data also indicate that the category 1 and 2 trees had higher levels of chlorophyll and bigger leaf sizes compared to the category 3 (sick) trees (Fig 4.4.2.2).

When comparing the soil analysis of Fig 4.4.2.3 with the newly grouped parameters of Fig 4.4.2.4 (Soil minus texture) no significant differences are visible. The same most important factors were identified. The factors are presented in Table 4.4.2.1.

Coinertia analysis (see last season's progress report) was done to determine if any associations between all the relevant factors monitored could be recorded. No significant conclusions could be made from all the analyses.

Step 3: 1. To project analysed factor on the field map.

Determine whether the (i) soil physical factors are evenly distributed across the field site and (ii) where the location of the different tree categories is on the field site map.

This was done to demonstrate how versatile and powerful the ADE 4 model is and that it is possible to apply the analysed data directly to the field plan. With this technique it is also possible to project interactions on the field map to hopefully determine if there are areas which are more subjective to trigger decline.

The first step was to project the different categories on the field map. The category 1 trees are represented by big squares, category 2 by smaller squares, category 3 trees by small dots and the category 4 trees by big dots exactly as seen on the original field plan to the right of the fig's (Fig 4.4.2.5). The next step was to project the interactions of the different analysed factors on the trial map which are a different means of presenting the data more visually on the trial map. The results clearly showed an increase in clay % and compaction in the 0 – 5 cm of the soil profile in the area where most of the diseased trees, category 4, were found and in contrast the category 1 trees were found to be more in the sandy soil part of the orchard (Fig 4.4.2.6).

Table 4.4.2.1. Results of the important factors initially identified and then after re-classification per parameter group for both sites 1 & 2.

Site 1 – Valley View - Alco		Site 2 - Friedenheim	
Initial groups	Re-grouped	Initial groups	Re-grouped
Soil Chemical <ul style="list-style-type: none"> • Ca, CEC, S - Value & Mg 	Soil minus texture <ul style="list-style-type: none"> • Ca, CEC, S- Value & Mg 	Soil Chemical <ul style="list-style-type: none"> • S- Value, CEC, Ca 	Soil minus texture <ul style="list-style-type: none"> • S-Value, CEC & Ca
Soil physical <ul style="list-style-type: none"> • Silt & sand 	-	Soil physical <ul style="list-style-type: none"> • Silt, sand & density 	-
Symptoms <ul style="list-style-type: none"> • Nem Soil, Nem roots, Free living & Blight 	Organism <ul style="list-style-type: none"> • Nem soil, Nem roots & Free-living 	Symptoms <ul style="list-style-type: none"> • Nem soil, leaf size & Blight 	Organism <ul style="list-style-type: none"> • Nem soil & Nem roots
Leaf <ul style="list-style-type: none"> • B, Mn, Ca & Na 	Plant <ul style="list-style-type: none"> • Blight, Mn, Na, P, Ca & Mo 	Leaf <ul style="list-style-type: none"> • Na, Mn, Ca, N, K & Mg 	Plant <ul style="list-style-type: none"> • N, K, Ca, Mg & leaf size
Yield <ul style="list-style-type: none"> • Brix, total yield, small fruit & big fruit 	•	Yield <ul style="list-style-type: none"> • Total yield & small fruit 	•

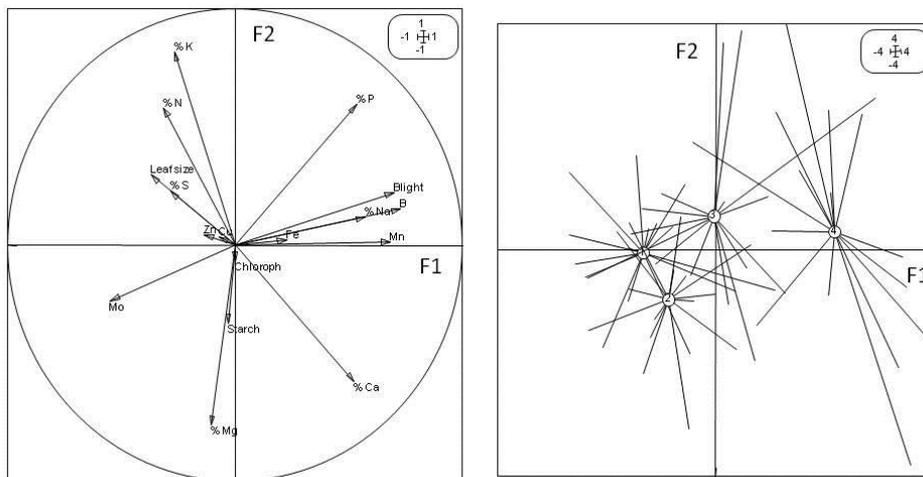


Fig 4.4.2.1. Correlation circle and Factorial plan of the PCA on the plant data for site 1 (Valley view).

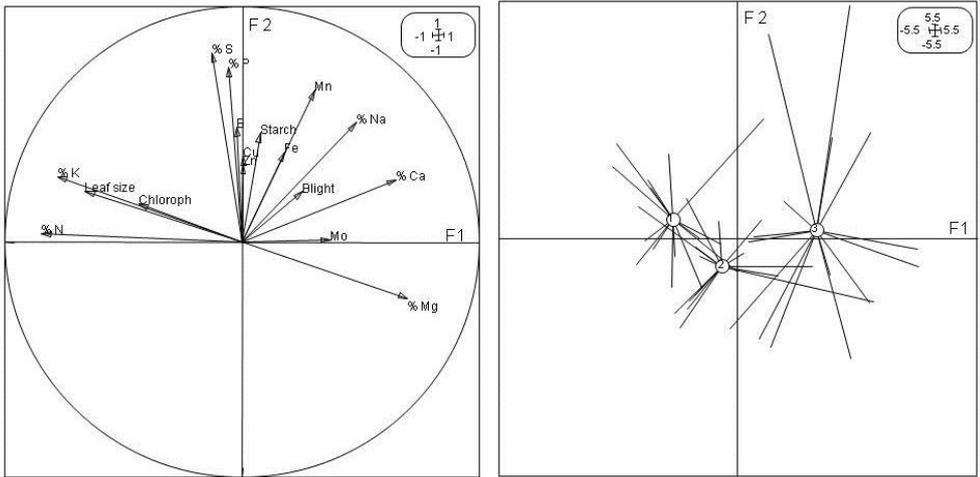


Fig 4.4.2.2. Correlation circle and Factorial plan of the PCA on the plant data for site 2 (Friedenheim).

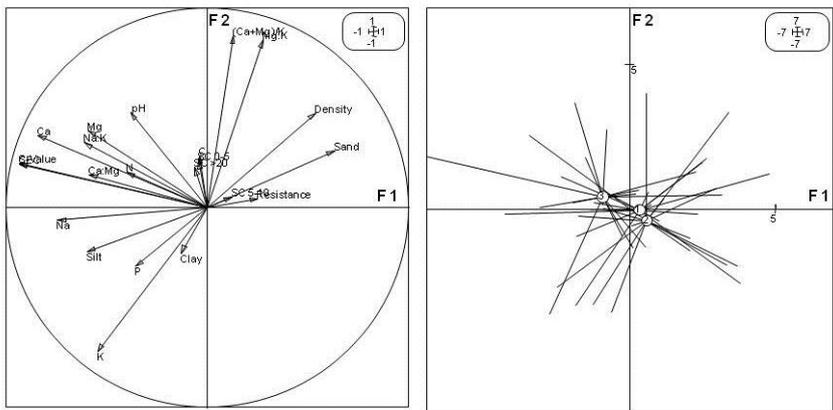


Fig 4.4.2.3. Correlation circle and Factorial plan of the PCA on all soil data for site 2 (Friedenheim).

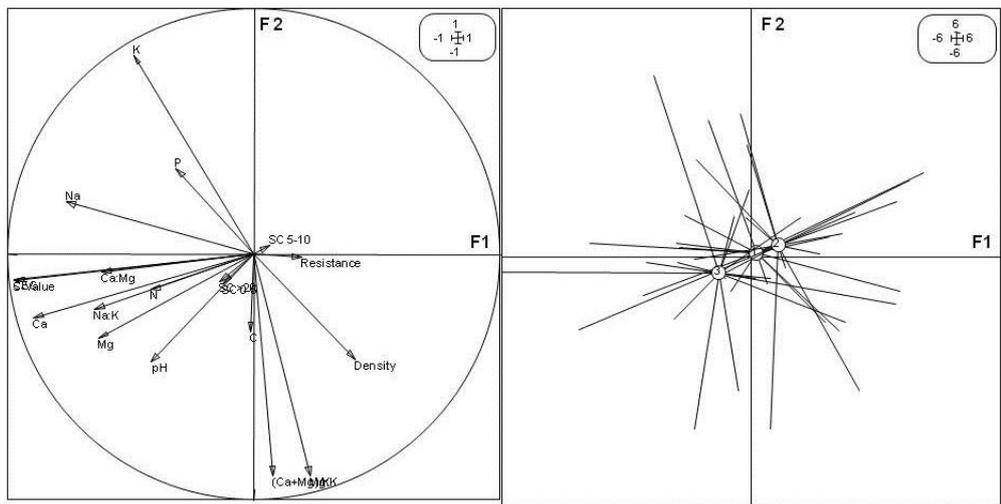


Fig 4.4.2.4. Correlation circle and Factorial plan of the PCA on the soil minus texture data for site 2 (Friedenheim).

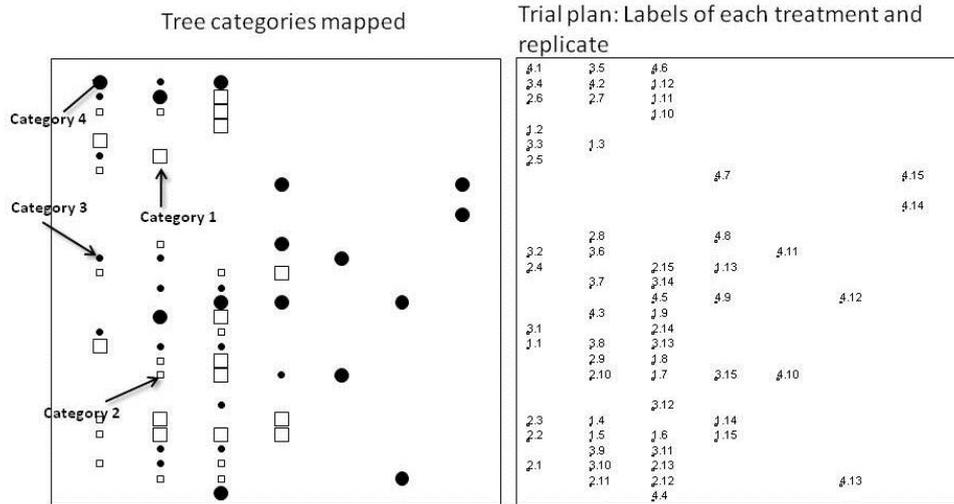


Fig 4.4.2.5. Trial map with coded trees and mapped tree categories for trial site 1.

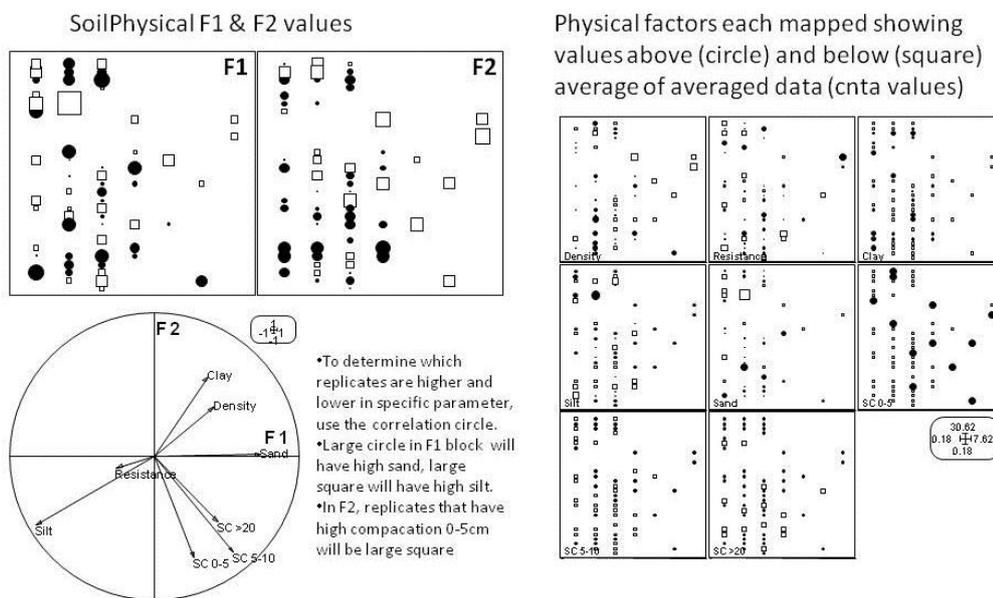


Fig 4.4.2.6. Soil physical factors, F-values and each physical factor mapped onto trial map for site 1.

Conclusion to date

- All the data (2010/11 and 2011/12) have now been collected.
- We have a technique that will enable us to identify interactions.
- According to the preliminary results we are convinced that the decline syndrome is the result of several factors interacting with one another.
- The objective is to influence the interactive balance to prevent the establishment of the detrimental environment which leads to the tree decline.
- Until now we were analyzing data vertically using parameters individually viz. nematodes, and Phytophthora or leaf and soil analysis.
- Another advantage is that we can project the interactions, which are factorial values, on the field trial map to determine if there are areas which are more subjective to trigger decline.
- It is known that organisms are in relation with the abiotic environment and by utilizing this mesological relationship it could be an easy technique to reduce the impact of decline.

Future research

1. Data will be utilised as part of a PhD study. The data collected from site 1 and 2 will be processed and prepared for a publication with the assistance of Drs A McLeod and P. Cadet.

2. Future experiments are planned as part of the PhD study but will be motivated in a new experiment proposal. The experiments would include:
 - Determine whether specific factors are progressively changing over the course of two seasons.
 - Determine whether citrus decline is caused by biological soil factor/s.
 - Identify the specific group/s of biological factors associated with severely declined trees.
 - Determine the pathogenicity of organisms associated with citrus decline.

Technology transfer

1. A talk was presented at the last CRI biennial symposium in August 2012.
2. A paper was presented at the Soilborne Plant Disease Interest Group meeting in Stellenbosch in September 2012 – The title of the talk was: "Investigating the potential association and interactions of edaphic factors with root disease-related citrus decline".
3. A talk with the same title was also presented at the International Citrus Congress in Spain during November 2012.

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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

Project 762 (2007 – 2014) by M.C. 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-sodium (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 were 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 regards 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 laer 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 as in beide die na-plant en onbehandelde kontrole handelings. Phytophthora is in meeste van die handelings gevind. Die algehele voorkoms van die bome is goed en visuele verskille tussen die berookte en onberookte handelings is sigbaar. Die aanvanklike resultate toon dat die berokings handelings uiters effektief was en heelwat potensiaal het vir toekomstige gebruik op herplant gronde maar die lang termyn effektiwiteit van die handelings 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**

Project 1030 (2008 – 2013) by M.C. 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. 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. 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 make final conclusions. The results of a new Bayer organic nematicide are very promising but the trial needs to be replicated to confirm the data. The glasshouse, pilot *Phytophthora* trial where alternative products and chemicals were evaluated for the control of *Phytophthora*, delivered promising results. The Bayer product and a number of other products controlled *Phytophthora* in the planting bags by 100%. Various other alternative products including the Bayer product will again be evaluated, whereafter a field trial will be established.

Opsomming

Die sitrusaalwurm, *Tylenchulus semipenetrans*, is die algemeenste aalwurm plaag wat bydra tot ekonomiese verliese in sitrusboorde. Internasionaal en plaaslik word al meer druk geplaas op die beperking/verwydering van die gebruik van toksiese middels. CRI het 'n pro-aktiewe benadering tot hierdie probleem en 'n verskeidenheid van alternatiewe produkte word huidiglik 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 standaard aalwurmdoder (cadusaphos), Foodprint en Diatomied. Alhoewel daar tans slegs die resultate van twee monsternemings beskikbaar is, dui dit tog aan dat sekere produkte wel 'n effek op die aalwurmpopulasies gehad het; die finale stel resultate is egter nodig om sinvolle afleidings hier uit te kan maak. 'n Nuwe Bayer, nie toksiese aalwurmdoder, het uiters belowende resultate gelewer maar sal herhaal word om die resultaat te bevestig. 'n Loodsproef met verskeie alternatiewe en chemiese produkte vir die beheer van *Phytophthora* het belowende resultate op gelewer en sal herhaal word gedurende die 2013/14 seisoen. Die nuwe Bayer produk het weereens *Phytophthora* 100% beheer.

4.4.5 **PROGRESS REPORT: Rootstock resistance against *Phytophthora nicotianae* root rot**

Project UP_CRR1-09 by N. Labuschagne, Z. Apostolides & M. Sakupwanya (UP)

Summary

Resistant rootstocks are one of the most cost effective ways of combating *Phytophthora* root rot of citrus. The main aims of the current project are screening of a wide range of rootstocks for tolerance and elucidating the biochemical mechanisms involved in resistance / tolerance of citrus rootstocks against *Phytophthora* root rot. More than eight separate greenhouse experiments have been conducted over two

seasons to assess the relative tolerance of 14 citrus rootstocks to Phytophthora root rot. Subsequently root samples from these experiments have been systematically analysed by means of thin layer chromatography and High performance Liquid Chromatography – Mass Spectroscopy (HPLC-MS) to determine the phytochemical profiles in the various rootstock samples. HPLC-MS results of crude extracts from a susceptible rootstock (Cairn RL) and a tolerant rootstock (Swingle citrumelo) indicated a large number of compounds of which several were significantly higher or lower in concentration when comparing inoculated plants with uninoculated controls, i.e. being either up or down regulated in pathogen infected plants. These compounds are being further tested in bioassays to determine whether they are inhibiting *P. nicotianae* and are therefore associated with rootstock resistance.

Opsomming

Weerstandbiedende onderstamme is een van die mees koste effektiewe maniere om Phytophthora wortelvrot van sitrus te bestry. Die hoof doelstellings van hierdie projek is die assessering van 'n wye reeks onderstamme vir weerstandbiedendheid en bepaling van die biochemiese meganismes betrokke by onderstam weerstandbiedendheid teen Phytophthora wortelvrot. Meer as agt afsonderlike glashuisproewe is oor twee seisoene uitgevoer om die relatiewe weerstandbiedendheid van 14 sitrus onderstamme teen Phytophthora wortelvrot te evalueer. Daaropvolgend is wortelmonsters vanuit lg. proewe sistematies ontleed d.m.v. dunlaag-chromatografie en HPLC-MS om die fitochemiese profiele in die onderskeie onderstam-wortelmonsters te bepaal. HPLC-MS resultate van kru-ekstrakte vanaf 'n vatbare onderstam (Cairn growweskil suurlemoen) en 'n weerstandbiedende onderstam (Swingle citrumelo) het 'n groot aantal verbindings gewys waarvan verskeie in betekenisvol hoër of laer konsentrasies voorgekom het wanneer geïnokuleerde en ongeïnokuleerde plante vergelyk is, m.a.w. opwaarts of afwaarts gereguleerd in patogeen geïnfecteerde plante. Hierdie verbindings word verder in bioessasings getoets om te bepaal of hulle *P. nicotianae* inhibeer en dus met onderstam weerstandbiedendheid geassosieer is.

4.4.6 **CONTRACT RESEARCH: Evaluation of a new nematicide for the control of the citrus nematode**

Project 950 by M.C. Pretorius & C. Kotze (CRI)

A contract trial for Makhteshim, Israel, was laid out in Komatipoort and in the Western Cape. The progress report with data collected over 2 years was sent to the company.

4.4.7 **CONTRACT RESEARCH: Evaluation of a new safer nematicide for the control of the citrus nematode**

Project 951 by M.C. Pretorius (CRI)

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 for a period of four years; one at Croc Valley Citrus Co. and the second in Citrusdal. A final report was sent to DevGem, Belgium. The contract was terminated.

4.4.8 **CONTRACT RESEARCH: Evaluation of a product with possible SAR characteristics for the control of citrus greening bacteria in citrus trees**

Project 971 by M.C. Pretorius (CRI)

A glasshouse trial was 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 and currently the final evaluations are completed where after the contract will be terminated.

4.5 **PROGRAMME: POST-HARVEST PATHOLOGY**

Programme coordinator: Arno Erasmus (CRI)

4.5.1 **Programme summary**

In the postharvest pathology programme, five projects are being funded. Project 123 provides an industry service to strategically evaluate products as potential alternatives for sanitisers or fungicides. Several products were evaluated in 2012, and some has been identified for further evaluation. A ring-test has been conducted between analytical laboratories which showed some variability in analyses of postharvest fungicide residues (4.5.2). 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. Short-duration hot water treatment showed good curative control and a yeast biocontrol agent showed some protective control (4.5.3). At Stellenbosch University (Project 936), residue loading and protective and curative green mould control following dip, wax and drench applications with imazalil (IMZ), thiabendazole (TBZ) and pyrimethanil (PYR) were studied. Residue benchmarks for effective control following dip application were determined, which showed excellent curative control of the fungicide sensitive strains, but relatively poor protective control with PYR and TBZ. An isolate resistant to IMZ and PYR 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 (4.5.4). The JBT heated flooder (JHF) was evaluated in Project 1050, and showed very good promise regarding residue loading and curative and protective control. Further optimisation of inline aqueous and drench applications is proposed as new projects (4.5.5). In Project 1034 at CRI-Nelspruit, the IMZ sensitivity of several *Penicillium digitatum* and *P. italicum* isolates were determined, as well as residue benchmarks for protective and curative control. Results confirm findings from Project 936, where only one resistant isolate of *P. digitatum* was used, that control failure occurs in cases of IMZ resistance. 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 (4.5.6). 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 (new project proposal).

Programopsomming

Vyf projekte word in die na-oespatologie program befonds. Projek 123 voorsien 'n industrie-diens om produkte strategies as potensiële alternatiewe vir saniteerders of swamdoders te evalueer. Verskeie produkte is in 2012 geëvalueer, en party is vir verdere evaluasie aanbeveel. 'n Ringtoets is met verskeie analitiese laboratoria gedoen, wat duidelike variasie in residu-resultate uitgewys het (4.5.2). 'n Alternatiewe benadering tot na-oes siekte- en koeskade-beheer, word by die Universiteit van KwaZulu-Natal bestudeer, waar silikonbemesting met na-oes hitte- en biobeheeragent-behandeling geïntegreer word. Kort hittebehandeling het goeie kuratiewe beheer gewys, terwyl 'n biobeheer gis beskermende beheer gewys het (4.5.3). By Stellenbosch Universiteit (Projek 936) is residulading en beskermende en genesende groenskimmelbeheer, volgende op doop-, waks- en storttoedienings met imazalil (IMZ), thiabendazole (TBZ) en pyrimethanil (PYR), bestudeer. Residu drempelwaardes vir effektiewe beheer na dipbehandeling is bepaal, wat uitstekende genesende beheer van die fungisiedsensitiewe isolate met al die fungisiedes getoon het, maar relatief swak beskermende beheer met PYR en TBZ. 'n Isolaat wat weerstandbiedend teen IMZ en PYR 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 (4.5.4). Die JBT verhitte vloed-toediener is in Projek 1050 evalueer en het baie goeie potensiaal gewys na aanleiding van residu-lading, genesende en geskermende beheer wat verkry is. Verdere optimisering van in-lyn pakhuis water toepassings asook stort-aanwending word in voortsettingsprojekte beplan (4.5.5). In Projek 1034 by CRI-Nelspruit is die IMZ sensitiwiteit van verskeie *Penicillium digitatum* en *P. italicum* isolate bepaal, asook residu drempelwaardes vir beskermende en genesende beheer. Resultate het bevindinge van Projek 936, waar slegs een weerstandbiedende isolaat van *P. digitatum* getoets is, bevestig, naamlik dat mislukte beheer in die geval van IMZ weerstand voorkom. 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 (4.5.6). In die ontwikkeling van 'n weerstandstoets, was blootgestelde plaattoets met 'n semi-selektiewe media nie-spesifiek en onbetroubaar, en 'n kwantitatiewe "real-time" PCR metode word tans ontwikkel.

4.5.2 PROGRESS REPORT: Provision of an industry service whereby new packhouse treatments are comparatively evaluated, fungicide resistance is monitored and standardised recommendations are provided

Project 123 (Ongoing) by Arno Erasmus and Paul H. Fourie (CRI)

Summary

Various pilot trials were conducted in this project. Products that showed promise as sanitising agents were hydrogen peroxide + parasitic acid (Citrocide and Tsunami), SOPP (SOPP 20) and electrolyte water (Neuthox and Ecolyte). As fungicides against green mould a combinations of azoxystrobin and fludioxonil (Graduate A⁺; Syngenta), propiconazole and cyproconazole (Artea; Syngenta), azoxystrobin and

cyproconazole (Amistar Extra; Syngenta) and propiconazole alone (Tilt; Syngenta) showed promise and will be investigated further. An inoculation protocol for sour rot has been developed to ensure more reliable results for future trials. A small trial was done to test the dip and total loss spray application of chlorine. The specific dip application inoculated fruit with sour rot whereas the total loss spray application was effective. The development of a protocol for assessment of fungicide resistance in *Penicillium* populations in packhouses has been initiated and is still in process. A ring test has been conducted to determine the variability and accuracy between different South African analytical laboratories. Variability is quite evident and further work will be done to reduce the variability and improve accuracy between laboratories. In future low-priority pilot trials will be diverted to QMS as far as possible to free up more time for work of higher priority.

Opsomming

Verskeie loods proewe is uitgevoer in hierdie projek. Produkte wat belofte gewys het as saniteermiddels was waterstofperoksied + parasitiese suur (Citrocide en Tsunami), SOPP (SOPP 20) en elektroliet water (Neuthox en Ecolyte). As swamdoders teen groenskimmel het die volgende kombinasies belofte getoon: azoxystrobin en fludioxonil (Graduate A +; Syngenta), propiconazole en cyproconazole (Artea; Syngenta), azoxystrobin en cyproconazole (Amistar Ekstra; Syngenta) en propiconazole alleen (Tilt; Syngenta). Hierdie produkte sal verder ondersoek word. 'n Inokuleringsprotokol is vir suurvrot ontwikkel om meer betroubare resultate vir toekomstige proewe te verseker. 'n Klein proef is gedoen om die dip en die totale verlies toediening van chloor te toets. Die spesifieke dip aanwending het vrugte met suurvrot besmet, terwyl die totale verlies bespuiting doeltreffend was. 'n Protokol vir die assessering van swamdoder weerstandbiedendheid in die *Penicillium* populasies in pakhuse word ontwikkel. 'n Vergelykende toets is uitgevoer om die variasie en akkuraatheid tussen die verskillende Suid-Afrikaanse analitiese laboratoriums te bepaal. Variasie was baie duidelik en verdere werk sal gedoen word om die wisselvalligheid en akkuraatheid tussen die verskillende laboratoriums te verbeter. In die toekoms sal nie-prioriteit loodsproewe so ver as moontlik na QMS verwys word met die doel om meer tyd beskikbaar te maak vir werk van 'n hoër prioriteit.

Pilot trials conducted

Nineteen different pilot trials were conducted during the season of 2012 (Table 4.5.2.1). Five were to test various product for fungicidal activity against green mould (one included sour rot) and fourteen to test products as sanitising agents against green mould (one included sour rot). Reports on these products are attached as addendums as shown in Table 4.5.2.1.

Table 4.5.2.1. Twenty different products tested for either fungicidal properties or as sanitising agents against citrus green mould (sour was included for one product).

Product	Company	Test	Disease	Addendum no.
SOPP 20	Advantage Agri Products	Fungicide	green mould	1
Biopepcide	ICA International Chemicals	Fungicide	green mould	2
Imaculent	ICA International Chemicals	Fungicide	sour rot	no report
Variety of products	Syngenta	Fungicide	green mould	3
Two products	Villa Crop Protection	Fungicide	green mould	4
C/A and F/S	Advantage Agri Products	Sanitatiser	green mould	5
SOPP 20 and Tsunami	Advantage Agri Products	Sanitatiser	green mould	6
767 Sept.	Advantage Agri Products	Sanitatiser	green mould	7
Oxy300	Aqueza	Sanitatiser	green mould	8
Citrocide	Citrosol	Sanitatiser	green mould	9
Essasol	Citrosol	Sanitatiser	green mould	10
Dutrition	Greenland Technologies SA	Sanitatiser	green mould	11
Biopepcide	ICA International Chemicals	Sanitatiser	green mould	12
Biopepcide	ICA International Chemicals	Sanitatiser	sour rot	13
Disease Shield	Kannar	Sanitatiser	green mould	14

Exolyte	McClean	Sanitiser	green mould	15
Neuthox	Procep chemicals	Sanitiser	green mould	16
Fv1	ViBacSan	Sanitiser	green mould	17
RBT	Pro Tech Services	Sanitiser	green mould	18
HAWA-SAN TR50	Nulandis	Sanitiser	green mould	19

Latent pathogens

An attempt was made to test different actives against the latent pathogens, but the trial was not successful due to the controls having no latent pathogen infection. This will be repeated in the 2013 season.

Sour rot

The protocol for sour rot inoculation was revised. A previous trial on this disease did not give satisfactory results in terms of disease development on untreated fruit. A few trials have been conducted to determine the optimum inoculation concentration and wound inducing protocol. Better results are expected for the 2013 season.

Phytophthora brown rot

No work has been done on this disease due to time constraints. With the reduction of pilot trials more time will be available to work on other diseases than green mould.

Fungicide resistance management

Swabs were received from a packhouse for assessment of imazalil resistance. Different media (enriched and none-enriched) were tested. Swabs were vortexed in sterile water to extract the spores and conidia. These suspensions were then diluted and plated out on various amended and unamended media. It was found that the assessment of resistance is not a simple process and development is ongoing. Findings so far are that bacterial and yeast contaminants influenced the growth of fungal cultures. It was also found that the colony count would differ from one replicate plate to another, which will hinder the quantification process. A portion of results can be seen in Addendum 20. This work was done as an exercise to develop a protocol for the CRI DC as a future service for commercial citrus packhouses in South Africa.

Packhouse sanitation assessment

Two different types of chlorine applicators were tested. A chlorine dip tank at Packhouse A and a total loss spraying system at Packhouse B were tested for its ability to inhibit green mould infection. Although it was a small trial and only conducted once it gives an indication of the efficacy and risks of the two types of applications. The two reports can be seen in Addendum 21 and 22. The chlorine dip tank in this case was not managed at an optimum and became a source of inoculum and new infections. The total loss spray system showed potential and was able in this case to eliminate inoculum and prevent new infections. More work will be done on this topic with the aim to confirm current recommendations or formulate new recommendations.

Residue analyses: Analytic laboratory ring test

A ring test was conducted to assess the accuracy between six different laboratories rendering a service to the South African citrus export industry. Substantial variation was found between different laboratories. This test will be repeated in the 2013 season and communication is ongoing with the different laboratories. The aim of this work is to assist the laboratories in improving accuracy and ensure that the citrus industry have more confidence in the results from analytical laboratories. The report to the laboratories can be seen in Addendum 23.

Technology transfer

Information on grower talks and presentations at conferences where data from CRI-funded research were presented.

Objective / Milestone	Achievement
1. New potential products will be tested as sanitation agents and/or fungicides	1. Seventeen products have been tested of which five showed potential (SOPP 20, Citrocide, Tsunami, Ecolyte and Neuthox) 2. Twelve products have been tested on green mould of which five showed potential (Tilt, Graduate A+, Amistar Xtra, Artea and SOPP20)
2. Potential products for the control of brown	1.The inoculation protocol for sour rot is under development

and sour rot will be actively pursued during the season with the aim to facilitate the registration of effective products against these two diseases	2. No trials were done on phytophthora brown rot
3. Ongoing focus will be to find an alternative for 2,4-D and alternatives for the control of green mould	1. No alternatives for 2,4-D have been presented during this season 2. Alternatives for green mould control was dealt with in Objective 1.2
4. Further work will also be done on the implementation of the use of GRAS chemicals in citrus packhouses	1. No trials were done during 2012

Further objectives (milestones) and work plan

1. New potential products will be tested as sanitation agents and/or fungicides, the bulk of the work will be done by QMS and CRI postharvest plant pathology will have limited involvement. CRI will be involved in setting up protocols and interpreting the data and findings
2. Seek and test alternative actives for the control of sour rot and phytophthora brown rot
3. Introduce and implement the application of GRAS chemicals into the citrus postharvest industry
4. Analytical lab focus – ring test with the aim to reduce variability
5. Develop and implement a DC protocol for assessing fungicide resistance in citrus packhouses

Quarterly milestones for Jan-Mar, Apr-Jun, Jul-Sep, Oct-Dec 2012 and Jan-Mar 2013

Apr-June 2014

- Objective 1: Connect with QMS and facilitate protocol development and client interaction
- Objective 2: Conduct trials investigating new actives against sour rot and phytophthora brown rot
- Objective 3: Do a trial run with sodium bicarbonate and potassium sorbate at 2 different packhouses
- Objective 4: Conduct a ring test
- Objective 5: Facilitate the implementation of the protocol into the DC system

Jul-Sep 2014

- Objective 1: Connect with QMS and facilitate protocol development and client interaction
- Objective 2: Conduct trials investigating new actives against sour rot and phytophthora brown rot. Analyse data.
- Objective 3: Do a trial run with sodium bicarbonate and potassium sorbate at 2 different packhouses. Analyse data
- Objective 4: Conduct a ring test. After first ring test and consultation with all role players conduct another ring test if deemed necessary.
- Objective 5: Facilitate the implementation of the protocol into the DC system. Analyse data and assist with reporting pack to packhouses

Oct-Dec 2014

- Objective 1: Connect with QMS and facilitate protocol development and client interaction. Assist with reporting back to clients
- Objective 2: Analyse data. Start writing report and plan future work on products that showed potential
- Objective 3: Analyse data. Start writing report. Plan CRI packhouse workshop presentation. Plan article for SAFJ
- Objective 4: Plan small conference with all role players, where data will be discussed, if necessary
- Objective 5: Analyse data and assist with reporting pack to packhouses. Assess the season and implement changes if necessary

Jan-Mar 2015

- Objective 1: Connect with QMS and facilitate protocol development and client interaction.
- Objective 2: Finish report and plan future work on products that showed potential
- Objective 3: Finish report. Prepare and present work on CRI packhouse workshops. Publish article in SAFJ
- Objective 4: Have small conference with all role players if necessary
- Objective 5: Plan for new season and finish report

Addendum 1

The *in vivo* screening of the fungicidal ability of SOPP Super 20 to control citrus green mould

Introduction

SOPP Super 20 (SS20) was evaluated for fungicidal ability. The products were compared to the fungicide imazalil. The curative and protective ability to control *Penicillium digitatum* (green mould) were assessed.

Materials and methods

Fruit

Untreated navel oranges (Joubert and sons, Nelspruit) 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. Thereafter the fruit was dried in the packline drying tunnel prior to inoculation.

Inoculation

Fruit was inoculated by dipping a wound inducer in a spore suspension (1×10^6 spores.mL⁻¹) prior to wounding the fruit. Each fruit was inoculated at 4 sites around the stem end.

Incubation

After inoculation and/or treatment fruit were placed in fruit cartons covered with polyethylene bags and incubated at 20°C.

Treatment

Fruit were treated curatively (24h after inoculation) and protectively (inoculated 4 h after treatment). The various treatments and concentrations can be seen in Table 1. Fruit were dipped for 3 min in a specific solution.

Evaluation

After incubation (7 days) the fruit was evaluated for decay and the results were recorded as percentage decay (number of infected wounds).

Table 1. Treatments and concentrations applied to citrus fruit inoculated either *P. digitatum*.

Treatment	Active ingredient	Concentration
Untreated control		
Treated control		Water (5 L)
SOPP Super 20	SOPP	1% (250 mL.5 L ⁻¹)
SOPP Super 20	SOPP	2% (500 mL.5 L ⁻¹)
SOPP Super 20	SOPP	4% (1000 mL.5 L ⁻¹)
Fungazil	imazalil	500 µg.mL ⁻¹

Results

SOPP Super 20 applied at 1, 2 and 4% was able to curatively control infections from *P. digitatum*, and was comparable to imazalil. The highest concentrations (4%) resulted to good protective control, but not comparable to imazalil. In this trial fruit was not rinsed after treatment and signs of phytotoxicity was observed. Results are shown in Table 2.

Table 2. Green mould infection (%) on navel oranges treated with SOPP or imazalil.

Treatment	Active ingredient/s	Concentration (µg.mL ⁻¹)	Action	Infection (%)
Untreated control			Curative	100.0
Treated control	water		Curative	100.0
SOPP Super 20	SOPP	1% (250 mL.5 L ⁻¹)	Curative	0.0
SOPP Super 20	SOPP	2% (500 mL.5 L ⁻¹)	Curative	0.0
SOPP Super 20	SOPP	4% (1000 mL.5 L ⁻¹)	Curative	0.0
Fungazil	imazalil	500 µg.mL ⁻¹	Curative	2.1

Untreated control			Protective	100.0
Treated control	water		Protective	100.0
SOPP Super 20	SOPP	1% (250 mL.5 L ⁻¹)	Protective	100.0
SOPP Super 20	SOPP	2% (500 mL.5 L ⁻¹)	Protective	83.3
SOPP Super 20	SOPP	4% (1000 mL.5 L ⁻¹)	Protective	8.3
Fungazil	imazalil	500 µg.mL ⁻¹	Protective	0.0

* Treatment was not conducted

Conclusion and recommendations

SOPP Super 20 shows potential in terms of the curative control of green mould. To avoid phototoxic damage fruit needs to be rinsed off after treatment and the effect of this exercise on curative ability is not known to this researcher. For use in citrus fruit, it needs to be registered under Act 36, which includes efficacy, suitability (phytotoxicity and compatibility with other fungicides) and food safety (chemical residues). Without this registration the product cannot be recommended by CRI.

Final comments

This was only a pilot trial. None of these results can be used in marketing or as CRI recommendation in any citrus chemical program.

Addendum 2

The *in vivo* screening of the fungicidal ability of the products Biopeptide G and Biopeptide T to control citrus green mould

Introduction

Biopeptide G and Biopeptide T, products from ICA International Chemicals, were evaluated for fungicidal ability. The products were tested against the fungicide imazalil. The curative and protective ability to control *Penicillium digitatum* (green mould) were assessed.

Materials and methods

Fruit

Untreated navel oranges (Joubert and sons, Nelspruit) 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. Thereafter the fruit was dried in the packline drying tunnel prior to inoculation.

Inoculation

Fruit was inoculated by dipping a wound inducer in a spore suspension (1×10^6 spores.mL⁻¹) prior to wounding the fruit. Each fruit was inoculated at 4 sites around the stem end.

Incubation

After inoculation and/or treatment fruit were placed in fruit cartons covered with polyethylene bags and incubated at 20°C.

Treatment

Fruit were treated curatively (24h after inoculation) and protectively (inoculated 4 h after treatment). The various treatments and concentrations can be seen in Table 1

Evaluation

After incubation (7 days) the fruit was evaluated for decay and the results were recorded as percentage decay (number of infected wounds).

Table1. Treatments and concentrations applied to citrus fruit inoculated either *P. digitatum*.

Treatment	Concentration
Untreated control	
Untreated inoculated control	Water (5 L)
BG	0.5X (2.85 mL.5 L ⁻¹)
BG	1X (5.70 mL.5 L ⁻¹)
BG	2X (11.4 mL.5 L ⁻¹)
BT	0.5X (2.025 mL.5 L ⁻¹)

BT	1X (4.050 mL.5 L ⁻¹)
BT	2X (8.100 mL.5 L ⁻¹)
Imazalil	500 µg.mL ⁻¹

Results

Biopeptide G had no effect against *P. digitatum*. Biopeptide T had a moderate effect in the highest concentration, but was not comparable to imazalil. Results are shown in Table 2.

Table 2. Green mould infection (%) on navel oranges treated with BG, BT or imazalil.

Treatment	Concentration	Action	Infection (%) ^a	
Untreated control		Curative	100.0	a
Untreated inoculated control	Water (5 L)	Curative	100.0	a
BG	0.5X (2.85 mL.5 L ⁻¹)	Curative	100.0	a
BG	1X (5.70 mL.5 L ⁻¹)	Curative	100.0	a
BG	2X (11.4 mL.5 L ⁻¹)	Curative	100.0	a
BT	0.5X (2.025 mL.5 L ⁻¹)	Curative	100.0	a
BT	1X (4.050 mL.5 L ⁻¹)	Curative	100.0	a
BT	2X (8.100 mL.5 L ⁻¹)	Curative	31.3	b
Imazalil	500 µg.mL ⁻¹	Curative	2.1	c
Untreated control		Protective	100.0	a
Untreated inoculated control	Water (5 L)	Protective	100.0	a
BG	0.5X (2.85 mL.5 L ⁻¹)	Protective	100.0	a
BG	1X (5.70 mL.5 L ⁻¹)	Protective	100.0	a
BG	2X (11.4 mL.5 L ⁻¹)	Protective	n/a	
BT	0.5X (2.025 mL.5 L ⁻¹)	Protective	100.0	a
BT	1X (4.050 mL.5 L ⁻¹)	Protective	100.0	a
BT	2X (8.100 mL.5 L ⁻¹)	Protective	100.0	a
Imazalil	500 µg.mL ⁻¹	Protective	0.0	c

^aMeans followed by the same letter do not differ significantly ($P < 0.0001$)

* Treatment was not conducted

Conclusion and recommendations

Biopeptide T shows potential and should be investigated further. Applying higher concentrations should be considered. For use in citrus fruit, it needs to be registered under Act 36, which includes efficacy, suitability (phytotoxicity and compatibility with other fungicides) and food safety (chemical residues). Without this registration the product cannot be recommended by CRI.

Recommendations

- Trials will need to be commenced on phytotoxicity on at least 3 different citrus kinds
- Trials will need to be commenced on the compatibility of BT with other postharvest fungicides
- Biopeptide T needs to be registered under Act 36

Final comments

This was only a pilot trial. None of these results can be used in marketing or as CRI recommendation in any citrus chemical program.

Addendum 3

The *in vivo* screening of the fungicidal ability of various products from Syngenta to control citrus green mould

Introduction

Amistar Xtra, Artea, Bravo, Graduate A+, Scholar, Switch and Tilt were evaluated for fungicidal ability. The products were compared to the fungicide imazalil. The curative and protective ability to control *Penicillium digitatum* (green mould) were assessed.

Materials and methods

Fruit

Untreated navel oranges (Joubert and sons, Nelspruit) 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. Thereafter the fruit was dried in the packline drying tunnel prior to inoculation.

Inoculation

Fruit was inoculated by dipping a wound inducer in a spore suspension (1×10^6 spores.mL⁻¹) prior to wounding the fruit. Each fruit was inoculated at 4 sites around the stem end.

Incubation

After inoculation and/or treatment fruit were placed in fruit cartons covered with polyethylene bags and incubated at 20°C.

Treatment

Fruit were treated curatively (24h after inoculation) and protectively (inoculated 4 h after treatment). The various treatments and concentrations can be seen in Table 1. Fruit were dipped for 3 min in a specific solution.

Evaluation

After incubation (7 days) the fruit was evaluated for decay and the results were recorded as percentage decay (number of infected wounds).

Table 1. Treatments and concentrations applied to citrus fruit inoculated either *P. digitatum*.

Treatment	Active ingredient	Concentration
Untreated control		
Treated control		Water (5 L)
Amistar Xtra	azoxystrobin / cyproconazole	300 µg.mL ⁻¹ (Azo; 7.5 mL.5 L ⁻¹)
Artea	propiconazole / cyproconazole	500 µg.mL ⁻¹ (Pro; 10.0 mL.5 L ⁻¹)
Bravo	chlorothalonil	300 µg.mL ⁻¹ (Chl; 2.1 mL.5 L ⁻¹)
Graduate A+	fludioxonil / azoxystrobin	500 µg.mL ⁻¹ (Flu; 10.5 mL.5 L ⁻¹)
Scholar	fludioxonil	500 µg.mL ⁻¹ (Flu; 10.9 mL.5 L ⁻¹)
Switch	cyprodinil / fludioxonil	300 µg.mL ⁻¹ (Flu; 6.0 mL.5 L ⁻¹)
Tilt	propiconazole	500 µg.mL ⁻¹ (Pro; 10.0 mL.5 L ⁻¹)
Fungazil	imazalil	500 µg.mL ⁻¹

Results

Artea (propiconazole) had a moderate curative effect against *P. digitatum*, but was not comparable to imazalil. None of the other products could curatively control *P. digitatum*. Amistar Xtra (azoxystrobin / cyproconazole), Graduate A+ (fludioxonil / azoxystrobin) and Tilt (propiconazole) had a moderate protective effect, but was not comparable to imazalil. None of the other products could protect the fruit from *P. digitatum* infections. Results are shown in Table 2.

Table 2. Green mould infection (%) on navel oranges treated with BG, BT or imazalil.

Treatment	Active ingredient/s	Concentration (µg.mL ⁻¹)	Action	Infection (%)
Untreated control			Curative	100.0
Treated control	water		Curative	100.0
Amistar Xtra	azoxystrobin / cyproconazole	300	Curative	89.6
Artea	propiconazole / cyproconazole	500	Curative	31.3
Bravo	chlorothalonil	300	Curative	100.0
Graduate A+	fludioxonil / azoxystrobin	500	Curative	83.3
Scholar	fludioxonil	500	Curative	97.9
Switch	cyprodinil / fludioxonil	300	Curative	100.0
Tilt	propiconazole	500	Curative	79.2
Fungazil	imazalil	500	Curative	2.1

Untreated control			Protective	100.0
Treated control	water		Protective	100.0
Amistar Xtra	azoxystrobin / cyproconazole	300	Protective	35.4
Artea	propiconazole / cyproconazole	500	Protective	*
Bravo	chlorothalonil	300	Protective	100.0
Graduate A+	fludioxonil / azoxystrobin	500	Protective	25.0
Scholar	fludioxonil	500	Protective	97.9
Switch	cyprodinil / fludioxonil	300	Protective	100.0
Tilt	propiconazole	500	Protective	39.6
Fungazil	imazalil	500	Protective	0.0

* Treatment was not conducted

Conclusion and recommendations

Artea showed potential in terms of curative control. Amistar Xtra, Graduate A+ and Tilt show potential in terms of protective control. These products can be investigated further. However, none of these products applied in the specific concentrations were comparable to imazalil. They could be useful as part of a resistance management program and in combination with imazalil. The effect of concentration, temperature, exposure time and solution pH should be investigated further.

Final comments

This was only a pilot trial. None of these results can be used in marketing or as CRI recommendation in any citrus chemical program.

Addendum 4

The *in vivo* screening for the fungicidal action of two products from Villa to control citrus green mould

Introduction

Two formulations, fludioxonil/azoxystrobin and pyrimethanil/azoxystrobin, were evaluated for the control of green mould (*Penicillium digitatum*) on citrus. The products were compared to the fungicide imazalil. The curative and protective abilities were assessed.

Materials and methods

Fruit

Untreated Valencia oranges (Karino, Nelspruit) 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 through a recirculating spray-on brush washing system with chlorine. Thereafter the fruit was dried in the packline drying tunnel prior to inoculation.

Inoculation

Fruit was inoculated by dipping a wound inducer in a spore suspension (1×10^6 spores.mL⁻¹) prior to wounding the fruit. Each fruit was inoculated at 4 sites around the stem end.

Incubation

After inoculation and/or treatment fruit were placed in fruit cartons covered with polyethylene bags and incubated at 20°C.

Treatment

Fruit were treated curatively (24h after inoculation) and protectively (inoculated 4 h after treatment). The various treatments and concentrations can be seen in Table 1

Evaluation

After incubation (7 days) the fruit was evaluated for decay and the results were recorded as percentage decay (number of infected wounds).

Results

The fludioxonil/azoxystrobin (F/A) combination had no curative effect on 24 h old infections, protectively the higher concentration showed promise. The pyrimethanil/azoxystrobin (P/A) combination gave poor curative control on the 24 h infections and similarly to F/A the higher concentration showed promise protectively. Results are shown in Table 1.

Table 1. Green mould infection (%) on Valencia oranges treated with two Villa products or imazalil.

Treatment	Concentration	Action	Infection (%)	
Untreated control		Curative	100.0	a
Fludioxonil/Azoxystrobin	500 µg.mL ⁻¹	Curative	100.0	a
Fludioxonil/Azoxystrobin	1000 µg.mL ⁻¹	Curative	95.8	a
Pyrimethanil/Azoxystrobin	500 µg.mL ⁻¹	Curative	50.7	b
Pyrimethanil/Azoxystrobin	1000 µg.mL ⁻¹	Curative	50.0	b
Imazalil	500 µg.mL ⁻¹	Curative	5.6	e
Untreated control		Protective	100.0	a
Fludioxonil/Azoxystrobin	500 µg.mL ⁻¹	Protective	61.1	b
Fludioxonil/Azoxystrobin	1000 µg.mL ⁻¹	Protective	26.4	cd
Pyrimethanil/Azoxystrobin	500 µg.mL ⁻¹	Protective	56.9	b
Pyrimethanil/Azoxystrobin	1000 µg.mL ⁻¹	Protective	29.2	c
Imazalil	500 µg.mL ⁻¹	Protective	13.9	de

* Means followed by the same letter do not differ significantly ($P = 0.05$)

Conclusion and recommendations

Both F/A and P/A showed promise as protective treatments at 1000 µg.mL⁻¹. The 24 h old infection for the curative treatments might be too old for these products and shorter incubation periods might be considered in future trials. The use of azoxystrobin as a postharvest treatment should be contemplated well, due to the fact that this active is part of the pre-harvest spray program against black spot. The risk of resistance development in *P. digitatum* orchard populations will be very high.

Recommendations

- Trials will need to be commenced on phytotoxicity on at least 3 different citrus kinds
- Trials will need to be commenced on the compatibility of F/A and P/A with other postharvest fungicides
- F/A and P/A need to be registered under Act 36

Final comments

This was only a pilot trial. None of these results can be used in marketing or as CRI recommendation in any citrus chemical program.

Addendum 5

The evaluation of C/A and F/S for potential use in a citrus packhouse dump tank washing system as sanitising agent against green mould

Product information

Product names: C/A and F/S

Active ingredients: Plant extracts bioflavonoid

Company: Advantage Agri Products

Trial date: 6 November 2012

Materials and methods

Test: Sanitation

Method:

- 1×10^4 spores.mL⁻¹ in 5L solution (*Penicillium digitatum* causal agent of green mould)
- Control treatment: Wounded citrus fruit dipped in spore suspension
- Product treatment: Wounded citrus fruit dipped in spore suspension 3 min after product has been added
- Incubation: 4 days at 20°C
- Evaluation: Infected fruit rated and converted to percentage infection (12 fruit per treatment)
- Products and treatment concentrations can be seen in Table 1

Results

Table 1. The percentage infection on Valencia oranges inoculated with *P. digitatum* during treatment in a solution with C/A, F/S or Sporekill.

Treatment	Concentration	Infection (%)
C/A	0.25%	100.0%
C/A	0.5%	83.3%
C/A	1%	100.0%
F/S	0.25%	100.0%
F/S	0.5%	not done
F/S	1%	83.3%
Sporekill	120 µg.mL ⁻¹	8.3%

Conclusion and recommendations

In comparison to Sporekill this product in the applied concentrations showed no potential for use as a possible sanitising agent against green mould.

Recommendations

- No further work to be done on C/A or F/S

Important comments

This was only a pilot trial. None of these results can be used in marketing or as CRI recommendation in any citrus chemical program.

Addendum 6

The evaluation of Tsunami and SOPP Super 20 in a citrus packhouse dump tank washing system as potential sanitising agents against *Penicillium digitatum*

Introduction

Tsunami (active ingredient: Peracetic acid 5% w/w + hydrogen peroxide 25% w/w) and SOPP Super 20, products from Advantage Agri Products, were evaluated in a simulated dump tank washing system for efficacy of sanitising the system and preventing possible infection of injured fruit moving through the system. The product was compared with Sporekill (DDAC). The simulated dump tank was seeded with a suspension of the post-harvest pathogen *Penicillium digitatum* (green mould) in this evaluation.

Materials and methods

The spore suspension of *P. digitatum* was made up by suspending spores in sterile deionised water containing the surfactant, Tween 20. The spore suspension was spectrophotometrically adjusted to a concentration of 1×10^6 spores.mL⁻¹. Good, sound, untreated Navel oranges from Joubert and sons (Nelspruit) 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 QAC. Thereafter the fruit was dried in the packline drying tunnel prior to treatment. The fruit was divided into lots of 6 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. For each treatment three replicates of six clean, surface sterilised navel oranges were injured 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 36 injury sites per treatment. The layout of treatments and concentrations can be seen in Table 1. Fruit were injured and dipped in water (untreated uninoculated control), in a 5 L spore suspension (1×10^4 spores.mL⁻¹; untreated inoculated control) or in a 5 L spore suspension amended with Tsunami, SOPP Super 20 or Sporekill.

Table 1. The various treatments and concentrations used in this trial.

Treatment	Concentration	Spores
Untreated uninoculated control	Water (5 L)	None
Untreated inoculated control	Water (5 L)	1×10^4 spores.mL ⁻¹
Tsunami	0.5X	1×10^4 spores.mL ⁻¹
Tsunami	1.0X	1×10^4 spores.mL ⁻¹

Tsunami	2.0X	1×10^4 spores.mL ⁻¹
SOPP Super 20	1%	1×10^4 spores.mL ⁻¹
SOPP Super 20	2%	1×10^4 spores.mL ⁻¹
SOPP Super 20	4%	1×10^4 spores.mL ⁻¹
Sporekill	1mL.L ⁻¹	1×10^4 spores.mL ⁻¹
Sporekill	2mL.L ⁻¹	1×10^4 spores.mL ⁻¹

All the treated fruit were placed in fruit cartons covered with polyethylene bags and incubated for 7 days at 20°C. After incubation the fruit was evaluated for decay and the results were recorded as percentage decay.

Results

Tsunami applied in concentrations 1X and 2X were comparable to Sporekill as a sanitation agent for use in aqueous solutions infested with spores from *P. digitatum*. Similarly SOPP Super 20 applied in concentrations of 2% and 4% were comparable to Sporekill. Results are shown in Table 2.

Table 2. The percentage infection on navel oranges inoculated with *P. digitatum* during treatment in a solution with Tsunami, SOPP Super 20 or Sporekill.

Treatment	Concentration	Infection (%)
<i>Tsunami trial</i>		
Untreated uninoculated control		0.0
Untreated inoculated control		100.0
Tsunami	0.5X	50.0
Tsunami	1.0X	0.0
Tsunami	2.0X	0.0
Untreated uninoculated control		0.0
Untreated inoculated control		88.9
Sporekill	1mL.L ⁻¹	0.0
Sporekill	2mL.L ⁻¹	0.0
<i>SOPP Super 20 trial</i>		
Untreated uninoculated control		0.0
Untreated inoculated control		53.7
SOPP Super 20	1%	7.4
SOPP Super 20	2%	0.0
SOPP Super 20	4%	0.0
Untreated uninoculated control		1.9
Untreated inoculated control		96.3
Sporekill	1mL.L ⁻¹	0.0
Sporekill	2mL.L ⁻¹	0.0

Conclusion and recommendations

The products (Tsunami and SOPP Super 20) show potential. For use in citrus fruit, it needs to be registered under Act 36, which includes efficacy, suitability (phytotoxicity and compatibility with other fungicides) and food safety (chemical residues). Without this registration the product cannot be recommended by CRI.

Recommendations

- Trials will need to be commenced on phytotoxicity on at least 3 different citrus kinds
- Trials will need to be commenced on the compatibility of Tsunami and SOPP Super 20 with other postharvest fungicides
- Tsunami and SOPP Super 20 need to be registered under Act 36

Final comments

This was only a pilot trial. None of these results can be used in marketing or as CRI recommendation in any citrus chemical program.

Addendum 7

The evaluation of 767 Sept for potential use in a citrus packhouse dump tank washing system as sanitising agent against green mould

Product information

Product name: 767 Sept
Company: Agri Challenge

Active ingredient: Benzalkonium Chloride (BAC)

Materials and methods

Test: Sanitation

Method:

- 1×10^4 spores.mL⁻¹ in 5L solution (*Penicillium digitatum* causal agent of green mould)
- Control treatment: Wounded citrus fruit dipped in spore suspension
- Product treatment: Wounded citrus fruit dipped in spore suspension 3 min after product has been added
- Incubation: 4 days at 20°C
- Evaluation: Infected wounds rated and converted to percentage infection
- Products and treatment concentrations can be seen in Table 1

Results

Table 1. The percentage infection on Valencia oranges inoculated with *P. digitatum* during treatment in a solution with 767 Sept, chlorine or Sporekill.

Treatment	Concentration	Infection (%)
767 Sept inoculated control	0%	72.9
767 Sept	0.5%	37.5
767 Sept inoculated control	0%	70.8
767 Sept	1%	52.1
767 Sept inoculated control	0%	81.3
767 Sept	2%	31.3
Chlorine inoculated control	0 µg.mL ⁻¹	95.8
Chlorine	100 µg.mL ⁻¹	6.3
Sporekill inoculated control	0 µg.mL ⁻¹	89.6
Sporekill	120 µg.mL ⁻¹	2.1

Conclusion and recommendations

In comparison to chlorine or Sporekill this product in the applied concentrations showed poor potential for use as a possible sanitising agent against green mould.

Recommendations

- Higher concentration should be tested
 - Financial viability will need to be assessed first
- If higher concentrations show potential
 - Trials will need to be commenced on phytotoxicity on at least 3 different citrus kinds
 - Trials will need to be commenced on the compatibility of 767 Sept with other postharvest fungicides
 - 767 Sept needs to be registered under Act 36

Due to the fact that 767 Sept contains the quaternary ammonium compound BAC, no further work will be done by CRI until such time that an export MRL for BAC is established on the export markets.

Important comments

This was only a pilot trial. None of these results can be used in marketing or as CRI recommendation in any citrus chemical program.

Addendum 8

The evaluation of Oxy 300 for potential use in a citrus packhouse dump tank washing system as sanitising agent against green mould

Product information

Product name: Oxy 300

Company: Aquaza

Active ingredient: Hydrogen peroxide

Trial date: 17 September 2012

Materials and methods

Test: Sanitation

Method:

- 1×10^4 spores.mL⁻¹ in 5L solution (*Penicillium digitatum* causal agent of green mould)
- Control treatment: Wounded citrus fruit dipped in spore suspension
- Product treatment: Wounded citrus fruit dipped in spore suspension 3 min after product has been added
- Incubation: 4 days at 20°C
- Evaluation: Infected wounds rated and converted to percentage infection
- Products and treatment concentrations can be seen in Table 1

Results

Table 1. The percentage infection on Valencia oranges inoculated with *P. digitatum* during treatment in a solution with Oxy 300, chlorine or Sporekill.

Treatment	Concentration	Infection (%)
Oxy 300 inoculated control	0 µg.mL ⁻¹	100.0
Oxy 300	1250 µg.mL ⁻¹	100.0
Oxy 300 inoculated control	0 µg.mL ⁻¹	75.0
Oxy 300	2500 µg.mL ⁻¹	68.8
Oxy 300 inoculated control	0 µg.mL ⁻¹	100.0
Oxy 300	5000 µg.mL ⁻¹	97.9
Chlorine inoculated control	0 µg.mL ⁻¹	95.8
Chlorine	100 µg.mL ⁻¹	6.3
Sporekill inoculated control	0 µg.mL ⁻¹	89.6
Sporekill	120 µg.mL ⁻¹	2.1

Conclusion and recommendations

In comparison to chlorine or Sporekill this product in the applied concentrations showed no potential for use as a possible sanitising agent against green mould.

Recommendations

- Higher concentration should be tested
 - Financial viability will need to be assessed first
- If higher concentrations show potential
 - Trials will need to be commenced on phytotoxicity on at least 3 different citrus kinds
 - Trials will need to be commenced on the compatibility of Oxy 300 with other postharvest fungicides
 - The specific act (5 or 36) under which Oxy 300 must be registered should be determined

It was stated that Oxy 300 is an encapsulated form of hydrogen peroxide; this may affect the potency of peroxide to kill green mould spores and may render this formulation unsuitable for use in citrus packhouses

Important comments

This was only a pilot trial. None of these results can be used in marketing or as CRI recommendation in any citrus chemical program.

Addendum 9

The evaluation of Citroside in a citrus packhouse dump tank washing system as potential sanitising agent against *Penicillium digitatum*

Introduction

Citroside (active ingredient: Peracetic acid 5% w/w + hydrogen peroxide 25% w/w), a product from Citrosol, 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 (DDAC). The simulated dump tank was seeded with a suspension of the post-harvest pathogen *Penicillium digitatum* (green mould) in this evaluation.

Materials and methods

The spore suspension of *P. digitatum* was made up by suspending spores in sterile deionised water containing the surfactant, Tween 20. The spore suspension was spectrophotometrically adjusted to a concentration of 1×10^6 spores.mL⁻¹. Good, sound, untreated Navel oranges from Joubert and sons (Nelspruit) 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 QAC. Thereafter the fruit was dried in the packline drying tunnel prior to treatment. The fruit was divided into lots of 6 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. For each treatment two replicates of six clean, surface sterilised navel oranges were injured 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 24 injury sites per treatment. The layout of treatments and concentrations can be seen in Table 1. Fruit were injured and dipped in water (untreated uninoculated control), in a 5 L spore suspension (adjusted to 1×10^4 spores.mL⁻¹; untreated inoculated control) or in a 5 L spore suspension amended with either Citrocide (0.25, 0.50 and 1.00%) or Sporekill.

Table 1. The various treatments and concentrations used in this trial.

Treatment	Concentration	Spores
Untreated uninoculated control	Water (5 L)	None
Untreated inoculated control	Water (5 L)	1×10^4 spores.mL ⁻¹
Citrocide	0.25%	1×10^4 spores.mL ⁻¹
Citrocide	0.50%	1×10^4 spores.mL ⁻¹
Citrocide	1.00%	1×10^4 spores.mL ⁻¹
Sporekill	120µg.mL ⁻¹	1×10^4 spores.mL ⁻¹

All the treated fruit were placed in fruit cartons covered with polyethylene bags and incubated for 7 days at 20°C. After incubation the fruit was evaluated for decay and the results were recorded as percentage decay.

Results

Citrocide in the applied concentrations was comparable to Sporekill as a sanitation agent for use in aqueous solutions infested with spores from *P. digitatum*. Results are shown in Table 2.

Table 2. The percentage infection on navel oranges inoculated with *P. digitatum* during treatment in a solution with either Citrocide or Sporekill.

Treatment	Concentration	Infection (%) ^a
Untreated uninoculated control	Water (5 L)	0.0 a
Untreated inoculated control	Water (5 L)	100.0 c
Citrocide	0.25%	0.0 a
Citrocide	0.50%	10.4 b
Citrocide	1.00%	0.0 a
Sporekill (1 mL.L ⁻¹)	120µg.mL ⁻¹	0.0 a

^aMeans followed by the same letter do not differ significantly ($P < 0.0001$)

Conclusion and recommendations

The product (Citrocide) shows potential. For use in citrus fruit, it needs to be registered under Act 36, which includes efficacy, suitability (phytotoxicity and compatibility with other fungicides) and food safety (chemical residues). Without this registration the product cannot be recommended by CRI.

Recommendations

- Trials will need to be commenced on phytotoxicity on at least 3 different citrus kinds
- Trials will need to be commenced on the compatibility of Citrocide with other postharvest fungicides
- Citrocide needs to be registered under Act 36

Final comments

This was only a pilot trial. None of these results can be used in marketing or as CRI recommendation in any citrus chemical program.

Addendum 10

The evaluation of Essasol for potential use in a citrus packhouse dump tank washing system as sanitising agent against green mould

Product information

Product name: Essasol

Active ingredient: Biodegradable detergent

Company: Wenkem

Materials and methods

Test: Sanitation

Method:

- 1×10^4 spores.mL⁻¹ in 5L solution (*Penicillium digitatum* causal agent of green mould)
- Control treatment: Wounded citrus fruit dipped in spore suspension
- Product treatment: Wounded citrus fruit dipped in spore suspension 3 min after product has been added
- Incubation: 4 days at 20°C
- Evaluation: Infected wounds rated and converted to percentage infection
- Products and treatment concentrations can be seen in Table 1

Results

Table 1. The percentage infection on Valencia oranges inoculated with *P. digitatum* during treatment in a solution with 767 Sept, chlorine or Sporekill.

Treatment	Concentration	Infection (%)
Essasol inoculated control	0%	100.0
Essasol	4%	91.7
Essasol inoculated control	0%	100.0
Essasol	8%	100.0
Essasol inoculated control	0%	100.0
Essasol	16%	97.9
Chlorine inoculated control	0 µg.mL ⁻¹	95.8
Chlorine	100 µg.mL ⁻¹	6.3
Sporekill inoculated control	0 µg.mL ⁻¹	89.6
Sporekill	120 µg.mL ⁻¹	2.1

Conclusion and recommendations

In comparison to chlorine or Sporekill this product in the applied concentrations showed no potential for use as a possible sanitising agent against green mould.

Recommendations

- No further work should be done on the effect of Essasol against green mould

Important comments

This was only a pilot trial. None of these results can be used in marketing or as CRI recommendation in any citrus chemical program.

Addendum 10

The evaluation of Essasol for potential use in a citrus packhouse dump tank washing system as sanitising agent against green mould

Product information

Product name: Essasol

Active ingredient: Biodegradable detergent

Company: Wenkem

Materials and methods

Test: Sanitation

Method:

- 1×10^4 spores.mL⁻¹ in 5L solution (*Penicillium digitatum* causal agent of green mould)
- Control treatment: Wounded citrus fruit dipped in spore suspension
- Product treatment: Wounded citrus fruit dipped in spore suspension 3 min after product has been added
- Incubation: 4 days at 20°C
- Evaluation: Infected wounds rated and converted to percentage infection
- Products and treatment concentrations can be seen in Table 1

Results

Table 1. The percentage infection on Valencia oranges inoculated with *P. digitatum* during treatment in a solution with 767 Sept, chlorine or Sporekill.

Treatment	Concentration	Infection (%)
Essasol inoculated control	0%	100.0
Essasol	4%	91.7
Essasol inoculated control	0%	100.0
Essasol	8%	100.0
Essasol inoculated control	0%	100.0
Essasol	16%	97.9
Chlorine inoculated control	0 µg.mL ⁻¹	95.8
Chlorine	100 µg.mL ⁻¹	6.3
Sporekill inoculated control	0 µg.mL ⁻¹	89.6
Sporekill	120 µg.mL ⁻¹	2.1

Conclusion and recommendations

In comparison to chlorine or Sporekill this product in the applied concentrations showed no potential for use as a possible sanitising agent against green mould.

Recommendations

- No further work should be done on the effect of Essasol against green mould

Important comments

This was only a pilot trial. None of these results can be used in marketing or as CRI recommendation in any citrus chemical program.

Addendum 11

The evaluation of Dutrion for potential use in a citrus packhouse dump tank washing system as sanitising agent against green mould

Product information

Product name: Dutrion

Company: Greenland Technologies SA

Active ingredient: Chlorine dioxide (in tablet form)

Trial date: 16 October 2012

Materials and methods

Test: Sanitation

Method:

- 1×10^4 spores.mL⁻¹ in 5L solution (*Penicillium digitatum* causal agent of green mould)
- Control treatment: Wounded citrus fruit dipped in spore suspension
- Product treatment: Wounded citrus fruit dipped in spore suspension 3 min after product has been added
- Incubation: 4 days at 20°C
- Evaluation: Infected fruit rated and converted to percentage infection (12 fruit per treatment)
- Products and treatment concentrations can be seen in Table 1

Results

Table 1. The percentage infection on Valencia oranges inoculated with *P. digitatum* during treatment in a solution with Dutrion or Sporekill.

Treatment	Concentration	Infection (%)
Dutrion inoculated control	0 $\mu\text{g.mL}^{-1}$	97.6
Dutrion	10 $\mu\text{g.mL}^{-1}$	50.0
Dutrion	20 $\mu\text{g.mL}^{-1}$	83.3
Dutrion	40 $\mu\text{g.mL}^{-1}$	100.0
Dutrion	1000 $\mu\text{g.mL}^{-1}$	45.5
Dutrion	2000 $\mu\text{g.mL}^{-1}$	63.6
Dutrion	4000 $\mu\text{g.mL}^{-1}$	58.3
Sporekill	120 $\mu\text{g.mL}^{-1}$	0.0

Conclusion and recommendations

In comparison to Sporekill this product in the applied concentrations showed poor potential for use as a possible sanitising agent against green mould.

This product released a very foul and uncomfortable smell and it irritated the respiratory systems and eyes of people that came in the vicinity of the solutions, this renders the product unsuitable for use in a citrus packhouse.

Recommendations

- No further work to be done on Dutrion

Important comments

This was only a pilot trial. None of these results can be used in marketing or as CRI recommendation in any citrus chemical program.

Addendum 12

The evaluation of Biopeptide T and Biopeptide G in a citrus packhouse dump tank washing system as potential sanitising agent against *Penicillium digitatum*

Introduction

Biopeptide T (BT) and Biopeptide G (BG), products from ICA International Chemicals, were evaluated in a simulated dump tank washing system for efficacy of sanitising the system and preventing possible infection of injured fruit moving through the system. The product was compared with Sporekill (DDAC). The simulated dump tank was seeded with a suspension of the post-harvest pathogen *Penicillium digitatum* (green mould) in this evaluation.

Materials and methods

The spore suspension of *P. digitatum* was made up by suspending spores in sterile deionised water containing the surfactant, Tween 20. The spore suspension was spectrophotometrically adjusted to a concentration of 1×10^6 spores.mL⁻¹. Good, sound, untreated Navel oranges from Joubert and sons (Nelspruit) 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 QAC. Thereafter the fruit was dried in the packline drying tunnel prior to treatment. The fruit was divided into lots of 6 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. For each treatment two replicates of six clean, surface sterilised navel oranges were injured 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 24 injury sites per treatment. The layout of treatments and concentrations can be seen in Table 1. Fruit were injured and dipped in water (untreated uninoculated control), in a 5 L spore suspension (adjusted to 1×10^4 spores.mL⁻¹; untreated inoculated control) or in a 5 L spore suspension amended with BG, BT (0.5X, 1X and 2X) or Sporekill.

Table 1. The various treatments and concentrations used in this trial.

Treatment	Concentration	Spores
Untreated uninoculated control	Water (5 L)	None
Untreated inoculated control	Water (5 L)	1×10^4 spores.mL ⁻¹
BG	0.5X (2.85 mL.5 L ⁻¹)	1×10^4 spores.mL ⁻¹
BG	1X (5.70 mL.5 L ⁻¹)	1×10^4 spores.mL ⁻¹
BG	2X (11.4 mL.5 L ⁻¹)	1×10^4 spores.mL ⁻¹
BT	0.5X (2.025 mL.5 L ⁻¹)	1×10^4 spores.mL ⁻¹
BT	1X (4.050 mL.5 L ⁻¹)	1×10^4 spores.mL ⁻¹
BT	2X (8.100 mL.5 L ⁻¹)	1×10^4 spores.mL ⁻¹
Sporekill	5mL.5 L ⁻¹	1×10^4 spores.mL ⁻¹

All the treated fruit were placed in fruit cartons covered with polyethylene bags and incubated for 7 days at 20°C. After incubation the fruit was evaluated for decay and the results were recorded as percentage decay.

Results

Biopeptide T in the applied concentrations had a moderate effect against *P. digitatum*, but was not comparable to Sporekill as a sanitation agent for use in aqueous solutions infested with spores from the pathogen. Biopeptide G in the applied concentrations had a weak effect. Results are shown in Table 2.

Table 2. The percentage infection on navel oranges inoculated with *P. digitatum* during treatment in a solution with BG, BT or Sporekill.

Treatment	Concentration	Infection (%) ^a
Untreated uninoculated control	Water (5 L)	0.0 a
Untreated inoculated control	Water (5 L)	100.0 d
BG	0.5X (2.85 mL.5 L ⁻¹)	83.3 ab
BG	1X (5.70 mL.5 L ⁻¹)	70.8 a
BG	2X (11.4 mL.5 L ⁻¹)	85.4 ab
BT	0.5X (2.025 mL.5 L ⁻¹)	29.1 c
BT	1X (4.050 mL.5 L ⁻¹)	26.3 c
BT	2X (8.100 mL.5 L ⁻¹)	31.3 c
Sporekill (1 mL.L ⁻¹)	5mL.5 L water ⁻¹	0.0 a

^aMeans followed by the same letter do not differ significantly ($P < 0.0001$)

Conclusion and recommendations

Biopeptide T shows potential. Higher concentrations should be investigated. For use in citrus fruit, it needs to be registered under Act 36, which includes efficacy, suitability (phytotoxicity and compatibility with other fungicides) and food safety (chemical residues). Without this registration the product cannot be recommended by CRI.

Recommendations

- Trials will need to be commenced on phytotoxicity on at least 3 different citrus kinds
- Trials will need to be commenced on the compatibility of BT with other postharvest fungicides
- Biopeptide T needs to be registered under Act 36

Final comments

This was only a pilot trial. None of these results can be used in marketing or as CRI recommendation in any citrus chemical program.

Addendum 13

The evaluation of Biopeptide T in a simulated citrus packhouse dump tank washing system as potential sanitising agent against *Geotrichum citri-aurantii*

Introduction

Biopeptide T (BT), a products from ICA International Chemicals, were evaluated in a simulated dump tank washing system for efficacy of sanitising the system and preventing possible infection of injured fruit moving

through the system. The product was compared with chlorine. The simulated dump tank was seeded with a suspension of the post-harvest pathogen *Geotricum citri-aurantii* (sour rot) in this evaluation.

Materials and methods

The spore suspension of *G.citri-aurantii* was made up by suspending spores in sterile deionised water containing the surfactant, Tween 20. The spore suspension was spectrophotometrically adjusted to a concentration of 1×10^6 spores.mL⁻¹. Good, sound, untreated Valencia oranges from Crocodile Valley (Nelspruit) 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 chlorine. Thereafter the fruit was dried in the packline drying tunnel prior to treatment. The fruit was divided into lots of 6 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. For each treatment two replicates of six clean Valencia oranges were injured by means of a wound inducer with four wounds equally spread around the stem end, giving a total of 24 injury sites per treatment. The layout of treatments and concentrations can be seen in Table 1. Fruit were injured and dipped in a 5 L spore suspension (adjusted to 1×10^4 spores.mL⁻¹; untreated inoculated control) or in a 5 L spore suspension amended with BT (4X) or Chlorine. Each product had an untreated inoculated control which was amended with the specific product and after 3 – 5 minutes injured fruit were submerged in the solution for 3 minutes.

Table 1. The various treatments and concentrations used in this trial.

Treatment	Concentration	Spores
Untreated inoculated control (Chlorine)	Water + spores	1×10^4 spores.mL ⁻¹
Chlorine	100 µg.mL ⁻¹	1×10^4 spores.mL ⁻¹
Untreated inoculated control (BT)	Water + spores	1×10^4 spores.mL ⁻¹
BT	4X (22.8 mL.5 L ⁻¹)	1×10^4 spores.mL ⁻¹

All the treated fruit were placed in fruit cartons covered with polyethylene bags and incubated for 7 days at 25°C. After incubation the fruit was evaluated for decay and the results were recorded as percentage decay.

Results

Biopeptide T in the applied concentrations had a moderate effect against *G. citri-aurantii*, but was not comparable to Chlorine as a sanitation agent for use in aqueous solutions infested with spores from the pathogen. Results are shown in Table 2.

Table 2. The percentage infection on Valencia oranges inoculated with *G. citri-aurantii* during treatment in a solution with BT or Chlorine.

Treatment	Concentration	Infection (%) ^a	
Untreated inoculated control (Chlorine)	Water + spores	83.33	a
Chlorine	100 µg.mL ⁻¹	0.00	c
Untreated inoculated control (BT)	Water + spores	75.00	a
BT	4X (22.8 mL.5 L ⁻¹)	41.67	b

^aMeans followed by the same letter do not differ significantly ($P = 0.002$)

Conclusion and recommendations

Biopeptide T shows potential. Higher concentrations should be investigated. For use in citrus fruit, it needs to be registered under Act 36, which includes efficacy, suitability (phytotoxicity and compatibility with other fungicides) and food safety (chemical residues). Without this registration the product cannot be recommended by CRI.

Recommendations

- Trials will need to be commenced on phytotoxicity on at least 3 different citrus kinds
- Trials will need to be commenced on the compatibility of BT with other postharvest fungicides
- Biopeptide T needs to be registered under Act 36

Final comments

This was only a pilot trial. None of these results can be used in marketing or as CRI recommendation in any citrus chemical program.

Addendum 14

The evaluation of KANNAR DiseaseShield™ for potential use in a citrus packhouse dump tank washing system as sanitising agent against green mould

Product information

Product name: KANNAR DiseaseShield™ (KDS)
Company: Kannar

Active ingredient: Unknown
Trial date: 16 October 2012

Materials and methods

Test: Sanitation

Method:

- 1×10^4 spores.mL⁻¹ in 5L solution (*Penicillium digitatum* causal agent of green mould)
- Control treatment: Wounded citrus fruit dipped in spore suspension
- Product treatment: Wounded citrus fruit dipped in spore suspension 3 min after product has been added
- Incubation: 4 days at 20°C
- Evaluation: Infected fruit rated and converted to percentage infection (12 fruit per treatment)
- Products and treatment concentrations can be seen in Table 1

Results

Table 1. The percentage infection on Valencia oranges inoculated with *P. digitatum* during treatment in a solution with KDS, chlorine or Sporekill.

Treatment	Concentration	Infection (%)
KDS inoculated control	0 mL.5 L. ⁻¹	100.0
KDS	5 mL.5 L. ⁻¹	91.7
KDS inoculated control	0 mL.5 L. ⁻¹	91.7
KDS	10 mL.5 L. ⁻¹	83.3
KDS inoculated control	0 mL.5 L. ⁻¹	100.0
KDS	20 mL.5 L. ⁻¹	100.0
Chlorine inoculated control	0 µg.mL ⁻¹	91.7
Chlorine	100 µg.mL ⁻¹	58.3
Sporekill inoculated control	0 µg.mL ⁻¹	100.0
Sporekill	120 µg.mL ⁻¹	0.0

Conclusion and recommendations

In comparison to chlorine or Sporekill this product in the applied concentrations showed no potential for use as a possible sanitising agent against green mould.

Recommendations

- No further work to be done on KDS

Important comments

This was only a pilot trial. None of these results can be used in marketing or as CRI recommendation in any citrus chemical program.

Addendum 15

The evaluation of Ecolyte for potential use in a citrus packhouse dump tank washing system as sanitising agent against green mould

Product information

Product names: Ecolyte

Active ingredients: Chlorine

Company: McClean

Trial date: 6 November 2012

Materials and methods

Test: Sanitation

Method:

- 1×10^4 spores.mL⁻¹ in 5L solution (*Penicillium digitatum* causal agent of green mould)
- Control treatment: Wounded citrus fruit dipped in spore suspension
- Product treatment: Wounded citrus fruit dipped in spore suspension 3 min after product has been added
- Incubation: 4 days at 20°C
- Evaluation: Infected fruit rated and converted to percentage infection (12 fruit per treatment)
- Products and treatment concentrations can be seen in Table 1

Results

Table 1. The percentage infection on Valencia oranges inoculated with *P. digitatum* during treatment in a solution with Ecolyte or Sporekill.

Treatment	Concentration	Infection (%)
Ecolyte	2.5%	58.3%
Ecolyte	5.0%	66.7%
Ecolyte	10.0%	25.0%
Ecolyte	Full dose	25.0%
Sporekill	120 µg.mL ⁻¹	8.3%

Conclusion and recommendations

In comparison to Sporekill this product in the applied concentrations showed moderate potential for use as a possible sanitising agent against green mould.

Recommendations

- pH was not measured and it might be that the efficacy of the product is pH dependant
- The product is unknown and factors such as phyto-toxicity should be investigated

Important comments

This was only a pilot trial. None of these results can be used in marketing or as CRI recommendation in any citrus chemical program.

Addendum 16

The evaluation of Neuthox in a citrus packhouse dump tank washing system as potential sanitising agent against *Geotrichum citri-aurantii*

Introduction

Neuthox, a product from Prosep Chemicals, 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 chlorine. The simulated dump tank was seeded with a suspension of the post-harvest pathogen *Geotrichum citri-aurantii* (sour rot) in this evaluation.

Materials and methods

The spore suspension of *G.citri-aurantii* was made up by suspending spores in sterile deionised water containing the surfactant, Tween 20. The spore suspension was spectrophotometrically adjusted to a concentration of 1×10^{12} spores.mL⁻¹. Good, sound, untreated Valencia oranges from Crocodile Valley (Nelspruit) 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 chlorine. Thereafter the fruit was dried in the packline drying tunnel prior to treatment. The fruit was divided into lots of 6 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. For each treatment two replicates of six clean Valencia oranges were injured by means of a wound inducer with four wounds equally spread around the stem end, giving a total of 24 injury sites per treatment. The layout of treatments and concentrations can be seen in Table 1. Fruit were injured and dipped in a 5 L spore suspension (adjusted to 2×10^{10} spores.mL⁻¹; untreated inoculated control) or in a 5 L spore suspension amended with BT (4X) or Chlorine. Spores were exposed to each product solution for 3 – 5 minutes before injured fruit were submerged in the solution for 3 minutes.

Table 1. The various treatments and concentrations used in this trial.

Treatment	Concentration	Spores
Untreated uninoculated control	Water (5 L)	None
Untreated inoculated control	Water (5 L)	1×10^4 spores.mL ⁻¹
Neuthox		1×10^4 spores.mL ⁻¹
Sporekill	120µg.mL ⁻¹	1×10^4 spores.mL ⁻¹

All the treated fruit were placed in fruit cartons covered with polyethylene bags and incubated for 7 days at 20°C. After incubation the fruit was evaluated for decay and the results were recorded as percentage decay.

Results

Neuthox in the applied concentration was less effective than Sporekill as a sanitation agent for use in aqueous solutions infested with spores from *P. digitatum*. Results are shown in Table 2.

Table 2. The percentage infection on navel oranges inoculated with *P. digitatum* during treatment in a solution with either Neuthox or Sporekill.

Treatment	Concentration	Infection (%) ^a	
Untreated uninoculated control	Water (5 L)	0.0	a
Untreated inoculated control	Water (5 L)	100.0	b
Neuthox		12.5	c
Sporekill	120µg.mL ⁻¹	0.0	a

^aMeans followed by the same letter do not differ significantly ($P < 0.0001$)

Conclusion and recommendations

Neuthox showed potential. The product needs to be tested at higher concentrations and needs to be more extensively tested prior to registration and subsequent recommendation for use. For use in citrus fruit, it needs to be registered under Act 36, which includes efficacy, suitability (phytotoxicity and compatibility with other fungicides) and food safety (chemical residues).

Recommendations

- Trials will need to be commenced on phytotoxicity on at least 3 different citrus kinds
- Trials will need to be commenced on the compatibility of Neuthox with other postharvest fungicides
- Neuthox needs to be registered under Act 36

Further concerns

Neuthox is a chlorine product that can be produced on sight. The cost of installing a plant at a packhouse and the volumes that can be produced should be comparable with the cost and availability of current chlorine products. The durability of the product in an industrial citrus chlorine applicator where tons of fruit pass through should also be determined.

Final comments

This was only a pilot trial. None of these results can be used in marketing or as CRI recommendation in any citrus chemical program.

Addendum 17

The evaluation of a ViBacSan™ product for potential use in a citrus packhouse dump tank washing system as sanitising agent against green mould

Product information

Product name: Fv1 Citrus hard skinned decontaminant sanitizer (VBS)

Company: ViBacSan™

Active ingredient: Citrus aurantium amara extract

Trial dates: 17 September and 16 October 2012

Materials and methods

Test: Sanitation

Method:

- 1×10^4 spores.mL⁻¹ in 5L solution (*Penicillium digitatum* causal agent of green mould)
- Control treatment: Wounded citrus fruit dipped in spore suspension
- Product treatment: Wounded citrus fruit dipped in spore suspension 3 min after product has been added
- Incubation: 4 days at 20°C
- Evaluation: Infected wounds rated and converted to percentage infection
- Products and treatment concentrations can be seen in Table 1 and 2

Results

Trial 1

The pH levels of the various solutions have not been tested during this trial.

Table 1. The percentage infection on Valencia oranges inoculated with *P. digitatum* during treatment in a solution with VBS, chlorine or Sporekill.

Treatment	Concentration	Infection (%)
VBS inoculated control	0%	93.8
VBS	1%	100.0
VBS	2%	79.2
VBS	4%	41.7
Sporekill	120 µg.mL ⁻¹	2.1

Trial 2

Trial 1 has been repeated in this trial together with the assessment of the pH level of the various solutions

Table 2. The percentage infection on Valencia oranges inoculated with *P. digitatum* during treatment in a solution with VBS or Sporekill.

Treatment	Buffer	pH	Concentration	Infection (%)
Inoculated control	none	7.6	0%	91.7
Inoculated control	Citric acid – 0.05%	3.0	0%	100.0
Inoculated control	NaOH – 0.02%	11.5	0%	64.8
Inoculated control	NaOH – 0.1%	12.4	0%	52.8
VBS	NaOH – 0.02%	3.2	1%	100.0
VBS	NaOH – 0.02%	3.9	2%	50.0
VBS	NaOH – 0.02%	2.52	4%	72.2
VBS	NaOH – 0.1%	6.9	1%	66.7
VBS	NaOH – 0.1%	3.8	2%	58.3
VBS	NaOH – 0.1%	3.1	4%	91.7
Sporekill			120 µg.mL ⁻¹	0.0

Conclusion and recommendations

In comparison to Sporekill this product in the applied concentrations showed poor potential for use as a possible sanitising agent against green mould.

Recommendations

- Higher concentration should be tested
 - Financial viability will need to be assessed first
- If higher concentrations show potential
 - Trials will need to be commenced on phytotoxicity on at least 3 different citrus kinds
 - Trials will need to be commenced on the compatibility of VBS with other postharvest fungicides
 - VBS have to be registered under Act 36

Important comments

This was only a pilot trial. None of these results can be used in marketing or as CRI recommendation in any citrus chemical program.

Addendum 18

The evaluation of RBT24/7–D-SAN-DIS in a citrus packhouse dump tank washing system as potential sanitising agent against *Penicillium digitatum*

Introduction

RBT24/7–D-SAN-DIS (RBT; active ingredient: combination of quaternary ammonium compounds [QAC] benzalkonium chloride [BAC] and the twin chain didecyl-dimethylammonium chloride [DDAC]) 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 (DDAC). The simulated dump tank was seeded with a suspension of the post-harvest pathogen *Penicillium digitatum* (green mould) in this evaluation.

Materials and methods

The spore suspension of *P. digitatum* was made up by suspending spores in sterile deionised water containing the surfactant, Tween 20. The spore suspension was spectrophotometrically adjusted to a concentration of 1×10^6 spores.mL⁻¹. Good, sound, untreated Navel oranges from Joubert and sons (Nelspruit) 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 QAC for 2 minutes. Thereafter the fruit was dried in the packline drying tunnel prior to treatment. The fruit was divided into lots of 6 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 navel 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 18 injury sites per treatment. The layout of treatments and concentrations can be seen in Table 1. Fruit were injured and dipped in water (untreated uninoculated control), in a 5 L spore suspension (1×10^4 spores.mL⁻¹; untreated inoculated control) or in a 5 L spore suspension amended with either RBT or Sporekill.

Table 1. The various treatments and concentrations used in this trial.

Treatment	Concentration	Spores
Untreated uninoculated control	Water (5 L)	None
Untreated inoculated control	Water (5 L)	1×10^4 spores.mL ⁻¹
RBT	10:1	1×10^4 spores.mL ⁻¹
Sporekill (1 mL.L ⁻¹)	5mL.5 L water ⁻¹	1×10^4 spores.mL ⁻¹

All the treated fruit were placed in fruit cartons covered with polyethylene bags and incubated for 7 days at 20°C. After incubation the fruit was evaluated for decay and the results were recorded as percentage decay.

Results

RBT was comparable with Sporekill as a sanitation agent for use in aqueous solutions infested with spores from *P. digitatum*. Results are shown in Table 2.

Table 2. The percentage infection on navel oranges inoculated with *P. digitatum* during treatment in a solution with either RBT or Sporekill.

Treatment	Concentration	Infection (%)
Untreated uninoculated control	Water (5 L)	0.0
Untreated inoculated control	Water (5 L)	100.0
RBT	10:1	0.0
Sporekill (1 mL.L ⁻¹)	5mL.5 L water ⁻¹	0.0

Conclusion and recommendations

The product (RBT) product shows potential, but needs to be more extensively tested prior to registration and subsequent recommendation for use. For use in citrus fruit, it needs to be registered under Act 36, which includes efficacy, suitability (phytotoxicity and compatibility with other fungicides) and food safety (chemical residues). The product contains QAC's and currently there is no export MRL (maximum residue limit) for QAC's. Postharvest QAC use on citrus fruit is not recommended, as the default MRL of 0.01 ppm will be exceeded.

Recommendations

- Trials will need to be commenced on phytotoxicity on at least 3 different citrus kinds
- Trials will need to be commenced on the compatibility of RBT with other postharvest fungicides
- RBT needs to be registered under Act 36

Further concerns

The label (Figure 1) on the RBT247 container does not mention any QAC as active ingredient, whereas the information (Appendix 1) send to CRI on the product does indicate the a.i. to be a combination of QAC's. This could be confusing to the industry. For the citrus export industry it is very important that a.i.'s should be displayed and declared clearly and accurately to avoid rejections on the overseas markets.

Final comments

This was only a pilot trial. None of these results can be used in marketing or as CRI recommendation in any citrus chemical program.

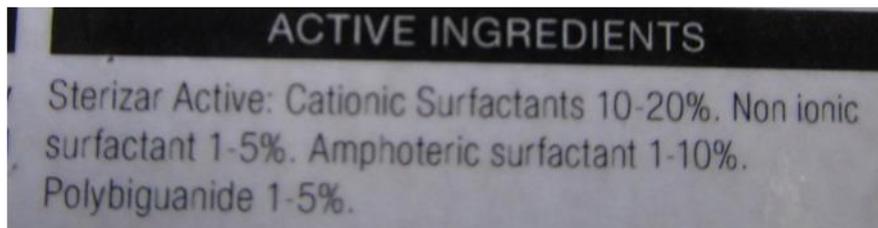


Figure 1. RBT247 label.

Addendum 19

The evaluation of HAWA-SAN TR50 for potential use in a citrus packhouse dump tank washing system as sanitising agent against green mould

Product information

Product name: HAWA-SAN TR50 (HS)

Active ingredients: Hydrogen peroxide
Ionic silver

Company: Nulandis

Trial date: 16 October 2012

Materials and methods

Test: Sanitation

Method:

- 1×10^4 spores.mL⁻¹ in 5L solution (*Penicillium digitatum* causal agent of green mould)
- Control treatment: Wounded citrus fruit dipped in spore suspension
- Product treatment: Wounded citrus fruit dipped in spore suspension 3 min after product has been added
- Incubation: 4 days at 20°C
- Evaluation: Infected fruit rated and converted to percentage infection (12 fruit per treatment)
- Products and treatment concentrations can be seen in Table 1

Results

Table 1. The percentage infection on Valencia oranges inoculated with *P. digitatum* during treatment in a solution with HS or Sporekill.

Treatment	Concentration	Infection (%)
HS Inoculated control	0.0%	90.3
HS	0.1%	91.7
HS	0.2%	100.0
HS	0.4%	83.3
HS	1.0%	91.7
HS	2.0%	50.0
HS	4.0%	83.3
Sporekill inoculated control	0 µg.mL ⁻¹	100.0
Sporekill	120 µg.mL ⁻¹	0.0

Conclusion and recommendations

In comparison to Sporekill this product in the applied concentrations showed no potential for use as a possible sanitising agent against green mould.

Recommendations

- No further work to be done on HS

Important comments

This was only a pilot trial. None of these results can be used in marketing or as CRI recommendation in any citrus chemical program.

Addendum 20

Analyses for imazalil resistance of *Penicillium* species on 30 swabs received from a packhouse.

Conidia and spores were extracted from each swab by means of a vortex in sterile water. The extraction was plated out where colonies were identified and *Penicillium* cultures were tested for imazalil resistance. Please refer to Table 1 for the results from this analysis.

Definitions

CRI no. – the number given to each swab by the CRI laboratory

Packhouse label – the label given to each swab by the packhouse

Colony identification – conidia and spores were extracted from each swab and plated out after which it was identified as bacterial (B) or fungal (F).

- B – Bacterial
- F – Fungal
- N – no conidia or spores were extracted from the specific swab

Penicillium identification – Penicillium colonies were identified as *Penicillium digitatum* (PD; green mould), *P. italicum* (PI; Blue mould) or other, furthermore the PD and PI cultures were tested for imazalil resistance.

P = present

- PD-S – *P. digitatum*; imazalil sensitive
- PI-S – *P. italicum*; imazalil sensitive
- PD-R – *P. digitatum*; imazalil resistant
- PI-R – *P. italicum*; imazalil resistant

Table 1. Swab numbers, labels and identification for cultures extracted from 30 swabs send to CRI from a packhouse.

CRI no	Packhouse label	Colony identification	Penicillium identification			
			PD-S	PI-S	PD-R	PI-R
1	Sorteer	B	0	0	0	0
2	sorteer	B	P ^a	0	0	0
3	sorteer	N	0	P	0	0
4	pakbak	B	0	0	0	0
5	pakbak	B	0	0	0	0
6	pakbak	B	0	0	0	0
7	sap krat	B,F	0	0	0	0
8	sap krat	F	P	0	0	0
9	sap krat	B,F	0	P	0	P
10	sap area	B	0	0	0	0
11	sap area	B,F	0	0	0	0
12	sap area	B,F	0	0	0	0
13	Tip 1	F	0	0	0	0
14	Tip 2	B	0	0	0	0
15	Tip 3	B,F	0	0	P	0
16	pakbak	B	0	0	0	0
17	pakbak	F	0	0	0	0
18	pakbak	B,F	0	0	0	0
19	Tip 1	B,F	P	0	0	0
20	Tip 2	B,F	P	0	0	0
21	Tip 3	B,F	0	0	0	0
22	Citrio pakhuis	B,F	0	P	0	0
23	Citrio pakhuis	B,F	0	0	0	0
24	Citrio pakhuis	B	0	0	0	0
25	Citrio tip& krat	B,F	0	P	0	0
26	Citrio tip& krat	B,F	P	P	0	0
27	Citrio tip& krat	B,F	0	0	0	0
28	Opkleur kamer(15) no 1	F	P	P	0	0
29	Opkleur kamer(15) no 2	F	0	0	0	0
30	Opkleur kamer(15) no 3	B,F	P	P	0	0

^a = Present; cultures for the specific Penicillium species were observed

Addendum 21

Pakhuis A Chloor bad toets

May 2012

Die chloor bad van Pakhuis A is ondersoek ten op sigte van vrug sanitasie.

Die invoer van vrugte na die paklyn begin by die chloor bad. Chloor word aangewend as sanitasie middel met die doel om enige oppervlakkige spore op die vrug skille te dood. So word risiko vir toekomstige infeksies verlaag. Die belangrikste siektes waarteen hierdie aanwending gerig is, is groen- en blouskimmel en suur vrot.

Die ideale chloor bad sal die volgende spesifikasies hê.

- Chloor konsentrasie: 75 – 100 ppm
- Vrug blootstellingstyd in die bad: 60 – 180 s
- pH van oplossing: 6 – 7

Op die dag van die ondersoek het die Pakhuis A chloor bad die volgende meetings gehad:

- Chloor konsentrasie: 62 ppm
- Vrug blootstellingstyd in die bad: gemiddeld 90 s
- pH van oplossing: 8.2

Sewe toetse is uitgevoer en word uitgelê hieronder.

Toets 1: Onbehandelde ongeïnokuleerde kontrole

- Metode
 - Vrugte was gewond, maar nie behandel
- Infeksie
 - Groenskimmel – 39%
 - Suur vrot – nie gedoen
- Bevindings
 - Hierdie wys dat daar 'n relatiewe hoë vlak van groen skimmel spore in die lug of reeds op vrugte vanaf die bord. Indien daar 10 gewonde vrugte is sal 4 vrot

Toets 2: Onbehandelde ongeïnokuleerde water kontrole

- Metode
 - Vrugte is gewond en gedoop in skoon kraan water. Hierdie water het gekom van die bron wat ook gebruik word vir die chloor bad.
- Infeksie
 - Groenskimmel infeksie – 57%
 - Suur vrot – 49%
- Bevindings
 - Die water het die effek van die hoë vlak van spore in die lug en op vrugte verhoog. As gevolg hiervan het meer vrugte gevrot. Indien daar 10 gewonde vrugte was wat nat geword het sal naby aan 6 groenskimmel kry en amper 5 suur vrot.

Toets 3: Onbehandelde geïnokuleerde water kontrole

- Metode
 - Vrugte is gewond en gedoop in skoon water waarby spore gevoeg is. Dit is gedoen om te toets of die spore wat ons gebruik wel vrugte sal kan vrot maak.
- Infeksie
 - Groenskimmel infeksie – 97%
 - Suur vrot infeksie – 25%
- Bevindings
 - Die groenskimmel spore is lewendig en kan vrot maak, daar kan egter fout wees met ons suur vrot spore.

Toets 4: Na chloor bad gewonde kontrole

- Metode
 - Vrugte wat nie gewond was nie is in 'n spoor suspensie gedoop. Daarna is die vrugte gewond en in bruin papiersakke geplaas. Hierdie toets is gedoen om te wys dat as daar spore op die vrug oppervlakte is siekte sal veroorsaak indien die vrug 'n wond opdoen.
- Infeksie
 - Groenskimmel – 100%
 - Suur vrot – 100%

- Bevindings
 - Al die vrugte het gevrot, wat bewys dat indien daar spore op die vrug oppervlakte is en die vroeg doen 'n wond op sal die spore infekteer en siekte veroorsaak.

Toets 5: Na chloor bad gewonde behandeling

- Metode
 - Vrugte wat nie gewond was nie is in 'n spoor suspensie gedoop en deur die Pakhuis A chloor bad gesit. Daarna is die vrugte gewond en in bruin papiersakke geplaas. Hierdie toets is om te toets of die chloor bad spore wat op die vrug oppervlakte is sal kan dood maak om so toekomstige infeksies te verhoed.
- Infeksie
 - Groenskimmel – 75%
 - Suur vrot – 78%
- Bevindings
 - 'n Hoë getal van die vrugte het gevrot. Wat wys dat die chloor bad nie totaal in staat is om al die spore op die oppervlakte van die vrugte te dood nie. Daar moet in ag geneem word dat hierdie toets baie streng is.

Toets 6: Chloor bad toets

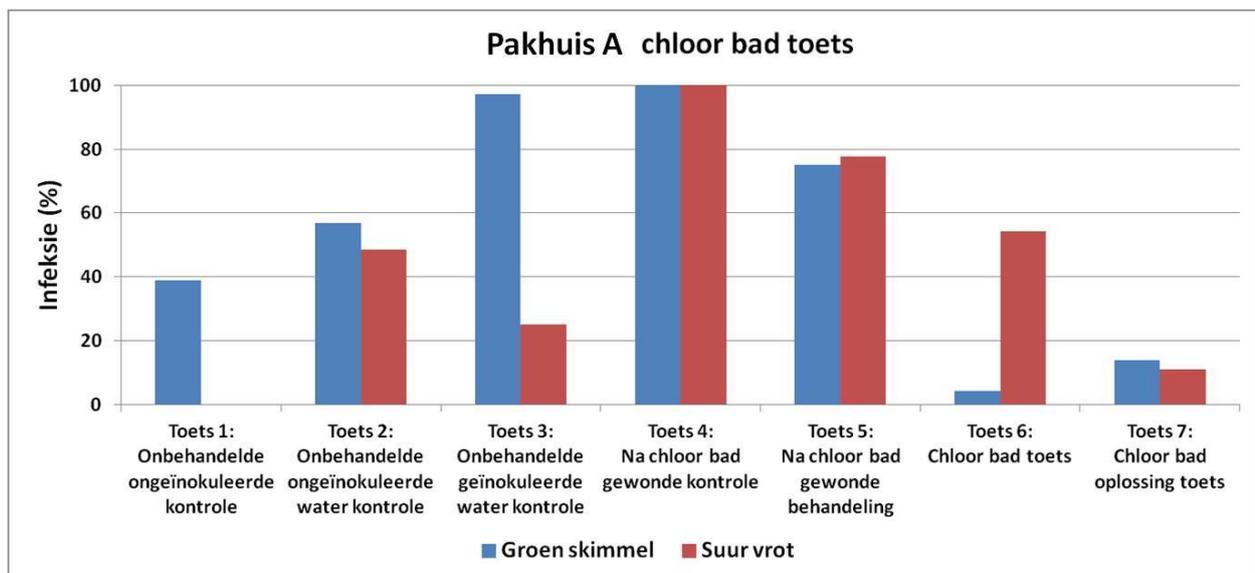
- Metode
 - Vrugte is gewond en deur die Pakhuis A chloor bad gesit. Daarna is die vrugte in bruin papiersakke geplaas. Hierdie is gedoen om te toets of die Pakhuis A chloor bad dien as infeksie bron.
- Infeksie
 - Infection – 4%
 - Suur vrot – 54%
- Bevindings
 - 'n Baie lae getal van die vrugte het gevrot aan groenskimmel. Dit wys dat die chloor bad nie dien as bron vir groenskimmel infeksie nie. Indien 10 gewonde vrugte deur die bad gaan sal minder as 1 vrot. Die omgekeerde is waar vir suur vrot, waar 'n redelike getal vrugte suur vrot opgedoen het. Indien 10 gewonde vrugte deur die bad gaan sal net meer as 5 suur vrot opdoen.

Toets 7: Chloor bad oplossing toets

- Metode
 - Van die Pakhuis A chloor bad oplossing is uitgeskep in 'n houer. Spore is gevoeg by die oplossing waarna gewonde vrugte in die oplossing gedoop is. Hierdie is gedoen om te toets of die chloor bad oplossing effektief is om spore dood te maak in oplossing sodat dit nie gewonde vrugte infekteer nie.
- Infeksie
 - Groenskimmel – 14%
 - Suur vrot – 11%
- Bevindings
 - 'n Baie lae getal van die vrugte het gevrot. Wat wys dat die chloor bad oplossing wel spore kan dood maak en so infeksies kan voorkom.

Gevolgtrekkings en aanbevelings

Tydens die ondersoek is gevind dat die Pakhuis A chloor bad redelike goeie sanitasie beheer teen groenskimmel vind



Figuur 1. Persentasie groenskimmel en suur vrot infeksie van navel lemoene gebruik in die toets van die Pakhuis A chloor bad.

Addendum 22

Packhouse B spray test

May 2012

Arno Erasmus and Vongani Rikhotso
Postharvest plant pathology
CRI Nelspruit

The chlorine spray application of Packhouse B was assessed in terms of its ability to sanitise (clean) fruit. Fruit are introduced to the packline through the chlorine spray applicator. Chlorine is applied as sanitiser with the purpose to kill any superficial spores on the fruit. By doing this the risk for future pathogen infections is reduced. The major pathogens that this treatment is directed to are green mould and sour rot. The applicator works on a total loss system and water is chlorinated by means of a Buccaneer system. The packhouse was packing lemons during the time of the assessment.

The ideal chlorine spray solution would have the following specifications:

- Chlorine concentration: 75 – 100 ppm
- Solution pH: 6 – 7

On the day of assessment the Packhouse B spray applicator had the following measurement:

- Chlorine concentration: 134 ppm
- Solution pH: 8.46

Seven tests were conducted and are explained below. A summary of the test can be seen in Figure 1.

Test 1: Untreated none-inoculated control

- Method
 - Fruit were wounded and not treated
- Infection – 0%
- Findings
 - This shows that there are relative low levels of spores in the air around the applicator and that our wound inducer is clean and does not cause any infections.

Test 2: Untreated none-inoculated water control

- Method
 - Fruit were wounded and dipped in clean water. This was the source water for the chlorine spray applicator.
- Infection – 11%
- Findings
 - This show that there is some level of green mould spores in the environment, weather it is from the water or the air is not known.

Test 3: Untreated inoculated water control

- Method
 - Fruit were wounded and dipped in clean water with added spores. This is to test the spores that we used in this assessment in terms of their ability to rot fruit.
- Infection – 94%
- Findings
 - Almost all of the fruit rotted, this means the spores is alive and able to cause disease.

Test 4: After chlorination wounded control

- Method
 - Fruit with no wounds were dipped in clean water with added spores. After the dip wounds were induced onto the fruit. This is to show that if there are spores on the surface of the fruit and a wound is induced it will cause infection.
- Infection – 89%
- Findings
 - A high level of the fruit was infected, this confirms that: spores + water + fresh wound = infection.

Test 5: After chlorination wounded treatment

- Method
 - Fruit with no wounds were dipped in clean water with added spores and allowed to run through the applicator. After the application wounds were induced onto the fruit. This to test if the chlorine applicator can remove and kill spores on the surface of fruit.
- Infection – 11%
- Findings
 - A very low level of fruit was infected. This means that the Packhouse B applicator is able to remove and kill spores on the surface of fruit and in so doing prohibit future infections of green mould.

Test 6: Chlorine solution treatment

- Method
 - A container was filled with chlorine solution from the applicator. Spores were added to this solution after which wounded fruit were dipped in it. This to test if the chlorine solution can kill spores and prohibit infection of wounded fruit.
- Infection – 3%
- Findings
 - A very low level of fruit was infected. This means that the chlorine of the Packhouse B applicator is able to kill spores and in so doing prohibit future infections of green mould.

Test 7: Chlorine spray applicator test

- Method
 - Wounded fruit were put through the Packhouse B spray applicator. This to test whether the applicator is a source of infection.
- Infection – 6%
- Findings
 - A very low level of fruit was infected. This means that the Packhouse B chlorine applicator is not a source of infection.

Conclusion and recommendation

The Packhouse B chlorine spray applicator is able to sanitise fruit, kill green mould spores and prohibit future infections. This test needs to be repeated on navel oranges. It must be noted that this test was done only on one batch of fruit during one day, and can therefore only serve as an indication of the abilities of the applicator.

Recommendations:

- The solution pH is too high and needs to be reduced to 6
- The chlorine concentration is also higher than the recommendation, this could be the reason for the higher pH

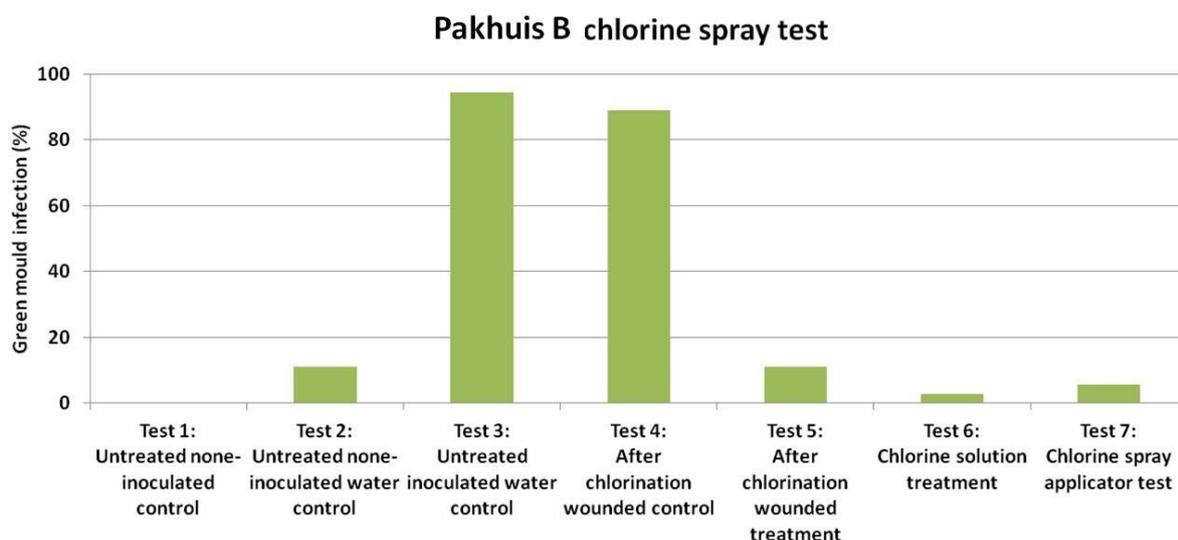


Figure 1. Green mould infection (%) on lemon fruit used to test the chlorine spray applicator at Packhouse B.

Addendum 23

2012 CRI ring test for analytical laboratories

18 April 2013

Arno Erasmus (CRI), Paul Fourie (CRI) Keith Lesar (CRI) and Paul Hardman (CGA)

The Exporters Technical Forum (ETF) requested CRI to conduct a ring test between the different analytical laboratories servicing the fresh citrus fruit export industry. At the end of the 2012 packing season, nine “spiked” citrus samples were sent to seven different analytical laboratories throughout South Africa. The samples were amended with specific concentrations of imazalil (IMZ), thiabendazole (TBZ) and pyrimethanil (PYR). Each laboratory received a portion of exactly the same sample batch. Analyses reports were received from six of the seven laboratories and were compared to the spiked concentrations as well as results from other laboratories.

Materials and methods

A 1 L stock solution containing 500 $\mu\text{g}\cdot\text{mL}^{-1}$ IMZ, TBZ and PYR was prepared. Untreated Valencia oranges were macerated by means of an electrical blender. To amend the active concentration in the pulp to 0.5 $\mu\text{g}\cdot\text{g}^{-1}$, 999 g of pulp were added to a blender jug on a scale before adding 1 mL from the stock solution. The pulp was then blended for 3 min before it was transferred to stainless steel mixing bowl. This was done 6 times to gain a pulp volume of approximately 6 kg. The pulp in the mixing bowl was mixed thoroughly by means of a hand-held electrical mixer. A similar protocol was followed for 2.5 and 8.5 $\mu\text{g}\cdot\text{g}^{-1}$. The amended pulp was divided into approximately 250 g portions in clean unused jars before it was deep frozen. The different samples were then sent via courier to the different laboratories.

Results and discussion

The results of all laboratories are summarised in Table 1. The variability between the different laboratories is concerning, and results differed too much from one lab to the other for the same samples.

Imazalil

0.5 $\mu\text{g}\cdot\text{g}^{-1}$ sample

The average deviation for each lab within samples varied from 0.00 - 0.09 $\mu\text{g}\cdot\text{g}^{-1}$, showing consistency in terms of each lab’s protocol. However, Lab 1 and Lab 6 were inaccurate giving mean IMZ residue results of 0.84 - 0.85 $\mu\text{g}\cdot\text{g}^{-1}$ and 2.11 – 2.33 $\mu\text{g}\cdot\text{g}^{-1}$, respectively. The other labs were more accurate and measured 0.39 – 0.56 $\mu\text{g}\cdot\text{g}^{-1}$ in this category.

2.5 $\mu\text{g}\cdot\text{g}^{-1}$ sample

The average deviation for each lab within samples varied from 0.04 - 0.20 $\mu\text{g}\cdot\text{g}^{-1}$. Lab 2, 3 and 6 had high levels of variation (0.13, 0.20 and 0.15 $\mu\text{g}\cdot\text{g}^{-1}$, respectively); the rest had levels of < 0.07 $\mu\text{g}\cdot\text{g}^{-1}$. For the 2.5 $\mu\text{g}\cdot\text{g}^{-1}$ sample, higher levels were measured by Lab 1 (\approx 3.88 $\mu\text{g}\cdot\text{g}^{-1}$) and Lab 6 (\approx 6.51 $\mu\text{g}\cdot\text{g}^{-1}$), while Lab 2 and 3 measured slightly lower levels (\approx 1.90 $\mu\text{g}\cdot\text{g}^{-1}$ and \approx 2.16 $\mu\text{g}\cdot\text{g}^{-1}$, respectively). Lab 4 and 5 were the closest to this amended level (2.56 and 2.23 $\mu\text{g}\cdot\text{g}^{-1}$, respectively).

7.5 $\mu\text{g.g}^{-1}$ sample

The average deviation for each lab within samples varied from 0.00 – 3.03 $\mu\text{g.g}^{-1}$. Lab 1, 3, 5 and 6 had high levels of variation (0.28, 0.34, 1.31 and 3.03 $\mu\text{g.g}^{-1}$, respectively). The other two labs were more consistent (0.00 and 0.08 $\mu\text{g.g}^{-1}$ for Lab 2 and 4, respectively). Lab 1 and 6 measured much higher levels than the 7.5 $\mu\text{g.g}^{-1}$ amended concentration (≈ 10.84 and $36.91 \mu\text{g.g}^{-1}$, respectively). Lab 2 and 3 measured the lowest levels (≈ 5.47 and $\approx 6.71 \mu\text{g.g}^{-1}$, respectively). Lab 4 and 5 measured close to the amended level (7.10 and 7.93 $\mu\text{g.g}^{-1}$, respectively).

Thiabendazole

Lab 1, 2 and 3 did not attempt to measure TBZ.

0.5 $\mu\text{g.g}^{-1}$ sample

The average deviation for each lab within samples was low ($\leq 0.02 \mu\text{g.g}^{-1}$). All three labs measure levels in close range to the amended level ($\approx 0.42 - \approx 0.49 \mu\text{g.g}^{-1}$).

2.5 $\mu\text{g.g}^{-1}$ sample

The average deviation within samples was low for Lab 4 (0.02 $\mu\text{g.g}^{-1}$) and high for Lab 5 and 6 (0.11 and 0.10, respectively). Lab 4 and 5 measured below (≈ 1.98 and $\approx 1.87 \mu\text{g.g}^{-1}$, respectively) and Lab 6 above (3.00 $\mu\text{g.g}^{-1}$) the amended level.

7.5 $\mu\text{g.g}^{-1}$ sample

The average deviation within samples was low for Lab 4 (0.01 $\mu\text{g.g}^{-1}$) and high for Lab 5 and 6 (1.33 and 0.15 $\mu\text{g.g}^{-1}$, respectively). Lab 4 measured below ($\approx 4.77 \mu\text{g.g}^{-1}$), Lab 5 close to (7.20 $\mu\text{g.g}^{-1}$) and Lab 6 above (9.89 $\mu\text{g.g}^{-1}$) the amended level.

Pyrimethanil

Lab 2 and 3 did not attempt to measure PYR.

0.5 $\mu\text{g.g}^{-1}$ sample

The average deviation for each lab within samples was very low ($\leq 0.01 \mu\text{g.g}^{-1}$). All labs measured far below the amended level ($\approx 0.08 - \approx 0.12 \mu\text{g.g}^{-1}$).

2.5 $\mu\text{g.g}^{-1}$ sample

The average deviation for each lab within samples was very low ($\leq 0.01 \mu\text{g.g}^{-1}$). All labs measured far below the amended level ($\approx 0.28 - \approx 0.38 \mu\text{g.g}^{-1}$).

7.5 $\mu\text{g.g}^{-1}$ sample

The average deviation for each lab within samples was very low ($\leq 0.06 \mu\text{g.g}^{-1}$). All labs measured far below the amended level (0.68 – 1.07 $\mu\text{g.g}^{-1}$).

Conclusion and recommendation

When considering the IMZ and TBZ results, Lab 1 and 6 should investigate why higher levels were consistently measured and Lab 2 and 3 should investigate why lower levels were consistently measured. It is unclear why all labs measured similar PYR levels, but far lower than the amended level. This could be due to a technical problem during the amending process.

The ring test will be repeated before the end of April 2013. Laboratories are welcome to interact with CRI to discuss and find ways on how the variation can be narrowed down to a minimum.

CRI thanks the six laboratories for taking part in the ring test. Through taking part in this exercise the laboratories show their willingness to be transparent and improve the very important service they are rendering to the fresh fruit export industry.

Table 1. Analytical results for imazalil (IMZ) amended fresh citrus samples sent to six different laboratories all over South Africa.

Amended concentration ($\mu\text{g.g}^{-1}$)	Sample no.	Measured concentration ($\mu\text{g.g}^{-1}$)					
		Laboratory no.					
		1	2	3	4	5	6
0.5	3	0.84	0.41	0.41	0.56	0.47	2.33
	5	0.84	0.43	0.39	0.54	0.48	2.16
	8	0.85	0.48	0.47	0.54	0.45	2.11
	Average level	0.84	0.44	0.42	0.55	0.47	2.20
	Average deviation	0.00	0.03	0.03	0.01	0.01	0.09
2.5	2	3.77	1.79	2.08	2.67	2.30	6.63
	4	3.90	2.09	1.94	2.46	2.20	6.29
	6	3.96	1.81	2.45	2.56	2.20	6.62
	Average level	3.88	1.90	2.16	2.56	2.23	6.51
	Average deviation	0.07	0.13	0.20	0.07	0.04	0.15
8.5	1	10.41	5.46	6.63	7.03	9.90	41.46
	7	11.03	5.47	7.22	7.06	7.70	34.64
	9	11.07	5.47	6.28	7.22	6.20	34.63
	Average level	10.84	5.47	6.71	7.10	7.93	36.91
	Average deviation	0.28	0.00	0.34	0.08	1.31	3.03

Table 2. Analytical results for thiabendazole (TBZ) amended fresh citrus samples sent to six different laboratories all over South Africa.

Amended concentration ($\mu\text{g.g}^{-1}$)	Sample no.	Measured concentration ($\mu\text{g.g}^{-1}$)					
		Laboratory no.					
		1	2	3	4	5	6
0.5	3				0.48	0.40	0.50
	5				0.47	0.45	0.46
	8				0.48	0.42	0.50
	Average level				0.48	0.42	0.49
	Average deviation				0.00	0.02	0.02
2.5	2				2.02	1.90	3.15
	4				1.95	1.70	2.94
	6				1.98	2.00	2.90
	Average level				1.98	1.87	3.00
	Average deviation				0.02	0.11	0.10
8.5	1				4.76	9.20	10.08

	7				4.77	7.00	9.66
	9				4.79	5.40	9.92
	Average level				4.77	7.20	9.89
	Average deviation				0.01	1.33	0.15

Table 3. Analytical results for pyrimethanil (PYR) amended fresh citrus samples sent to six different laboratories all over South Africa.

Amended concentration ($\mu\text{g.g}^{-1}$)	Sample no.	Measured concentration ($\mu\text{g.g}^{-1}$)					
		Laboratory no.					
		1	2	3	4	5	6
0.5	3	0.13			0.09	0.10	0.08
	5	0.12			0.09	0.10	0.08
	8	0.12			0.11	0.10	0.09
	Average level	0.12			0.10	0.10	0.08
	Average deviation	0.00			0.01	0.00	0.00
2.5	2	0.38			0.34	0.34	0.29
	4	0.39			0.31	0.36	0.28
	6	0.38			0.32	0.34	0.27
	Average level	0.38			0.32	0.35	0.28
	Average deviation	0.00			0.01	0.01	0.01
8.5	1	0.95			0.94	1.10	0.77
	7	1.01			0.94	1.10	0.62
	9	1.03			0.96	1.00	0.66
	Average level	1.00			0.95	1.07	0.68
	Average deviation	0.03			0.01	0.04	0.06

4.5.3 PROGRESS REPORT: Use of potassium silicate and biocontrol agents to reduce postharvest disease and chilling injury in citrus fruit

Project UKZN1 (2010/10-2014/4) by Mark Laing, Nicolette du Rand and Iona Basdew (UKZN)

Summary

The aim of this research is 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) evaluate 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, which is consistent with prior research by Abraha and Laing. The optimal time and temperature for the hot water treatment of navel oranges was unclear and this trial may need to be repeated. Hot water treatments for the Valencias still need to be determined. B13 yeast as a preventative treatment was tested at the end of 2012. It had been found that the B13 yeast does deliver a curative protection against infection of *P. digitatum*, hence the need for a curative hot water treatment first. Trials were not successful on navels and trials need to be conducted on Valencias. The first cold storage injury trials were conducted in January 2012. Fruit from silicon-fertilized trees do have a lower level of chilling injury than control fruit.

Opsomming

Die doel van die eksperiment was om die integrasie van kalium silikaat bemesting, warm water behandeling en die van biologiese agente, 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) om beskermende effek van warm water behandeling gekombineer met 'n gis biobeheer agent B13 te optimaliseer, en (3) evaluasie van die buffer gevolge van die voor-oes toedenings van kalium silikaat bemesting in die voorkoming van koue-skade in suurlemoene. Gedurende die afgelope seisoen, is warm water behandelinge op vier verkillende soorte sitrus vrugte uitgevoer (suurlemoene, nawels, Valencias en minneolas). 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 die warm water behandeling van nawels was onduidelik en sal herhaal moet word. Warm water tyd en temperatuur kombinasies vir die behandelinge vir die Valencias moet nog bepaal word. B13 gis was toegedien op suurlemoene as 'n beskerming teen *P. digitatum* en was gevind dat daar wel beskerming teen die na-oes siekte was. Die gis was nie so suksesvol op nawels nie en moet nog op Valencias getoets word in 2013. Die eerste koue stoor proewe was in Januarie 2012 gedoen. Die resultate wat verkry is, dui daarop dat die suurlemoene van silikon behandelde bome 'n laer hoeveelheid van koue skade opgedoen het as vrugte van die bome wat nie met silikon behandel was nie.

4.5.4 FINAL REPORT: Optimisation of fungicide application in citrus packhouses

Project 936 (April 2008 – March 2014) by Paul Fourie (CRI – SU)

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.

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 thiabendasool (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. Beskermdende beheer met IMZ was beter na toediening in waks, terwyl dubbele aanwending in doop- en waksbehandelings uitstekende genesende asook beskermdende 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; beskermdende beheer was relatief swak en geen beheer van die TBZ-weerstandbiedende isolaat kon verkry word nie. TBZ in waks het beter beskermdende 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 beskermdende 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% beskermdende 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.

Introduction

Excessive financial losses occur every year due to decay caused by postharvest pathogens such as *Penicillium digitatum* (causal agent of citrus green mould). Excellent fungicides such as imazalil are available and effective to control diseases such as Citrus green mould. An investigation was conducted to determine why this fungicide is not as effective as it should be; the main focus was on the application method and the subsequent residue loading in practice. Laboratory studies were conducted to study ways and methods in an effort to optimise fungicide application. If fungicide applications could be effective less resistance and waste would occur.

Management of post-harvest diseases of citrus involves several fungicide applications in packhouses, such as in drenches, dips, sprays and wax applications. Biological efficacy of the fungicides is, however, directly related to the adequacy of deposition of the active ingredient on the hydrophobic citrus fruit surfaces. By using green mould caused by *Penicillium digitatum* and the fungicide imazalil sulphate (IMZ) as model system optimisation of fungicide application in citrus packhouses was studied. Maximum residue level (MRL) for IMZ on citrus fruit is set at 5 $\mu\text{g}/\text{ml}$, whereas 2-3 $\mu\text{g}/\text{ml}$ is regarded as a biologically effective residue level that should at least inhibit green mould sporulation. Standard compliance auditing of residue levels of citrus fruit, however, indicated that citrus fruit from the majority of packhouses have residue levels below 1 $\mu\text{g}/\text{ml}$. Poor control of green mould as a result of insufficient residue loading might further be compounded by the presence of IMZ-resistant strains of *P. digitatum* in packhouses. The aims of this study are to determine the adequate residue levels needed for control and sporulation inhibition of IMZ-sensitive and -resistant strains, to investigate IMZ application and resultant residue levels in commercial citrus packhouses, and to study optimisation of IMZ application in citrus packhouses. Factors that will be studied include application type (spray vs. dip), exposure time, bath temperature, wound size, solution temperature and solution pH on the control of *Penicillium digitatum* with IMZ.

The following aims were defined in the 2010/11 project proposal and will be reported on below:

1. Improve the deposition assessment protocol for use on mature citrus fruit.
2. Determine benchmark values for biologically effective fungicide residue loading [Imazalil sulphate and *Penicillium digitatum* (green mould) will be used as the model system].
3. Evaluate residue loading as effected by drench, dip, spray and wax applications in citrus packhouses.
4. Optimise fungicide residue loading in terms of biological efficacy, MRLs and fruit quality.
5. *New objective*: Development of a user-friendly fungicide resistance assay for packhouses.

Materials and methods

1. Determine benchmark values for biologically effective fungicide residue loading
2. Evaluate residue loading as effected by drench, dip, spray and wax applications in citrus packhouses.
3. Optimise fungicide residue loading in terms of biological efficacy, MRLs and fruit quality.

Research has focussed on residue loading and green mould control using imazalil, pyrimethanil and thiabendazole and has mostly been concluded. Ongoing research focuses on drench application (residue loading, stripping and green mould control in single or mixed applications) and the effects of temperature on residue loading and control in bath application.

Imazalil:

Initial work on imazalil application in the fungicide bath was completed and two articles published:

1. Arno Erasmus, Cheryl L. Lennox, Hennie Jordaan, Joseph L. Smilanick, Keith Lesar, Paul H. Fourie. 2011. Imazalil residue loading and green mould control in citrus pack-houses. *Postharvest Biology and Technology* 62: 193–203. This work was comprehensively reported in 2011.
2. Arno Erasmus, Cheryl L. Lennox, Joseph L. Smilanick, Keith Lesar, Paul H. Fourie. 2013. Imazalil residue loading on citrus fruit as affected by formulation, solution pH and exposure time in aqueous dip treatments. *Postharvest Biology and Technology* 77: 43–49. This work was comprehensively reported in 2012.

Imazalil application in wax coatings was completed and one article was published, while another was accepted for publication:

1. Ncumisa S. Njombolwana, Arno Erasmus, Paul H. Fourie. 2013. Evaluation of curative and protective control of *Penicillium digitatum* following imazalil application in wax coating. *Postharvest Biology and Technology* 77: 102-110. This work was comprehensively reported in 2012.
2. Ncumisa S. Njombolwana, Arno Erasmus, J. Gideon van Zyl, Paul J.R. Cronje Wilma du Plooy and Paul H. Fourie. Effects of citrus coating and brush type on imazalil residue loading, green mould control and fruit quality retention of sweet oranges *Postharvest Biology and Technology* (accepted with minor revision).

Continuing work focuses on dip application and forms part of Arno Erasmus's PhD studies, of which aspects have been included in project number 1034. Research that investigated imazalil residue loading in different sized wounds as well as the effects of post-dip brushing on residue loading has been concluded and will be written up in the final report of this project.

Thiabendazole: Dip and wax application work forms part of Mareli Kellerman's MSc.Agric. study. This work has been completed and is reported in addendum A.

Pyrimethanil: Research on pyrimethanil residue loading and green mould control following dip application was initiated in the honours project of Elbie Liebenberg. This has been reported in 2012. Follow-up work on wax and drench application has been conducted and are reported in Addendum B.

Commercial drench evaluation

The drench system was extensively evaluated at a reputable commercial packhouse. Fruit was inoculated 24 h before treatment and incubated in boxes covered with polyethylene bags at ambient temperature. Treatment consisted of two drench mixtures: the first contained thiabendazole ($1000 \mu\text{g.mL}^{-1}$), guazatine ($1000 \mu\text{g.mL}^{-1}$), pyrimethanil ($1000 \mu\text{g.mL}^{-1}$) and 2.4D ($250 \mu\text{g.mL}^{-1}$), while the second contained the same mixture with Sporekill (1 mL/L). In each mixture, a yellow fluorescent pigment was added at 1 mL/L to visualise fungicide deposition (Van Zyl et al., 2012). On the day of treatment, the inoculated fruit was placed at the top of each bin. For both drench mixtures, three bins were stacked on top of each other and drenched for 2 min in a commercial drench at a rate of 800 L/bin. Twelve fruit from each position was sampled for residue analysis and fluorescent pigment analysis from the following positions: top centre, middle far left, middle centre, middle right and bottom centre of the each bin. Following treatments, fruit was allowed to dry overnight and digital photos of the top and bottom of each fruit was taken under near-UV light illumination for fluorescent pigment deposition analysis (Van Zyl., 2012). Fruit sampled for residue analysis was prepared by the method of Erasmus et al. (2011), with the exception that each fruit was halved and the top and bottom halves analysed separately. The number of rotted fruit was recorded after 7 days and disease incidence was calculated. Statistical analysis was done using XLstat software.

2. New objective: Development of a user-friendly fungicide resistance assay for packhouses.

Filter paper spore trap were prepared using the methods described by (Schweigkofler *et al.*, 2004). Filter papers (Whatman, No 1) were dipped into TE buffer (4 M tris, 1 M EDTA) and secured into a Petri dish using Vaseline. Three dishes were placed open with the filter paper facing upward at various positions in packhouses: at the fruit tip, wax applicator and packing area. Dish was left open for 7 days; the date and time it was opened and closed was noted. Each dish was replaced by a second dish after 7 days to trap spores for two consecutive weeks from each packhouse. The Petri dishes was stored at -20°C . A total of 27 packhouses participated.

Isolates of *P. digitatum* from the culture collection (University of Stellenbosch) were tested for IMZ sensitivity by growing the isolates on PDA for 14 days. For inoculation, 500 μL of 0.2 % agar solution was

pipetted onto each culture and spores harvested and stored in a 2 mL Eppendorf tube. Three microliter of spore suspension was dropped onto a PDA plate amended with 2 µg.mL⁻¹ IMZ and also onto a non-amended PDA. Isolates were classified as resistant to IMZ if they grew on both the non-amended and IMZ-amended PDA.

DNA from mycelia of 19 *P. digitatum* isolates from the culture collection grown on PDA was extracted using the Wizard® SV Genomic DNA Purification system. The type of resistance (R1, R2 or R3) prevalent in South African *P. digitatum* isolates was determined using the method of Sun *et al.* (2011). The PCR reaction was adjusted to the following: 2 µl buffer, 0.5 µl dNTP (10mM), 0.4 µl of each primer (B1, B2, CYP51A1 and CYP51A2 at 5 µM each), 0.2 µl Taq, 0.1 µl BSA, 1.2 µl MgCl₂ (50 mM), 9.4 µl H₂O and 5 µl DNA (20 ng/µl). Real time primers were designed using PrimerBlast (NCBI) to be specific to the IMZ-R3 genotype. Primer sequences were blasted on Genbank to indicate their level of species specificity.

Results

Objective / Milestone	Achievement
1. Improve the deposition assessment protocol for use on mature citrus fruit	The deposition assessment protocol was optimised and is being used to study drench application, and was also configured to quantify shine on waxed fruit. Residue loading is studied through determination of fungicide residues using analytical methods. An in-house IMZ residue analysis protocol was developed, but to increase throughput and the scope of research, samples are analysed in a commercial laboratory.
2. Determine benchmark values for biologically effective fungicide residue loading	<u>Imazalil</u> : IMZ application in dip and wax provides good curative and protective control and sporulation inhibition. Various factors influencing residue loading in dip and wax applications were studied to optimise and manage IMZ application. However, the IMZ resistant strain could not be controlled. Outcomes from this study were extensively communicated to industry.
3. Evaluate residue loading as effected by drench, dip, spray and wax applications in citrus packhouses	<u>Pyrimethanil</u> : PYR provided sufficient curative control and moderate protective control against green mould following dip applications, and was able to control an isolate of <i>P. digitatum</i> resistant to commonly used IMZ and TBZ. Sporulation was poorly and inconsistently inhibited. PYR provided relatively poor green mould control when applied as drench or with wax coating. Optimisation of PYR drench application is still in progress.
4. Optimise fungicide residue loading in terms of biological efficacy, MRLs and fruit quality	<u>Thiabendazole</u> : TBZ in dip application provided excellent curative control and poor protective control of the sensitive isolate. TBZ in wax provided moderate protective control, and sporulation inhibition. The TBZ resistant isolate could not be controlled. TBZ applied in dip or wax provided chilling injury control. TBZ applied in the drench lead to poor control on soft citrus and moderate to good control on oranges. Drench exposure time did not have an influence on TBZ residue, but it did have an effect on the efficacy of TBZ to control green mould. Optimisation of TBZ drench application is still in progress.
2. New objective: Development of a user-friendly fungicide resistance assay for packhouses	An exposed-plate assay using published semi-selective media was studied but found to be insufficiently species-specific. Presently, we are developing a real-time PCR assay to quantify IMZ resistance in <i>P. digitatum</i> populations.

1. Determine benchmark values for biologically effective fungicide residue loading
2. Evaluate residue loading as affected by drench, dip, spray and wax applications in citrus packhouses.
3. Optimise fungicide residue loading in terms of biological efficacy, MRLs and fruit quality.

See Addenda for complete papers/reports on these objectives specifically for imazalil (previous reports), thiabendazole (Addendum A) and pyrimethanil (Addendum B). Progress / outcomes are briefly summarised below:

Imazalil

Abstracts of papers.

Imazalil residue loading and green mould control in citrus pack-houses

Arno Erasmus, Cheryl L. Lennox, Hennie Jordaan, Joseph L. Smilanick, Keith Lesar, Paul H. Fourie. 2011. *Postharvest Biology and Technology* 62: 193–203.

Imazalil (IMZ) is commonly applied in South African citrus packhouses for the control of green mould, caused by *Penicillium digitatum*, yet the disease still causes significant postharvest losses. Maximum residue limit (MRL) for IMZ on citrus fruit is $5 \mu\text{g.g}^{-1}$, whereas $2\text{--}3 \mu\text{g.g}^{-1}$ is regarded as a biologically effective residue level that should at least inhibit green mould sporulation. Standard compliance auditing of residue levels of citrus fruit, however, indicated that fruit from the majority of packhouses have residues of $\approx 1 \mu\text{g.g}^{-1}$. Poor disease control from insufficient residue loading might further be compounded by the presence of IMZ-resistant isolates of *P. digitatum* in packhouses. This study was conducted to assess the current status of IMZ application in South African packhouses, to determine the adequate residue levels needed to control green mould and inhibit its sporulation using both IMZ sensitive and resistant isolates, to investigate IMZ application methods and resultant residue levels in commercial citrus packhouses, and to study optimisation of modes of IMZ application in citrus packhouses. Factors studied were IMZ concentration, application type (spray vs. dip and drench), exposure time, solution temperature and pH, as well as curative and protective control of *P. digitatum*. The packhouse survey showed that the majority of packhouses applied IMZ in a sulphate salt formulation through a fungicide dip tank, and loaded an IMZ residue of $\approx 1 \mu\text{g.g}^{-1}$. In dip applications, IMZ had excellent curative and protective activity against *Penicillium* isolates sensitive to IMZ. However, curative control of IMZ resistant isolates was substantially reduced and protective control was lost, even at twice the recommended concentration, nor was sporulation inhibited. The use of sodium bicarbonate (2%) buffered imazalil sulphate solutions at pH ± 8 , compared with pH ± 3 of the unbuffered solutions, markedly increased IMZ residue loading on Navel and Valencia oranges and improved curative and protective control of IMZ resistant isolates. Exposure time did not affect IMZ residue loading in IMZ sulphate solutions at pH 3, although the MRL was exceeded after 45 s exposure in pH 8 solutions. Imazalil applied through spray or drench application improved residue loading, but green mould control was less effective than after dip application.

Imazalil residue loading on citrus fruit as affected by formulation, solution pH and exposure time in aqueous dip treatments

Arno Erasmus, Cheryl L. Lennox, Joseph L. Smilanick, Keith Lesar, Paul H. Fourie. 2013. *Postharvest Biology and Technology* 77: 43–49.

Green mould, caused by *Penicillium digitatum*, is responsible for major postharvest fruit losses on the South African fresh citrus export market. Some of these losses as well as fungicide resistance development can be attributed to sub-optimal imazalil (IMZ) residue loading on citrus fruit ($<2 \mu\text{g.g}^{-1}$), which is commonly the case in South African packhouses. This will result in loss of control and sporulation inhibition on decayed fruit. IMZ formulation [IMZ sulphate and emulsifiable concentrate (EC)], solution pH (IMZ sulphate at $500 \mu\text{g.mL}^{-1}$ buffered with NaHCO_3 or NaOH to pH 6 and 8) and exposure time (15 to 540 s) were investigated in order to improve IMZ residue loading and the green mould control on Clementine mandarin, 'Eureka' lemon, and navel and Valencia orange fruit. Exposure time had no significant effect on residue loading in the unbuffered IMZ sulphate solution (pH 3). No differences were observed between the pH buffers used, but residue loading improved with increase in pH. The maximum residue limit (MRL) of $5.0 \mu\text{g.g}^{-1}$ was exceeded following dip treatment in the IMZ EC (after 75 s exposure time), and IMZ sulphate at pH 8 using NaHCO_3 (77 s) or NaOH (89 s) as buffer. The MRL was exceeded after 161 s in IMZ sulphate solutions buffered at pH 6 with either NaHCO_3 or NaOH . An IMZ residue loading curve was prepared from which residue levels can be predicted for the control of IMZ-sensitive and IMZ-resistant isolates of *P. digitatum*. From this model the benchmark residue level for 95% control of an IMZ-sensitive isolate and of an IMZ-resistant isolate were predicted to be 0.81 and $2.64 \mu\text{g.g}^{-1}$, respectively. Residue loading can be improved by adjusting the pH upward of an IMZ sulphate solution or by using the IMZ EC formulation, but exposure time should be restricted so as not to exceed the MRL. The resistant isolate could not be controlled adequately with residue levels below the MRL, therewith indicating the practical relevance of IMZ resistance.

Thiabendazole

Abstract of work presented in addendum A:

Thiabendazole (TBZ) residue loading was studied with application methods typically used in South African citrus packhouses, and resulting residue levels correlated with the level of green mould (*Penicillium digitatum*) control and chilling injury incidence. TBZ was applied curatively and protectively to fruit of various citrus types, which were inoculated with *P. digitatum* in dip (60 s in 0 to $2000 \mu\text{g.mL}^{-1}$ TBZ at 22°C), drench (1000 and $2000 \mu\text{g.mL}^{-1}$ TBZ, drenched for 30, 60 or 90 s) and wax coating treatments ($4000 \mu\text{g.mL}^{-1}$ TBZ,

at 0.6, 1.2 or 1.8 L wax /ton fruit). Waxed fruit were also evaluated for chilling injury after storage at -0.5°C for 40 days. Dip treatments resulted in residues of 0.50 – 1.70 $\mu\text{g.g}^{-1}$ at 1000 $\mu\text{g.mL}^{-1}$ TBZ and gave excellent curative control of the TBZ sensitive isolate (98 – 100%). Benchmark residue levels of 0.06 to 0.22 $\mu\text{g.g}^{-1}$ were predicted for 75% curative control, while unreliable protective control of the sensitive isolate (20 – 81%) and very poor control of the resistant isolate was observed (3 – 44%). Wax coating application showed better protective than curative control on Valencia orange (14 – 20% vs. 27 – 40% infection), but poor control on satsuma and Clementine mandarins. Drenching 6 hours after inoculation showed good curative control (63 – 94%), but poor control when drenched after 24 hours, as well as for protective treatments. The resistant isolate could not be controlled in any treatment. The 5 $\mu\text{g.g}^{-1}$ maximum residue limit was sometimes exceeded in wax and drench treatments. Chilling injury on Valencia oranges were reduced by wax coatings, and more so when combined with a TBZ dip prior to waxing, or the inclusion of TBZ in the coating.

Pyrimethanil

Pyrimethanil (PYR) is a newly registered fungicide for postharvest use against green mould caused by *Penicillium digitatum* on citrus. It belongs to a class of fungicides called anilinopyrimidine and its mode of action is to inhibit germ tube elongation and mycelia growth. Development of resistant strains against effective fungicides like imazalil and thiabendazole prompted the registration of PYR to specifically form part of management of disease resistance.

Baseline sensitivity of *P. digitatum* against PYR was determined and reported on in 2011. Likewise, the efficacy of PYR in dip applications was reported in 2011 annual report. Outcomes from this honours study are summarised in the next paragraph.

Baseline sensitivity for PYR against 44 *P. digitatum* isolates and *in vivo* benchmark PYR residue values for effective green mould control following curative and protective dip applications were determined. Effective concentrations where *in vitro* mycelial growth was inhibited by 50% (EC_{50}) and 95% (EC_{95}) was 0.354 and 0.515 $\mu\text{g.mL}^{-1}$, respectively. *In vivo* trials involved infection studies on fruit following curative (24-h-old wound infections) and protective dip-treatment (60 s at 18°C) in range of PYR solutions (0 to 1000 $\mu\text{g.mL}^{-1}$) and were repeated on satsuma, Clementine and twice on navel fruit. PYR residue loading and levels of control varied between fruit types (1.404 to 2.954 $\mu\text{g.g}^{-1}$ for 1000 $\mu\text{g.mL}^{-1}$ treatment) and also within the two navel batches, but trends were generally similar, but with predicted maximum levels of 1.636 to 7.177 $\mu\text{g.g}^{-1}$. Curative control was significantly better than protective control. Mean benchmark PYR residue values for 50% and 75% curative control were 0.268 and 0.905 $\mu\text{g.g}^{-1}$, respectively, while 50% protective control was predicted at 1.181 $\mu\text{g.g}^{-1}$. Sporulation was poorly and inconsistently inhibited. PYR provided sufficient curative control and moderate protective control against green mould, and was able to control an isolate of *P. digitatum* resistant to commonly used imazalil and thiabendazole.

In past season (see Addendum B), the single application of PYR with wax coating and in the drench was evaluated. The application of PYR at 2000 $\mu\text{g.ml}^{-1}$ in wax coating in navel oranges resulted in lower residue loading (2.31 to 3.44 $\mu\text{g.g}^{-1}$) than when applied at 4000 $\mu\text{g.ml}^{-1}$ (3.18 to 5.46 $\mu\text{g.g}^{-1}$). Similar trends were observed in Valencia oranges (2.21 to 3.56 $\mu\text{g.g}^{-1}$) and (4.22 to 8.39 $\mu\text{g.g}^{-1}$). Generally, in both curative and protective treatments, there was poor control of green mould and sporulation inhibition in all the treatments on navel oranges. In Valencia oranges, poor curative control was observed at 2000 $\mu\text{g.ml}^{-1}$ (10 to 21%) and slightly better at 4000 $\mu\text{g.ml}^{-1}$ (22 to 27%). However, protective treatments resulted in 33 to 38% at 2000 $\mu\text{g.ml}^{-1}$ and significantly improved control with increasing coating load (41 to 54%) at 4000 $\mu\text{g.ml}^{-1}$. Sporulation was reduced in the protective treatments, resulting in decreasing level of sporulation with increasing coating load (79 to 61%) and increasing concentration (68 to 51%).

Preliminary results from laboratory drench application of PYR clearly showed that the drench application was not as good as dip or wax application. Compared with previous experience with dip and wax applications, protective control following drench application with PYR was relatively poor despite comparable residue levels loaded on treated fruit. Curative control was significantly better than protective control, but only when treated 6 hours after infection; treatment after 24 hours gave relatively poor control. Further drench trials are planned for the coming season to investigate means of improving green mould control with this application.

Commercial drench evaluation

Factors that were considered for effects on green mould control and residue loading in the trial were drench with/without Sporekill (adjuvant effect), bin position in stack and fruit orientation (facing up or down). Analyses of variance for residues of 2,4-D, thiabendazole, pyrimethanil and fluorescent pigment coverage indicated no significant interactions. The addition of Sporekill had a significant effect on residues ($P < 0.05$)

with lower 2,4-D (0.30 vs. 0.46 $\mu\text{g.g}^{-1}$), thiabendazole (0.30 vs. 0.45 $\mu\text{g.g}^{-1}$), pyrimethanil (0.80 vs. 1.54 $\mu\text{g.g}^{-1}$) residues loaded when the drench mix included Sporekill. This reduction was not noticeable for the fluorescent pigment (5.34 vs. 5.79 %FPC), but a meaningful interaction ($P=0.131$) was observed with fruit orientation: for drenches without Sporekill, significantly lower pigment levels were recorded on the bottom-facing halves of fruit (4.52 %FPC) compared with the top-facing halves (7.07 %FPC), while Sporekill-containing drenches resulted in similar levels between top and bottom halves (5.67 and 5.01 %FPC, respectively). This observation indicates the potential value of the adjuvant in Sporekill, which resulted in improved quality of deposition. However, fruit orientation proved to be a significant factor in 2,4-D residue loading but with bottom halves loading more residue than top halves (0.40 vs. 0.36 $\mu\text{g.g}^{-1}$); a similar but not significant effect was observed in the case of thiabendazole residue loading (0.40 vs. 0.34 $\mu\text{g.g}^{-1}$), but not for pyrimethanil loading (1.19 vs. 1.15 $\mu\text{g.g}^{-1}$). Bin position did not have a significant effect ($P > 0.590$), which indicated that similar residue levels were loaded in all bins, whether stacked in the bottom, middle or top. However, green mould was more effective in top bins and improved with the addition of Sporekill (5.1 and 4.3% disease incidence, respectively), compared with bins in the middle (57.9 and 35.9%, respectively) and bottom of stacks (61.5 and 50.0%, respectively).

These as yet un-replicated results provide valuable insights into the efficacy and possibilities for optimisation of drench applications, but will have to be studied in greater detail. From packhouse trials, it was also clear that residue loading on the fruit decreased over time, which could be attributed to stripping of the active ingredients from the drench mixture, or precipitation in cases where agitation of the mixture is inadequate. This warrants further investigation and we will focus on developing a safe and effective top up protocol.

2. **New objective: Development of a user-friendly fungicide resistance assay for packhouses**

Genotyping of South African isolates

All resistant isolates were found to be of the R3 genotype, showing 2 bands of 506 and 600bp each (Fig. 4.5.4.4). The sensitive isolates all showed 2 bands of 401 and 506bp when using the primers of Sun *et al.* (2011). Subsequently, species specific real time PCR primers were designed for detecting the R3 genotype. The development of the real time PCR is still in progress.

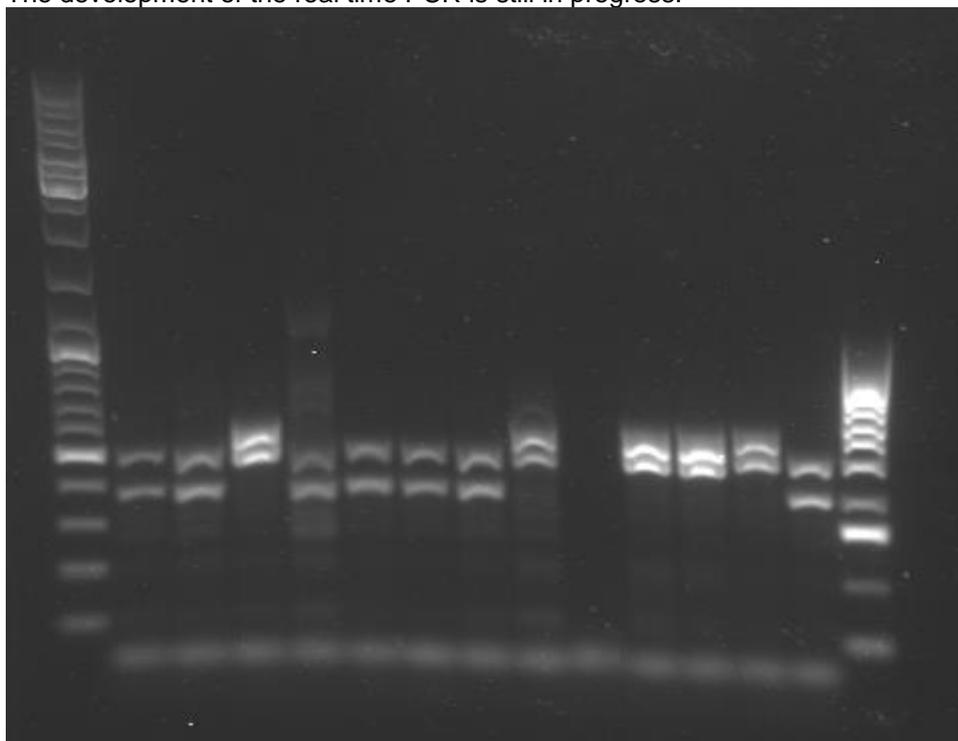


Figure 4.5.4.4.

PCR with primers of (Sun, Wang, Feng, Ma & Li, 2011:1) on *P. digitatum* isolates. Lanes 2, 3, 5, 6, 7, 8 and 14 are IMZ-sensitive isolates with band sizes of 401 and 506 bp. Lanes 4, 9, 11, 12 and 13 are IMZ-resistant and show band sizes of 506 and 600 bp which is expected for the R3 genotype.

Genotyping international isolates

A large collection of 223 IMZ-resistant *P. digitatum* isolates were obtained from Janssen PMP. These isolates were obtained from green moulded citrus fruit from South Africa, USA, Uruguay, Spain, Israel, Cyprus, Chile, Australia and Argentina. The resistance genotypes were identified using a multiplex PCR assay employing previously published primers. A total of 148 isolates yielded PCR products that could be separated and visualised using gel electrophoresis. Isolates from the USA showed the most diversity with 20% identified as R1, 14% as R2 and 66% as R3 type resistance. All the isolates from the other countries were classified as R3 type resistance. The R3 gene (199 bp insertion into the CYP51B gene) therefore could be used as target gene to quantify IMZ resistance in *P. digitatum* populations using real-time PCR. The results furthermore indicate that other uncharacterised resistant genotypes might be involved in IMZ resistance in *P. digitatum*, as 75 resistant isolates could not be genotyped during this assay.

This objective is still in progress.

Conclusions to date

South African citrus packhouses rely extensively ($\approx 78\%$) on the fungicide dip tank to apply IMZ in the sulphate salt formulation. The fungicide dip tank is the most commonly used method to apply imazalil (IMZ) in South African citrus packhouses; either alone or in combination with other application methods. The IMZ sulphate formulation is exclusively used in dip tanks and the IMZ EC (emulsifiable concentrate) formulation in wax. The median fruit exposure time in dip tanks was 47.08 s and medians for solution temperature, pH and IMZ residue level was 33.4°C, 5.37 and 1.02 $\mu\text{g}\cdot\text{mL}^{-1}$, respectively. However, lots of variation existed between packhouses with regard to these parameters. No significant correlation could be made between exposure time, solution pH and solution temperature and other (capacity, length, concentration, age and brushes after dip tank) factors and IMZ residue loading. This indicates the complexity of fungicide application.

Imazalil

In dip tank studies with IMZ sulphate not amended or amended with 2% sodium bicarbonate, it was found that IMZ had a better curative action than protective action against IMZ sensitive (S) or resistant (R) isolates of *Penicillium digitatum*. By amending the pH of the IMZ sulphate solution it was possible to increase the IMZ residue loaded and better control the R isolate. However, a residue of 3.50 $\mu\text{g}\cdot\text{mL}^{-1}$ could only reduce R isolate infection to 7.4 and 19.1% (for curative and protective treatment, respectively). The unamended IMZ sulphate formulation can be regarded as a safe application due to the fact that residue loading could not be increased to higher than 2.06 $\mu\text{g}\cdot\text{mL}^{-1}$ after 540 s exposure to a 500 $\mu\text{g}\cdot\text{mL}^{-1}$ solution, which is well below the MRL of 5 $\mu\text{g}\cdot\text{mL}^{-1}$. This will be sufficient to control IMZ sensitive strains of *Penicillium digitatum*, but not for the resistant strains. This residue can be increased by amending the pH of the IMZ sulphate solution or by using the IMZ EC formulation. When these options are followed, exposure time will become important and needs to be strictly limited in order not to exceed the MRL. The IMZ concentration in the bath will also be sooner depleted in comparison to an unamended IMZ sulphate solution, and should be topped-up more frequently.

Alternative application systems (spray-on and drench) resulted in comparable or better residue levels, although green mould control was not as effective as following dip application. Furthermore it was found that even though exposure time had no effect on IMZ residue loading in an unamended IMZ sulphate solution, it did have an effect on controlling green mould development. Fruit exposed for longer had less green mould infection. Therefore, residue loading should not be regarded as the only factor to measure the effectiveness of a specific application, but the biological efficacy on sensitive and resistant strains of the pathogen following various applications should also be evaluated.

Imazalil rapidly started to lose its efficacy to control green mould when infections were older than 12 hours. This indicated the importance to reduce the time from harvest to first fungicide treatment to as short as possible. When this period becomes longer than 24 hours, curative control was diminished, and the resistant strain could not be controlled.

Brushes after the dip tank decreases IMZ residue loading by 50 to 80%. For curative control, residue loading in the wound sites is most important, and these lower whole-fruit residue levels were sufficient to curatively control the S isolate. However, the R isolate could not be controlled. It is therefore extremely important to limit / manage the development of IMZ resistance. Further work needs to be done on alternatives to brushes and roller doughnuts.

Work done on IMZ application with wax, showed that IMZ is more protective than curative when applied through this application. This is contrary to the dip application as mentioned previously. Imazalil residue levels were significantly increased through a second application in the wax. Through the double application the curative benefits of the aqueous dip and the protective benefits of the wax applications can be utilised.

IMZ application in polyethylene or carnauba wax coating as applied by horsehair or synthetic brushes was studied. Although not conclusively demonstrated, it was shown that horsehair brushes applied a better quality coating, which led to reduced sporulation and/or improved quality retention. Differences were observed between wax types, specifically regarding shine and weight loss control. However, such differences can be expected as formulations differ, but clearly indicate that packhouses should scrutinise the coating options for their specific needs.

Pyrimethanil

PYR provided sufficient curative control and moderate protective control against green mould, and was able to control an isolate of *P. digitatum* resistant to commonly used IMZ and TBZ. Sporulation was poorly and inconsistently inhibited. Mean benchmark PYR residue values for 50% and 75% curative control were 0.268 and 0.905 $\mu\text{g.g}^{-1}$, respectively, while 50% protective control was predicted at 1.181 $\mu\text{g.g}^{-1}$. Research on PYR drench and wax application yielded non-satisfactory results.

Thiabendazole

For TBZ, benchmark residue levels of 0.06 to 0.22 $\mu\text{g.g}^{-1}$ 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 on Clementine and Valencia fruit, but Satsuma fruit showed poor results. TBZ reduced chilling injury on Valencia oranges. Applied in the drench, TBZ gave moderately good control on navel oranges and poor to moderate control on Clementine fruit.

IMZ, PYR and TBZ form the integral part of the green mould postharvest disease management. However, PYR and TBZ appear to be inferior to IMZ in terms of green mould control. It is therefore advised that packhouses focus the use of IMZ in dip and wax applications to maximise protection on exported fruit, while PYR and TBZ is used in drench application to cure harvest and transit infections and protect fruit during the period of degreening and prior to packing. Research on optimising drench application with these fungicides is ongoing.

Technology transfer

Research papers published:

1. Arno Erasmus, Cheryl L. Lennox, Hennie Jordaan, Joseph L. Smilanick, Keith Lesar, Paul H. Fourie. 2011. Imazalil residue loading and green mould control in citrus pack-houses. *Postharvest Biology and Technology* 62: 193–203.
2. Arno Erasmus, Cheryl L. Lennox, Joseph L. Smilanick, Keith Lesar, Paul H. Fourie. 2013. Imazalil residue loading on citrus fruit as affected by formulation, solution pH and exposure time in aqueous dip treatments. *Postharvest Biology and Technology* 77: 43–49.
3. Ncumisa S. Njombolwana, Arno Erasmus and Paul H. Fourie. 2013. Evaluation of curative and protective control of *Penicillium digitatum* following imazalil application in wax coating. *Postharvest Biology and Technology* 77: 102–110.
4. Ncumisa S. Njombolwana, Arno Erasmus, J. Gideon van Zyl, Wilma du Plooy, Paul J.R. Cronje and Paul H. Fourie. 2013. Effects of citrus wax coating and brush type on imazalil residue loading, green mould control and fruit quality retention of sweet oranges. *Postharvest Biology and Technology* (Accepted).

Presentations at international congresses:

1. Paul Fourie, Ncumisa Njombolwana, Arno Erasmus and Keith Lesar. 2011. Postharvest citrus green mould control in South Africa: integration of aqueous and wax fungicides application systems. Invited presentation at Citrus Postharvest Pest Control meeting, 4-5 April 2011, Santa Barbara, USA.
2. Ncumisa Njombolwana, Arno Erasmus and Paul Fourie. 2011. Evaluation of protective and curative control of *Penicillium digitatum* following imazalil application in wax. Invited presentation at Citrus Postharvest Pest Control meeting, 4-5 April 2011, Santa Barbara, USA.
3. Arno Erasmus, Cheryl Lennox, Joseph L. Smilanick, Keith Lesar and Paul H. Fourie. 2011. Aqueous imazalil sulphate application in citrus packhouses, residue loading and green mould control. Invited presentation at Citrus Postharvest Pest Control meeting, 4-5 April 2011, Santa Barbara, USA.
4. Paul H. Fourie, GC Schutte, S Serfontein, SH Swart. 2011. Modelling of *Guignardia pseudothecium* maturation and ascospore dispersal in citrus orchards. Oral presentation at the American

Phytopathological Society Congress, 6-10 August 2011, Waikiki, Hawaii, USA (Phytopathology Vol. 101, No. 6 (Supplement), 2011 S54).

5. Erasmus A., Rikhotso V., Lesar K.H., Lennox C.L., and Fourie P.H. 2012. Practical impact of imazalil resistance on control of postharvest citrus green and blue mould. Poster presentation at XII International Citrus Congress, 18-23 November 2012, Valencia, Spain.
6. Njombolwana N.S., Erasmus A., and Fourie P.H. 2012. Curative and protective control of *Penicillium digitatum* following imazalil application in aqueous dip and wax coating. Poster presentation at XII International Citrus Congress, 18-23 November 2012, Valencia, Spain.
7. Mareli Kellerman, Ncumisa S. Njombolwana, Arno Erasmus, Paul J.R. Cronjé and Paul H. Fourie. 2012. Thiabendazole residue loading for control of green mould and chilling injury on citrus. Oral presentation at the 7th CIGR International Technical Symposium, 25-29 November 2012, Stellenbosch, South Africa.
8. Njombolwana, N.S., Erasmus, A., and Fourie, P.H. 2012. Evaluation of curative and protective control of *Penicillium digitatum* following imazalil application in wax coating. Oral presentation at the 7th CIGR International Technical Symposium, 25-29 November 2012, Stellenbosch, South Africa.
9. Arno Erasmus, Vongani Rikhotso, Cheryl L. Lennox, Keith Lesar and Paul H Fourie. 2012. Practical impact of imazalil resistance on control of postharvest citrus green and blue mould. Oral presentation at the 7th CIGR International Technical Symposium, 25-29 November 2012, Stellenbosch, South Africa.

Presentations at local congresses:

1. Arno Erasmus, Cheryl Lennox, Joseph L. Smilanick, Keith Lesar and Paul H. Fourie. 2010. Citrus green mould control by improved imazalil residue loading in fruit dip applications. Oral presentation at CRI Citrus Research symposium (Drakensberg, Aug 2010).
 2. Ncumisa S. Njombolwana, Arno Erasmus and Paul H. Fourie. 2010. Imazalil *versus* green mould: effect of incubation time on curative control. Poster presentation at CRI Citrus Research symposium (Drakensberg, Aug 2010).
 3. Arno Erasmus, Cheryl Lennox, Joseph L. Smilanick, Keith Lesar and Paul H. Fourie. 2011. The efficacy of imazalil sulphate dip applications for the control of citrus green mould. Oral presentation at 47th SASPP conference (Kruger Park, Jan 2011).
 4. Ncumisa S. Njombolwana, Arno Erasmus and Paul H. Fourie. 2011. Evaluation of protective and curative control of *Penicillium digitatum* following imazalil application in wax. Oral presentation at 47th SASPP conference (Kruger Park, Jan 2011).
 5. Arno Erasmus, Vongani Rikhotso, Cheryl L. Lennox, Keith Lesar and Paul H Fourie. 2012. Practical impact of imazalil resistance on control of postharvest citrus green and blue mould. Oral presentation at the 7th Citrus Research Symposium, Champagne Sports Resort, Drakensberg, 19-22 August 2012.
 6. N.S. Njombolwana, A. Erasmus, P.H. Fourie. 2012. Evaluation of curative and protective control of *Penicillium digitatum* following imazalil application in wax coating. Oral presentation at the 7th Citrus Research Symposium, Champagne Sports Resort, Drakensberg, 19-22 August 2012.
 7. Mareli Kellerman, Ncumisa S. Njombolwana, Arno Erasmus, Paul J.R. Cronjé and Paul H. Fourie. 2012. Thiabendazole residue loading for control of green mould and chilling injury on citrus. Oral presentation at the 7th Citrus Research Symposium, Champagne Sports Resort, Drakensberg, 19-22 August 2012.
 8. E. Liebenberg, A. Erasmus, P.H. Fourie. 2012. Optimal use of pyrimethanil, a new postharvest fungicide, for the control of green mould on citrus in South Africa. Oral presentation at the 7th Citrus Research Symposium, Champagne Sports Resort, Drakensberg, 19-22 August 2012.
 9. W. du Plooy, A. Erasmus, C. Jewell, P. Fourie. 2012. A Heated Imazalil Flooder – New technology for South African packing houses. Oral presentation at the 7th Citrus Research Symposium, Champagne Sports Resort, Drakensberg, 19-22 August 2012.
 10. Ncumisa S. Njombolwana, Arno Erasmus, Paul Cronje, Wilma du Plooy and Paul H. Fourie. 2013. The effect of wax coating and brush type on imazalil residue loading and citrus green mould control. Poster presentation at the 48th Congress of the South African Society for Plant Pathology, ATKV Klein Kariba, 20-24 January 2013.
 11. Mareli Kellerman, Ncumisa S. Njombolwana, Arno Erasmus, Paul J.R. Cronjé and Paul H. Fourie. 2012. Thiabendazole residue loading for control of green mould and chilling injury on citrus. Oral presentation at the 48th Congress of the South African Society for Plant Pathology, ATKV Klein Kariba, 20-24 January 2013.
- Three presentations (1.Sour rot, guazatine and burn; 2. The fungicide bath: Temperature and pH; 3. Optimised drench applications) at the five regional packhouse workshops.

Further objectives (milestones) and work plan

1. Improve the protocol for use on mature citrus fruit.
Completed.
2. Determine benchmark values for biologically effective fungicide residue loading

3. Evaluate residue loading as affected by drench, dip, spray and wax applications in citrus packhouses.
4. Optimise fungicide residue loading in terms of biological efficacy, MRLs and fruit quality. To date, commercial packhouse application systems (drench, dip, wax) were investigated. These studies will be concluded and scientifically published. Future work will focus on optimisation strategies (such as pH and temperature) and management systems (such as top-up and replacement).
5. Development of a user-friendly fungicide resistance assay for packhouses
The ambitious objective of developing a quantitative real-time PCR assay to determine resistance frequencies in packhouses has taken longer than initially expected. Once developed and optimised, it will be validated and implemented at selected packhouses at different times during the season.

Quarterly milestones for Apr-Jun, Jul-Sep, Oct-Dec 2013 and Jan-Mar 2014

Apr-Jun:

- Finalise annual reports
- Objective 2-4: Treatments on early-season cultivars
- Objective 5: Early-season spore sampling

Jul-Sep:

- Objective 2-4: Treatments on mid- and late-season cultivars
- Objective 5: Mid- and late-season spore sampling

Oct-Dec:

- Conclude final treatments and samplings, residue analyses and statistical analyses

Jan-Mar:

- Write-up of final report and scientific articles

Addendum A

Thiabendazole residue loading in dip, drench and wax coating applications to control green mould and chilling injury on citrus fruit

Abstract

Green mould is a major cause of postharvest losses in citrus, and is caused by *Penicillium digitatum*. TBZ is one of the few fungicides registered against this pathogen in South Africa. The aim of the study was to determine the amount of TBZ residues loaded with application methods typically used in South African packhouses, and correlate the residue to the level of control obtained against green mould. TBZ was applied curatively and protectively to fruit inoculated with *P. digitatum* in dip, drench and wax coating treatments. The dip treatments consisted of TBZ concentrations of 0 – 2000 µg.mL⁻¹ and fruit was dipped for 60 s at 22°C at a pH of 6.99. Drench treatments consisted of TBZ concentrations of 1000 and 2000 µg.mL⁻¹ and exposure times of 30, 60 and 90 s. Wax was applied at 0.6, 1.2 and 1.8 L.ton⁻¹ at 4000 µg.mL⁻¹ TBZ. Waxed fruit was also evaluated for chilling injury after storage at -0.5°C for 40 days. Residues loaded in dip treatments differed between fruit batches and ranged from 0.5 – 1.7 µg.g⁻¹ at application of 1000 µg.mL⁻¹ TBZ. Curative dip treatments were very effective. The residues needed for 75% control ranged from 0.06 to 0.22 µg.g⁻¹ depending on citrus type. Average TBZ residues following drench treatments were 2.14 µg.g⁻¹ for Clementines and 3.50 µg.g⁻¹ for navel oranges following 1000 µg.mL⁻¹ TBZ treatments. Exposure time did not have an effect on residue loading, but influenced disease control. Disease control on navel fruit resulted in 77.2 – 92.1%, 67.7 – 89.7% and 31.8 – 37.9% control for curative treatment after 6 h, 24 h and protective treatments respectively. On Clementines it resulted in 66.3 – 84.4%, 33.9 – 55.8% and 9.3 – 17.1% respectively. Average TBZ residues loaded on fruit at a concentration of 4000 µg.mL⁻¹ TBZ in wax was 1.3, 1.3 and 2.7 µg.g⁻¹ at 1.2 L.ton⁻¹ for Satsuma, Clementine and Valencia fruit, respectively. In wax application, protective treatments showed lower infection (14.1 – 19.8%) than curative treatments (26.8 – 40.4%) for Valencia oranges. The same trend was observed with Satsuma (92.2 – 95.3% curative; 86.7 – 90.1% protective) and Clementine fruit (81.5 – 90.4% curative; 58.9 – 87.8% protective). Dip and drench treatments loaded residues far below the MRL, but wax treatments exceeded it at higher wax loads (1.2 and 1.8L/ton). Wax treatments showed a significant reduction in chilling injury. Dip treatments gave better overall control of green mould, but wax application showed a better protective treatment as well as a small amount of sporulation inhibition. Drench treatment was much less effective than dip treatments. It is clear that TBZ is more effective when applied as a dip, although this is not a common application in packhouses. TBZ resistant isolates could not be controlled.

1. Introduction

Thiabendazole (4-(1*H*-1,3-benzodiazol-2-yl)-1,3-thiazole) is a benzimidazole fungicide and can be used curatively (Schirra *et al.*, 2008) and protectively (Brown, 1977) against *Penicillium digitatum*, which causes green mould on citrus, and leads to 90% of postharvest losses (Eckert and Eaks, 1989), and also controls its sporulation (Ladaniya, 2008). The maximum residue level (MRL) of thiabendazole on citrus according to *Codex alimentarius* is 7 µg.g⁻¹, but in European Union member countries it is 5 µg.g⁻¹ (FAO, 2012).

Postharvest TBZ treatment has also been shown to reduce chilling injury, a physiological disorder that reduces the quality of citrus fruit stored at 12°C or below (Eckert and Eaks, 1989). Certain importers require cold sterilisation treatment of South African citrus as a mitigating control measure against certain insect pests. For example, fruit from South Africa exported to China and the USA is kept at -0.6°C for 22 days, and these measures increase the risk of chilling injury (Paul Cronjé, pers. comm.).

Although imazalil (IMZ) is the preferred fungicide for control of green mould (Ladaniya, 2008; Lesar, 2008), IMZ resistance in *P. digitatum* populations have also been widely observed (Holmes and Eckert, 1999; Kinay *et al.*, 2007; Sánchez-Torres and Tuset, 2011), therefore sole reliance on IMZ is not advised.

There are several methods of applying TBZ to fruit, and different methods influence the efficacy of TBZ to control disease. Drenching fruit before degreening is a common practice in South Africa, and since degreening conditions favour the development of green mould, effective fungicide application is crucial (Smilanick *et al.*, 2006). Fruit can be dipped in TBZ, but this is not a popular application method because TBZ tends to precipitate (McCornack, 1970). Schirra *et al.* (2008) did extensive studies with TBZ in the dip tank, and stated that the location of the fungicide is more important than the amount of residue present on the fruit for control of *P. digitatum*. Their study found that different application parameters led to TBZ being loaded at different depths into the fruit skin, and this influenced the fungicide's bio-efficacy, even to control TBZ-resistant isolates. It is therefore important that the application of TBZ is optimal to ensure efficacy and prevent the development of resistance. TBZ can also be applied in a wax coating, and this is a popular method because it saves costs in fungicide application (Gutter, 1970), and it loads higher residues on fruit (Hayward and McCornack, 1971), but it was shown to be less effective in controlling disease than other application methods (Brown, 1984).

Erasmus *et al.* (2011) did a survey to determine the most commonly used fungicide application methods and parameters in South African citrus packhouses. No work has been done to evaluate TBZ efficacy under these conditions. Therefore the aim was to study TBZ residue loading and its efficacy in controlling green mould on citrus in three different applications: dip, wax coating and drench as commonly applied by South African citrus packhouses. Also, the efficacy of TBZ to reduce chilling injury incidence when applied through a wax coating was studied.

2. Materials and methods

2.1 Fruit

Satsuma and Clementine mandarins (*Citrus reticulata* Blanco), and navel and Valencia oranges (*Citrus sinensis* (L.) Osbeck) were transported from packhouses in Franschoek and Citrusdal. It was ensured that fruit was harvested at most 2 days before collection and not treated with any post-harvest chemicals. Fruit was washed over rotating brushes using a 1 mL/L didecyl dimethyl ammonium chloride (Sporekill, ICA International, Stellenbosch, South Africa), dried at ambient temperature in a drying tunnel and stored at 4°C. One day before the trial, the fruit was transferred from cold storage to 22°C.

2.2. Fungal cultures and spore suspensions

Two isolates of *P. digitatum* were used in the trials: isolate STE-U 6560 that is sensitive to TBZ and IMZ, and isolate STE-U 6590 that is resistant to TBZ and IMZ (Erasmus *et al.*, 2011). The isolates were plated out from -80°C storage cultures onto PDA (DIFCO, Becton, Dickinson and Company, USA and Le Pont de Claix, France) and grown for 7 – 14 days at 25°C before each trial. Spore suspensions were freshly prepared each day of inoculation by filtering the culture grown on PDA through two layers of cheesecloth with distilled water with Tween 20 (Sigma-Aldrich, St Louis, MO, USA) added at a concentration of 0.01 mL/L. Spores were counted with a haemocytometer and final spore concentration adjusted to 10⁶ spores per mL. Viability of spores was verified after each trial by plating out the used spore suspension on PDA.

2.3. Fruit Inoculation

Fruit were inoculated by dipping a sterilized wound inducer into the spore suspension and making a 2 mm deep wound into the peel, piercing both the flavedo and albedo. Four wounds were made on each fruit equidistantly around the calyx. Wound inducers used for the dip trials consisted of three insect needles placed in a needle clamp to create three small wounds of 0.5 mm wide and 2 mm deep at a triangular distance of 1.5 mm apart. Wound inducers used for wax and drench trials consisted of a 7-mm diameter cylindrical rod with a protruding tip 2 mm long and 1 mm in diameter.

2.4. TBZ residue loading and effective residue values for curative and protective control of *P. digitatum* using dip treatments

For Clementine mandarins, a 6×2×2 factorial experiment was done where fruit was dipped in one of 6 different concentrations of TBZ (0, 50, 100, 250, 500 or 1000 µg.mL⁻¹). For navel and Valencia oranges, a 7×2×2 factorial experiment was done where fruit was dipped in one of 7 different concentrations of TBZ (0, 50, 100, 250, 500, 1000 or 2000 µg.mL⁻¹). Fruit were inoculated with either the sensitive or resistant isolate of *P. digitatum* and treated curatively or protectively. Fruit treated curatively were inoculated 24 hours before the dip treatment. Control fruit were not dipped but only inoculated with the TBZ-sensitive (S) or TBZ-resistant (R) isolate. The inoculated fruit were placed in cartons and covered with polyethylene bags and incubated at 22°C. Four replications were done for each treatment.

Dip baths were prepared by adding municipal water into 25-L plastic baths the night before to allow the water to reach ambient temperature (22°C) overnight. TBZ (Thiazole 500 SC, Villa Crop Protection) was added to each bath to make up concentrations of 50, 100, 250, 500, 1000 or 2000 µg.mL⁻¹. One litre of water from each bath was replaced with 1 L of hot water into which TBZ has been dissolved. One of the baths contained water only. Fruit were dipped for 60 seconds in the bath. Baths were stirred frequently during the trial in order to prevent any precipitation of TBZ. Six randomly selected non-inoculated fruit were sampled from each dip treatment for TBZ residue analysis. The fruit for the protective trial were allowed to dry and inoculated on the same day they were dipped with a freshly prepared spore suspension. Different TBZ suspensions were used to treat fruit inoculated with sensitive and resistant isolate.

Fruit were placed in cartons and wrapped with polyethylene bags and incubated at 22°C for 11 days. Infection was rated with a UV light (UV-A at 365 nm, Labino Mid-light; www.labino.com) after 4 and 5 days' incubation for curative and protective treatments, respectively. The number of infected wounds was recorded, where the UV light made a yellow fluorescent lesion visible that could not be seen with the naked eye (Erasmus *et al.*, 2011). Sporulation was rated after 10 or 11 days on a scale of 0 – 6 (0 = no sign of disease; 1 = visible lesion but no sporulation; 2 = sporulating area on lesion smaller than a quarter of the fruit; 3 = sporulating area larger than a quarter of the fruit, but smaller than half of the fruit; 4 = sporulating area larger than half of the fruit, but smaller than three quarters of the fruit; 5 = sporulating area larger than three quarters of the fruit, but smaller than the whole fruit; 6 = sporulating area covering the whole fruit). Incidence of sporulation inhibition was calculated for infected fruit only, with sporulation index of 1 to 3 regarded as inhibition and 4 to 6 regarded as sporulation.

Dip trials were conducted 6 times; twice each on Clementine, navel and Valencia fruit.

2.5. Wax coating trials

Fruit was treated on a custom-built experimental packline (Dormas, Johannesburg, South Africa) resembling those at commercial packhouses. It consisted of four modular units: an elevator feeding fruit into the line, a re-cycling spray-on washing system over 8 brushes, a coating applicator with 8 rotating synthetic brushes and coating applicator (JBT Foodtech, Brackenfell, South Africa), and a drying tunnel that uses high volume air at low speeds (ambient temperature) to dry the fruit. The wax coating applicator was calibrated using one pulsating nozzle (0.5 s on, 2 s off) at 22 mL.min⁻¹ (3 bar). The whole packline is speed-controlled and the wash and coating units have brush-sweep paddles to move fruit across the unit at a set speed. The rotating brushes were adjusted to 100 rpm for Satsuma and Clementine mandarins, and to 120 rpm for treatment of Valencia fruit.

Wax was prepared by adding 0.04 mL/L anti-foaming adjuvant (Chempac, Paarl, South Africa) to a carnauba wax coating (Citrashine, John Bean Technologies, Brackenfell, South Africa). Wax coating was applied on the rotating brushes for 15 s to saturate them with wax before the fruit was treated. Buffer fruit was put through the applicator before and after each replication's treatment fruit. Brushes were flushed for 5 s before increasing the wax load. Different brush sets were used for the fruit treated with wax only and fruit treated with TBZ-amended wax. Six non-inoculated fruit were added to each treatment to be used for residue analysis.

A factorial experiment was done where fruit were treated with the non-amended carnauba wax coating or wax coating amended with 4000 µg.mL⁻¹ TBZ (ICA Thiabendazole 500SC, ICA International, Stellenbosch, South Africa). Wax was applied at three different wax loads: 0.6 L/ton of fruit (10 s exposure on wax applicator), 1.2 L/ton of fruit (20 s exposure) and 1.8 L/ton of fruit (30 s exposure); in each case the line speed was adjusted. After applying the wax coating, fruit was dried in the drying tunnel.

Curative green mould control was evaluated by treating fruit that was inoculated with freshly prepared spore suspensions of either the sensitive or the resistant isolate 24 h prior to wax coating treatment, while protective control was evaluated by inoculating fruit 1- 3 hours after waxing. Fruit were incubated and rated in the same manner as with the dip trials.

The remainder of treated fruit (12 fruit per treatment) was not inoculated but stored at -0.5°C for 40 days for evaluation of the effects of TBZ-incorporated wax treatment on chilling injury. To evaluate the effects of TBZ dip treatment followed by non-amended wax treatment, fruit were dipped in 1000 µg.mL⁻¹ TBZ (Valencia oranges), or 2000 µg.mL⁻¹ TBZ (Satsuma and Clementine mandarins) and then treated with non-amended wax at a load of 1.2 L/ton fruit. To evaluate the effects of no dip, water or TBZ dips, fruit were dipped either in water or a 1000 µg.mL⁻¹ aqueous TBZ suspension (Valencia oranges) or 2000 µg.mL⁻¹ TBZ (Clementine and Satsuma mandarins) or left untreated before cold storage. After 40 days, fruit was taken out of storage and left at 22°C for 4 days. Chilling injury was rated on a scale of 0 to 3, where 0 = no pitting, 1 = slight pitting, 2 = medium pitting and 3 = severe pitting (Sala, 1998). Additionally, the effect of treatments on the preservation of fruit buttons was analysed by rating the incidence (%) of green buttons.

Four replications were done for each treatment combination, using 12 fruit per treatment combination. Controls consisted of non-waxed or waxed fruit that was inoculated 24 hours before or 1 – 3 hours after waxing of treatment fruit. Wax trials were repeated 6 times, twice per citrus type.

2.6. Drench trials

Unwashed fruit were packed into black plastic drench crates (480 x 310 x 280 mm; closed sides) according to replicates. Each drench crate had evenly spaced holes in the bottom to allow the TBZ suspension to run through. The holes comprised the same surface area compared to the holes in the bottoms of wooden commercial bins commonly used in South Africa (CHEP). To prevent fruit blocking holes, three PVC pipes were placed at the bottom of each crate and a layer of non-inoculated buffer fruit was placed in the bottom of each drench crate. Inoculated fruit was placed in the crate with half of it facing button-up and the other button-down. Fruit was inoculated 24 hours before drenching, 6 hours before drenching (curative treatments) or 1 day after drenching (protective treatment), as described previously. A layer of non-inoculated fruit was placed over the inoculated fruit in the top of each crate. Fruit in each crate were drenched using an experimental drench system designed to simulate industry best-practice. The drench fungicide mixture was pumped through a spray manifold at 52 L/min, which simulated the industry recommended standard of 260 L/bin, into an empty drench crate and showered evenly through the evenly spaced holes onto the fruit in the drench crate below. The fungicide mixture was collected in and re-circulated from a larger container in which the fruit crate was placed; in an elevated position so as to prevent fruit from standing in fungicide mixture. Different exposure times (30 sec, 60 sec and 90 sec) and different TBZ concentrations (1000 or 2000 µg.mL⁻¹) were evaluated. After drenching, fruit was left to dry overnight before being packed into boxes. Four replications were done in each trial and the trials were repeated 4 times on Clementine and navel fruit.

2.7. Residue analysis

For each treatment combination, six non-inoculated fruit per replication were sampled for residue analysis, and stored at -20°C until they were prepared for residue analysis. Fruit were chopped, then weighed and pulped for 2 minutes using a blender (Salton Elite, Almagamated Appliance Holdings Limited, Reuven, South Africa). Pulped fruit samples were stored at -20°C. Samples from the first and last replication of each treatment were sent for TBZ residue analyses by an accredited analytical laboratory (Hearshaw and Kinnes Analytical Laboratory, Westlake, Cape Town). Samples were extracted by using acetonitrile followed by a matrix solid phase dispersion extraction. Analysis of the extracts was conducted in liquid chromatography mass spectrometry (LCMS/MS; Agilent 6410, Agilent Technologies Inc., Santa Clara, CA, USA).

2.8. Statistical analysis

For the green mould control trials (dip, drench and wax), wound infection data were normalized by calculating the percentage control relative to the untreated control treatment. For the chilling injury trial, data was used unchanged. Data were subjected to analysis of variance, means were separated using Fisher's least significant difference test at 95% confidence interval, and trends described using appropriate non-linear regression analyses in XLSTAT version 2011.4.02 (www.xlstat.com).

- **Results**

3.1. TBZ residue loading and effective residue values for curative and protective control of *P. digitatum* using dip treatments

Analysis of variance for residue data was done separately for each citrus type (Table 1). For Clementine mandarins, there was a significant interaction between trial and TBZ concentration ($P < 0.0001$). Fisher's LSD showed that there were no differences in terms of residue loading between the two trials at all TBZ concentrations used except at the lowest and highest concentrations (50 and 1000 µg.mL⁻¹ TBZ). These differences were less than 0.15 µg.g⁻¹, and trends were similar, thus the trial factor was ignored in further analysis of the data. For navel oranges, an interaction between TBZ concentration and trials was not

observed ($P = 0.9254$), and only TBZ concentration had an effect ($P < 0.0001$). For Valencia oranges, there was also an interaction between trial and TBZ concentration ($P < 0.0001$), and differences occurred between trials at all the TBZ concentrations used, but to describe a more robust residue loading trend, data was combined. TBZ residue loading on Clementine, navel and Valencia fruit followed linear trends (R^2 values = 0.77, 0.93, 0.76, respectively). Clementine fruit loaded TBZ residues at a slower rate and had a predicted residue of $0.5 \mu\text{g.g}^{-1}$ following dips at $1000 \mu\text{g.mL}^{-1}$ for 60 s. Navel and Valencia fruit showed markedly faster loading rates, with residues of 1.5 and $1.4 \mu\text{g.g}^{-1}$ respectively, predicted following a 60 s dip in $1000 \mu\text{g.mL}^{-1}$ TBZ (Fig. 1).

Analysis of variance of percentage control data showed a significant interaction between trial, TBZ concentration, isolate and action for all citrus types ($P = 0.0015$, $P = 0.0036$ and $P < 0.0001$ for Clementine, navel and Valencia fruit, respectively; Table 2). Differences between trials were largely ascribed to susceptibility differences between fruit batches and trial effects were ignored in further analyses. Non-linear regression was done with curative and protective control of the sensitive and resistant isolate as response variables and TBZ residue as dependent variable. Effective TBZ residue levels indicating 50% and 75% curative or protective green mould was calculated from the cultivar-specific model equations (Table 3). Low residue levels of TBZ were needed to obtain 75% curative control of the sensitive isolate on all citrus types (0.22 , 0.13 and $0.06 \mu\text{g.g}^{-1}$ for Clementine, navel and Valencia fruit respectively).

No accurate models could be fitted to protective treatment data, and also not for control of the resistant isolate ($R^2 = 0.01 - 0.53$). At the recommended concentration of $2000 \mu\text{g.mL}^{-1}$ TBZ, the control obtained from protective treatments of the sensitive isolate was considerably lower for Valencia and navel oranges than the curative treatments (50.0 and 55.0% vs. 94.0 and 98.0%, respectively). Both the curative and protective treatments of the resistant isolate at the recommended concentration led to 0.0 – 4.0% control for both orange types. These did not differ significantly from untreated control treatments. Protective control of the sensitive isolate on Clementine mandarins was very low (16.9 – 17.0%) compared to curative treatments (90.9 – 91.7%). The resistant isolate was controlled by 9.3 – 17.3% on Clementine mandarins with no significant difference between curative and protective control. Interestingly, protective control of the sensitive isolate increased marginally (6.2% to 25.1%) when the TBZ concentration was raised from 1000 to 2000 $\mu\text{g.mL}^{-1}$ in the case of Valencia oranges, but not Clementine mandarins and navel oranges.

For sporulation inhibition, analysis of variance showed a significant interaction between action, isolate and treatment for navel and Valencia oranges ($P < 0.0001$ and $P = 0.0022$ respectively; Table 4). Generally, protectively treated fruit had greater sporulation inhibition than curatively treated fruit, and sporulation inhibition increased with increasing TBZ concentration. For Clementine fruit, a significant interaction was observed between trial, treatment and isolate ($P = 0.0096$). Greater sporulation inhibition was seen in the second trial compared to the first trial. Generally, increasing TBZ concentration led to increased sporulation inhibition. For navel oranges, sporulation inhibition was only seen at 1000 and 2000 $\mu\text{g.mL}^{-1}$ TBZ, with curative and protective control of the sensitive isolate. At 2000 $\mu\text{g.g}^{-1}$ TBZ, the best results were seen for sporulation inhibition (48% incidence for both curative and protective, compared to 99.5 – 100% on untreated control fruit). On Valencia oranges, data was variable, but the best results were seen at 2000 $\mu\text{g.g}^{-1}$ TBZ for curative and protective control of the sensitive isolate (24% and 48 % respectively, compared to 92 – 99.5% on control fruit). On Clementine mandarins, the only significant lower sporulation incidences were at 500 and 1000 $\mu\text{g.g}^{-1}$ TBZ for the sensitive isolate of the second trial. (38% for curative and 94% for protective treatments at 1000 $\mu\text{g.mL}^{-1}$ TBZ, compared to 99.5 – 100% for control fruit).

3.2. Wax coating trials

Analysis of variance of TBZ residue data showed a significant interaction ($P = 0.0009$) between wax coating load and citrus type (Table 5). Valencia oranges loaded significantly higher residues than Satsuma and Clementine fruit in TBZ amended wax (Table 6) and frequently exceeded the MRL of $5 \mu\text{g.g}^{-1}$ at wax loads of 1.2 and 1.8 L.ton^{-1} . Residue loading on the two soft citrus types did not differ significantly. TBZ residues increased with increasing wax load (from 0.40 to 3.10 $\mu\text{g.g}^{-1}$ for Clementine fruit, 0.40 – 2.90 $\mu\text{g.g}^{-1}$ for Satsuma, and 2.70 – 7.60 $\mu\text{g.g}^{-1}$ for Valencia oranges. TBZ residue loading following increasing wax loads followed linear trends ($R^2 = 0.66$, 0.60 and 0.80 for Clementine, Satsuma and Valencia fruit respectively). For Satsuma fruit, the rate of residue loading follows the equation $y = 1.3893x$, for Clementine fruit $y = 1.4591x$ and for Valencia fruit $y = 4.4032$ (where y is the residue loaded in $\mu\text{g.g}^{-1}$ and x is the wax load in L.ton^{-1}). Valencia fruit had a much greater rate of residue loading than the soft citrus types.

Given the variable infection data obtained on untreated control fruit, percentage infection data was used rather than percentage control data. Analysis of variance showed a significant interaction between citrus type, isolate (sensitive or resistant isolate of *P. digitatum*), action (curative or protective) and treatment (wax load combined with TBZ concentration) ($P < 0.0001$; Table 7). High infection levels were observed with the resistant isolate and no significant difference was generally observed between control, non-amended and

TBZ amended wax treatments on all citrus types. For the sensitive isolate, better protective than curative control was generally observed with TBZ applied in wax (Table 8). For Valencia oranges, protective treatment with TBZ led to 14.1 – 19.8% infection compared with 89.6% on control fruit and 91.1 – 94.7% on waxed control fruit; no significant difference was observed between TBZ amended wax coating loads. Curative treatment resulted in somewhat higher infection levels, with significantly better control at 1.2 and 1.8 L wax/ton fruit compared with 0.6 L wax/ton fruit (26.8 – 27.1% vs. 40.4% respectively). For the two soft citrus types, infection levels were only slightly reduced by TBZ amended wax treatments. Satsuma fruit showed the highest infection of all citrus types, with protective TBZ wax treatments ranging from 87.0 – 90.1% infection compared with 91.1% on control fruit and 92.1 – 96.3% on waxed control fruit. Curative treatment resulted in 92.1 – 95.3 % infection. There was no significant difference between wax coating loads or between treated and control fruit. For Clementine fruit, protective TBZ wax treatments led to 58.6 – 87.8% infection compared with 90.1% on control fruit and 83.3 – 91.1% on waxed control fruit. Curative treatment resulted in 81.5 – 90.4% infected fruit and infection decreased significantly with increasing wax load.

For sporulation incidence, analysis of variance showed a significant interaction between citrus type, isolate (resistant and sensitive), action (curative or protective) and treatment (wax load combined with TBZ concentration) ($P < 0.0001$; Table 7). Sporulation incidence was near 100% for all infected waxed or non-amended waxed control fruit. For the resistant isolate, no sporulation inhibition was observed. For the sensitive isolate, significantly lower incidence of sporulation was observed when Clementine or Satsuma fruit was treated curatively (0.0 – 41.7% and 0.0 – 22.8% for Clementine and Satsuma fruit, respectively) compared to protective treatment (12.6 – 73.3% and 8.7 – 54.8%; Table 9), especially at the higher wax loads. To the contrary, sporulation incidence of the sensitive isolate on Valencia fruit was lower on protectively (8.3 - 43.6%) than curatively treated fruit (9.2 - 61.1%). Treatment with TBZ amended wax generally resulted in a decreasing sporulation incidence with increasing wax load.

Analysis of variance showed that the trial \times treatment interaction was significant ($P < 0.0001$; Table 10) for chilling injury incidence. Trials with the same citrus type were compared to each other and differences were observed between the two Valencia trials, where treatments in the second trial led to much lower CI incidence than in the first trial. Also control fruit of the Satsuma trials had different levels of CI and therefore all the treatments differed between the two trials. The two Clementine trials showed similar results. Since each fruit batch will have a different susceptibility to CI and trends were the same, this interaction was ignored, and data for each citrus type was grouped and analysed together. The Satsuma and Clementine fruit showed a low incidence of chilling injury on control fruit, and none of the treatments lowered this incidence significantly. Control fruit from the Valencia trials showed a high amount of chilling injury (79.2%; Table 11), and this was significantly lowered by all the treatments, except for dipping fruit into cold water. The non-amended wax treatments at 0.6 and 1.2 L/ton and the TBZ dip treatment led to a significantly higher incidence of CI than the other TBZ-amended wax coating treatments (44.8 – 47.2 vs. 18.8 - 27.1%). The TBZ wax at 1.8 L/ton and the TBZ dip followed by non-amended wax treatments at 1.2 L/ton showed the lowest mean CI incidence (18.8 and 18.1%, respectively).

Analysis of variance showed a significant interaction between treatment and citrus type for amount of buttons that remained green during storage ($P < 0.0001$; Table 12). Clementine fruit had the highest incidence of green buttons (77.8% on control fruit; Table 13), followed by Valencia oranges (83.3%), and Satsuma mandarins had a very low incidence (8.3%). Non-amended wax had a beneficial effect on button appearance of soft citrus (36.5 – 37.5% for Satsuma fruit at 0.6 – 1.8 L/ton and 96.9% for Clementine fruit at both 1.2 and 1.8 L/ton, but significantly lower levels at 0.6 L/ton (76.7%). On Valencia oranges, dip treatments seemed to have a detrimental effect (47.2%). TBZ amended wax led to similar incidence than control fruit on Satsuma mandarins and Valencia oranges, while on Clementines at 1.2 – 1.8 L/ton an increase was observed (92.7 – 93.8 % compared to controls which was 77.8%).

3.3. Drench trials

Analyses of variance for TBZ residue values on navel oranges showed no interaction between TBZ concentration and exposure time ($P = 0.7257$); TBZ concentration had a significant effect ($P = 0.0023$), but the effect of exposure time was not significant ($P = 0.5498$; Table 14). A TBZ concentration of 2000 $\mu\text{g.mL}^{-1}$ loaded significantly higher residues than 1000 TBZ $\mu\text{g.mL}^{-1}$, (mean of 5.40 vs. 3.50 $\mu\text{g.g}^{-1}$) and often exceeded the MRL of 5 $\mu\text{g.g}^{-1}$.

On Clementine fruit, exposure time did not have a significant effect with residues increasing only marginally from 30 to 90 s exposure at 1000 $\mu\text{g.g}^{-1}$ (2.02 to 2.25 $\mu\text{g.g}^{-1}$; Table 15). TBZ concentration appeared to have some effect as 45 s exposure to 2000 $\mu\text{g.g}^{-1}$ resulted in higher residues (3.06 $\mu\text{g.g}^{-1}$).

Analysis of variance of control data from navel oranges showed a significant interaction between orientation and action ($P = < 0.0001$; Table 16), and between TBZ concentration and action ($P = 0.0035$). Disease was

controlled significantly better on fruit facing to the top (button-up) than fruit facing down, for 24-h curative (89.7 vs. 67.7%, respectively), 6-h curative (92.1 vs. 77.2%) as well as protective treatments (37.9 vs. 31.8%). For the curative treatments, there was no significant difference in the percentage control obtained whether 1000 or 2000 $\mu\text{g}\cdot\text{mL}^{-1}$ TBZ was used (77.7 - 79.6% and 83.9 - 85.4% for 6-h and 24-h curative treatments, respectively). However, for the protective treatments, 2000 $\mu\text{g}\cdot\text{mL}^{-1}$ TBZ resulted in a higher percentage control (39.8 vs. 29.9%). Exposure time had a significant effect ($P = 0.0002$): drenching for 30 s led to significantly poorer control (62.0%) than drenching for 60 or 90 s (67.2 - 69.0%).

On Clementine mandarins, analysis of variance of control data showed a significant interaction between orientation and action ($P = 0.0183$; Table 17), and between treatment and action ($P = 0.0010$). Fruit facing button-up led to better disease control than fruit facing button-down for 24-h curative (55.8 vs. 33.9%), 6-h curative (84.4 vs. 66.3%) and protective treatments (17.1 vs. 9.3%). There was no significant difference among treatments (consisting of fruit drenched with 1000 $\mu\text{g}\cdot\text{mL}^{-1}$ TBZ for 30 s, 1000 $\mu\text{g}\cdot\text{mL}^{-1}$ TBZ for 60 s, 1000 $\mu\text{g}\cdot\text{mL}^{-1}$ TBZ for 90 s, or 2000 $\mu\text{g}\cdot\text{mL}^{-1}$ TBZ for 45 s) for the 6-h curative action (71.6 - 77.6% control); for curative 24h action the only treatments that differed from each other were 1000 $\mu\text{g}\cdot\text{mL}^{-1}$ 30s and 2000 $\mu\text{g}\cdot\text{mL}^{-1}$ 45s (36.2 vs. 54.9 %) and for protective treatments the 1000 $\mu\text{g}\cdot\text{mL}^{-1}$ 30s treatment led to the highest control (21.6%) and differed from the other treatments (6.0 - 12.9%).

Analysis of variance of sporulation incidence data from navel oranges indicated interactions between action and exposure time ($P = 0.0100$; Table 18) and an interaction between exposure time and TBZ concentration ($P = 0.0366$). At 1000 $\mu\text{g}\cdot\text{mL}^{-1}$, 90 s exposure time led to a significantly higher sporulation incidence than 60 s or 30 s (53.5 vs. 37.2 - 38.9% respectively). At 2000 $\mu\text{g}\cdot\text{mL}^{-1}$ there were significant differences between all the exposure times (20.1, 32.6 and 43.1% for 30, 60 and 90 s respectively). There was no significant difference between exposure times for 24-h curative treatments (27.1 - 39.6%), but for 6-h curative treatments 30s exposure time led to significantly less sporulation incidence than 60 and 90 s (28.6 vs. 42.2 - 50.5% respectively). For protective treatments, drenching for 90 s led to significantly higher sporulation (54.7%) than drenching for 30 or 60 s (28.6 - 32.8%).

On Clementine mandarins, analysis of variance showed a significant interaction between treatment and action ($P = 0.0003$; Table 19) for sporulation incidence. For 24-h curative treatments, similar sporulation incidence was observed for the 1000 $\mu\text{g}\cdot\text{mL}^{-1}$ drench for 30 s and 2000 $\mu\text{g}\cdot\text{mL}^{-1}$ drench for 45 s (39.4 - 48.2%), and this differed significantly from the 1000 $\mu\text{g}\cdot\text{mL}^{-1}$ drenches for 60 s and 90 s (65.8 - 70.0%). For 6-h curative treatments, sporulation incidence was less after the 2000 $\mu\text{g}\cdot\text{mL}^{-1}$ drench for 45 s (35.1%) than the other treatments (53.8 - 57.4%). Protective treatments showed markedly lower sporulation incidence at the 2000 $\mu\text{g}\cdot\text{mL}^{-1}$ drench for 45 s (25.0%) compared to the other treatments (72.6 - 90.4%).

4. Discussion

This study clearly indicated differences in TBZ residue loading and green mould control between different application methods commonly used in South Africa. TBZ applied as a dip treatment loaded the lowest residues, but it gave the best curative control of green mould, and relatively poor protective control on all citrus types. When TBZ was applied as a wax coating, protective treatments led to better control than curative treatments, although control was relatively poor. The drench application of TBZ was most effective if fruit was drenched 6 h after inoculation, and its efficacy diminished when drenched 24 h after inoculation, especially on Clementine mandarin fruit. Protective drench treatments were not effective. Protective control seemed especially dependant on the amount of residue loaded on fruit in all applications (except in the wax treatments), but TBZ does not seem to have a good, reliable protective action in any of the applications. TBZ was also shown to reduce chilling injury incidence in wax coating application. The study shows that controlling TBZ resistant isolates will be problematic in South African packhouses.

For all three citrus types, the residue levels loaded when dipped in 1000 $\mu\text{g}\cdot\text{mL}^{-1}$ TBZ for 60 s at 22°C and pH 6.99 were well below the MRL of 5 $\mu\text{g}\cdot\text{g}^{-1}$, and ranged from 0.50 - 1.60 $\mu\text{g}\cdot\text{g}^{-1}$. At the recommended concentration of 2000 $\mu\text{g}\cdot\text{mL}^{-1}$ TBZ the MRL was not exceeded and ranged from 2.90 - 3.10 $\mu\text{g}\cdot\text{g}^{-1}$ on Valencia and navel oranges. A linear relationship was found between TBZ concentration and the amount of TBZ residue loaded on fruit. Smilanick *et al.* (2005) also found a linear relationship between TBZ concentration and residues loaded on lemons. Navel fruit loaded significantly higher residues than other citrus types. This might be due to the navel end providing a larger surface area to adsorb TBZ. Schirra *et al.* (2008) loaded higher residues with lower TBZ concentrations at 20°C on Valencia oranges, and found that these fruit loaded more residues than Salustiana oranges, which demonstrates the influence of citrus type on residue loading. Residue loading with pyrimethanil was also found to be influenced by citrus type (Smilanick *et al.*, 2006; Liebenberg, 2011). However, imazalil residue loading was not influenced by type according to Erasmus *et al.* (2011), except when imazalil sulphate was applied at an increased pH (Erasmus *et al.*, 2013). Smilanick *et al.* (1997) found that different citrus types did indeed load different IMZ residues.

Nordby and Nagy (1977) showed that the epicuticular wax of different citrus cultivars differed significantly with regards to the type of hydrocarbons found in them. A change in epicuticular wax of citrus fruit was observed by Palma *et al.* (2013) after treatment with TBZ. The use of adjuvants alter the epicuticular wax by solubilizing certain components (Tamura *et al.*, 2001), and it was suggested that the change in epicuticular wax appearance is due to the adjuvants in the TBZ formulation, and not necessarily due to TBZ itself (Palma *et al.*, 2013). This might explain why higher residues were loaded on fruit in other studies; for example, Schirra *et al.* (2008), D'Aquino *et al.* (2013), Hordijk (2013) and Palma *et al.* (2013) all used Tecto® (thiabendazole, Syngenta, South Africa) in their studies and loaded higher TBZ residues than found in this study. The location of TBZ deposition on the fruit skin was found to be mostly on the outer surface (Schirra *et al.*, 2008) which contributed to the hypothesis that the condition and composition of the epicuticular wax influences residue loading. Schirra *et al.* (2008) also found that the location of the TBZ residue on the fruit is very important, *i.e.* whether it is superficially on the skin, absorbed into the cuticle or even deeper into fruit tissue.

Schirra *et al.* (1998) also found that the fruit peel condition at harvest influenced TBZ residue loading, which might explain differences between fruit batches of the same type. In our study, different fruit types were treated at different times, and it would be interesting to simultaneously investigate residue loading on different fruit types of similar maturity standards. Also, the organic acids contained in fruit may lead to different degradation rates of the fungicide in different fruit types, and it is known that the half-life of pesticides differ among plant species (Fantke and Juraske, 2013), and since samples were not processed immediately this might have played a role in this study.

When applied as a dip treatment, TBZ performed best when used curatively. This confirmed the work of Smilanick *et al.* (2006). Effective residue levels for 50% control ranged from 0.03 – 0.11 $\mu\text{g.g}^{-1}$ and were 0.06 – 0.22 $\mu\text{g.g}^{-1}$ for 75% control depending on citrus type. This is much lower than effective residue levels of IMZ: 0.23 $\mu\text{g.g}^{-1}$ IMZ was needed to obtain 50% control (Erasmus *et al.*, 2013). Gutter (1970) found that infection in curatively treated Valencia oranges only lowered from 8.1 to 4.6% when TBZ concentration was increased from 500 to 2000 $\mu\text{g.mL}^{-1}$. This corresponds with the results of this study that showed a maximum level of control is already obtained at very low residues. Even though navel oranges loaded the highest residue, Valencia oranges generally showed better control (and therewith lower effective residue values), even with protective treatments. Schirra *et al.* (2008) and Erasmus *et al.* (2011) also found that Valencia fruit were naturally less susceptible to green mould than other citrus types used in their studies. Different citrus cultivars are known to produce different flavonones which may influence their resistance to green mould and have different susceptibility levels to *P. digitatum* (Del Rio *et al.*, 1998; Ortuño *et al.*, 2006). It must be kept in mind that different preharvest practices may occur for different fruit batches, such as choice of rootstock, irrigation, fertilization and harvesting practices, and this may also influence the fruit's susceptibility to green mould.

Our methodology evaluated TBZ protection of intact citrus fruit rinds against green mould infection from freshly made wounds. In this case, TBZ in the dip did not perform well as a protective treatment. The reason for the low protective action is probably due to the limited penetration of TBZ into the fruit rind, meaning that any new wounds would be unprotected. It is anticipated that TBZ protection of non-infected fresh rind wounds against subsequent infection would be very effective, as was seen for the curative treatments. However, this hypothesis should still be tested. Electron micrographs showed that the epicuticular wax on citrus fruit becomes 'patchy' after TBZ treatment (D'Aquino *et al.*, 2013; Palma *et al.*, 2013). Whether the uneven distribution of epicuticular wax influence the uniformity of TBZ deposition is not known, but it is anticipated that improved deposition uniformity should lead to improved control (van Zyl *et al.*, 2010). Contrary to our findings, El-Tobshy *et al.* (1987) found that dipping fruit in aqueous TBZ did give protective control. It should be noted that they used dry spores for inoculum and the disease pressure was probably lower than in this study.

Imazalil was also found to have a better curative than protective action in the dip application (Dore *et al.*, 2009; Erasmus *et al.*, 2011), but protective treatments with imazalil were more effective than the protective treatments with TBZ in this study. Pyrimethanil was also found to have an excellent curative action (Smilanick *et al.*, 2006), and less effective protective action (Liebenberg, 2011), although it was still more effective than the protective action of TBZ in this study. When applied as a curative treatment, pyrimethanil and imazalil were found to reduce decay completely, while azoxystrobin and fludioxonil only reduced decay up to approximately 40% and 20%, respectively (Kanetis *et al.*, 2007). On naturally infected fruit, the curative action of TBZ was found to be similar to that of imazalil and fludioxonil (Zhang, 2007). Guazatine was found to be more effective than TBZ as a curative dip treatment by Kassim and Khan (1996), but Wild and Spohr (1989) found that its efficacy was less than TBZ when incubation times were prolonged. SOPP reduced

decay up to 20% and sporulation inhibition was observed when fruit was treated curatively (Hall, 1988). TBZ was more effective in reducing natural decay compared to SOPP (Smoot and Melvin, 1970). Sporulation inhibition following dip treatments was generally very low. No sporulation inhibition was seen on Clementines, and little inhibition was seen for the protective dip treatments of navel and Valencia fruit.

When TBZ was incorporated in wax coating application, it led to much higher residues on all citrus types. TBZ applied in wax at $4000 \mu\text{g}\cdot\text{mL}^{-1}$ resulted in exceedence of the MRL when applied at higher wax loads (1.2 and 1.8 L wax/ton fruit) on Valencia oranges. The fact that Valencia oranges loaded higher residues than the soft citrus may be due to various factors, including the size of the fruit, and also the faster brush speed used.

TBZ in wax coating gave better protective control than curative control of the sensitive isolate. The opposite was found by Brown (1984), i.e. better curative control in the wax, but different inoculation techniques were used. At a lower spore concentration (10^4 spores/mL) Kouassi *et al.* (2012) found that TBZ in wax at a 0.4% concentration gave 100% protective control. Unfortunately they did not specify which citrus type was used and only referred to fruit as oranges. The observation that TBZ in wax applications did not give such excellent control as in the dip, even though much higher TBZ residues were loaded was also found by Brown (1984) and El-Tobshy *et al.* (1987) who suggested that TBZ becomes encapsulated in the wax and thus its bioavailability is limited. Non-amended wax did not have any beneficial effect on green mould control. Ansari and Feridoon (2008) also found that non-amended wax did not control decay.

In our study, very poor green mould control was observed on Satsuma fruit. Njombolwana *et al.* (2013) also found that IMZ applied in a wax coating on soft citrus, especially Satsuma fruit, showed lower control than other citrus types. Valencia oranges loaded TBZ residues that exceeded the MRL at higher wax loads, and curative control increased with the increased TBZ residue resulting from increasing wax load. Njombolwana (2012) observed through SEM imaging that the wax coating on Valencia fruit was not evenly distributed at 0.6 L/ton. Applying wax at this load may therefore lead to unreliable control.

Hall *et al.* (1978) found that protective treatment of fruit with $5000 \mu\text{g}\cdot\text{mL}^{-1}$ TBZ in a wax treatment inhibited sporulation of TBZ sensitive isolates to some extent. In our study, sporulation inhibition was better on fruit following curative than protective treatments.

TBZ inhibited chilling injury on Valencia fruit and benefits were similar irrespective of whether fruit was dipped in TBZ and then waxed, or whether TBZ was applied in the wax. Schiffman-Nadel *et al.* (1975) found that incorporating TBZ in wax was more beneficial than dipping grapefruit in TBZ and then waxing. In our study, wax had an additive effect in reducing chilling injury compared to TBZ dip treatment alone, most likely due to inhibition of moisture loss and prevention of transpiration (Wang, 1993; Wild, 1993). Likewise, wax only treatments also inhibited chilling injury, although at lower levels than when TBZ was included. Schirra *et al.* (2000) also found that dipping grapefruit in TBZ reduced chilling injury, while Dou (2004) found that carnauba wax reduced chilling injury on its own. The addition of wax seemed to have a preserving effect on fruit buttons, but the condition of fruit at harvest probably plays a greater role, as can be concluded from the poor results on Satsuma fruit.

Compared with TBZ in dip and wax application, drench treatments were not very effective. Higher residues were loaded than in the dip application, and on navel fruit the MRL was sometimes exceeded. However, lower levels of curative control were achieved than in the dip application, as well as lower levels of protective control than in the wax coating application. Increased residues led to increased protective control, but not increased curative control. Erasmus *et al.* (2011) also found that different citrus types loaded different amounts of IMZ residues in a drench and that this was higher than residues loaded by dip application. However, it can be expected that deposition of the residue on the fruit rind is not as evenly distributed as would be expected from dip treatments. The finding that fruit facing button-up, i.e. inoculated wounds facing upward, had less disease incidence is evidence of this statement. Highly variable coverage of fungicide on fruit following drench treatment was observed using fluorescent image analysis (unpublished results), which might explain why no correlation could be drawn between residue loading and green mould control following drench treatment. Interestingly, sporulation inhibition seemed to increase with decreasing drench exposure time. However sporulation incidence was not negligible, making reliance solely on TBZ for sporulation control unlikely. Erasmus *et al.* (2011) also found that applying imazalil in the drench was also not as effective as applying it in the dip tank.

The TBZ resistant isolate was very poorly controlled in all applications. Extra measures were needed to control the resistant isolate, such as modification of pH and temperature of the dip tank (Schirra *et al.*, 2008). Little work has been done on modification of wax coating application parameters in order to control resistant

isolates. Nelson (1984) found that adding potassium sorbate to TBZ amended wax controlled resistant isolates of *P. digitatum*, but dipping fruit in an aqueous suspension of potassium sorbate followed by TBZ amended wax had the lowest amount of infection. No sporulation inhibition was seen for the resistant isolate in the dip application. For wax coating application, sporulation actually increased when TBZ was applied at increasing wax loads.

TBZ is classified as a high risk for development of fungicide resistance (Brent and Hollomon, 1998). TBZ resistance in *P. digitatum* populations has been isolated with varying frequencies around the world (Holmes and Eckert (1999); Kinay *et al.* (2007); Lee *et al.* (2011); Sánchez-Torres and Tuset (2011)). The current status of resistance in South Africa is unknown. Due to a zero tolerance to citrus black spot (CBS) by the European Union (EU), related benzimidazole compounds, such as benomyl, carbendazim and thiophanate-methyl, may be applied in citrus orchards to control CBS (Kellerman and Kotze, 1979). Smilanick *et al.* (2006) warned that frequent preharvest application of benzimidazole fungicides may lead to resistant *P. digitatum* isolates in orchards, to which postharvest application of TBZ in the packhouse will be ineffective.

The question remains as to which application of TBZ would be most beneficial in a packhouse. TBZ in the dip tank showed the highest efficacy for both green mould control (curatively) and chilling injury (if followed by a non-amended wax treatment), but it is not a popular ingredient in dip tank mixtures. In fungicide dip tanks of neutral pH and in water-based wax, TBZ tends to precipitate (McCornack, 1970; Ladaniya, 2008). In South Africa, TBZ is often added to pre-packhouse drench mixtures as it is also registered for controlling latent pathogens during degreening. However, since TBZ does not optimally control green mould in the drench, there will be a high risk of TBZ resistance development in *P. digitatum* populations if TBZ drenched fruit are submitted to the conditions of degreening which are very favourable for green mould development. Therefore, if it is decided to use TBZ in the drench to control latent pathogens, further reliance on TBZ after degreening to control green mould should be discouraged. Moreover, use of another green mould fungicide in combination with TBZ should be advised in the drench

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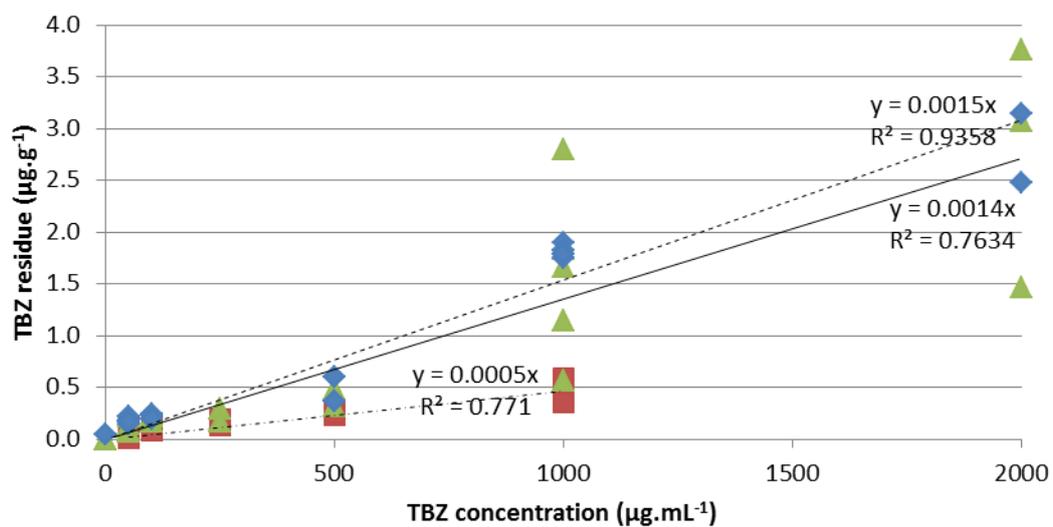


Figure 4.5.4.1. Thiabendazole (TBZ) residues and linear regression lines fitted indicating trends of residue loading for Clementine(■; $y = 0.0005x$), navel (◆; $y = 0.0015x$) and Valencia (▲; $y = 0.0014x$) fruit dipped for 60s in TBZ suspension at concentrations ranging from 0 to 2000 $\mu\text{g.mL}^{-1}$ at 22°C.

Table 4.5.4.1. Analysis of variance for thiabendazole (TBZ) residues loaded on fruit in different trials where Clementine, navel and Valencia fruit were dipped in TBZ concentrations ranging from 0 – 2000 µg.mL⁻¹ for 60 seconds.

Source	Clementine			Navel			Valencia		
	DF ^a	MS ^b	P ^c	DF ^a	MS ^b	P ^c	DF ^a	MS ^b	P ^c
Model	9	0.0806	< 0.0001	8	2.5025	< 0.0001	9	2.855844	<.0001
Trial	1	0.0176	0.0004	1	0.0002	0.9227	1	4.210634	<.0001
TBZ concentration	4	0.1670	< 0.0001	5	3.6383	< 0.0001	6	3.3137	<.0001
Trial* TBZ concentration	4	0.0100	< 0.0001	2	0.0014	0.9254	2	0.804881	0.0137
Error	30	0.0011		17	0.0180		25	0.157193	
Corrected Total	39			25			34		

^aDF = Degrees of freedom

^bMS = Mean sum of squares

^cP = Probability

Table 4.5.4.2. Analysis of variance of control (%) data of a sensitive or resistant isolate of *Penicillium digitatum* obtained following curative and protective dip treatments of Clementine mandarins, navel and Valencia oranges in different thiabendazole (TBZ) concentrations (0 to 2000 µg.mL⁻¹) and incubation at 22°C for 4-5 days.

Source	Clementine			Navel			Valencia		
	DF ^a	MS ^b	P ^c	DF ^a	MS ^b	P ^c	DF ^a	MS ^b	P ^c
Model	47	35168.8737	< 0.0001	43	73477.0327	< 0.0001	43	259106.1537	< 0.0001
Trial^d	1	3548.3007	0.0086	1	2735.8156	0.0016	1	206580.6421	< 0.0001
Treatment^e	5	97384.7298	< 0.0001	6	114073.0387	< 0.0001	6	111104.8782	< 0.0001
Action^f	1	167168.4578	< 0.0001	1	268429.7316	< 0.0001	1	1095880.5001	< 0.0001
Isolate^g	1	411369.7871	< 0.0001	1	1453576.0292	< 0.0001	1	40357.6347	< 0.0001
Trial*<i>Treatment</i>	5	510.7485	0.4196	3	451.1744	0.1747	3	64548.7871	< 0.0001
Trial*<i>Action</i>	1	2433.0535	0.0296	1	2508.6700	0.0024	1	6899.6958	0.0008
Trial*<i>Isolate</i>	1	1082.5772	0.1467	1	9785.4298	< 0.0001	1	7766.5266	< 0.0001
Treatment*<i>Action</i>	5	26782.9858	< 0.0001	6	17730.2743	< 0.0001	6	69819.2894	< 0.0001
Treatment*<i>Isolate</i>	5	36760.3443	< 0.0001	6	85545.4981	< 0.0001	6	188185.6697	< 0.0001
Action*<i>Isolate</i>	1	290266.7430	< 0.0001	1	427564.6706	< 0.0001	1	12263.0197	< 0.0001
Trial*<i>Treatment</i>*<i>Action</i>	5	991.2015	0.0862	3	572.9410	0.0980	3	1643.4896	0.0441
Trial*<i>Treatment</i>*<i>Isolate</i>	5	978.6607	0.0902	3	1543.1948	0.0007	3	31597.6192	< 0.0001
Trial*<i>Action</i>*<i>Isolate</i>	1	9479.3154	< 0.0001	1	2307.9258	0.0037	1	19021.4124	< 0.0001
Treatment*<i>Action</i>*<i>Isolate</i>	5	26033.1786	< 0.0001	6	26254.4474	< 0.0001	6	6120.9132	< 0.0001
Trial*<i>Treatment</i>*<i>Action</i>*<i>Isolate</i>	5	2019.4120	0.0015	3	1231.4042	0.0036	3	81071.2810	< 0.0001
Error	2640	513.7076		2452	272.6300		2452	608.2720	
Corrected Total	2687			2495			2495		

^aDF = Degrees of freedom

^bMS = Mean sum of squares

^cP = Probability

^dTrial= Each experiment was repeated twice

^eTreatment = concentrations of TBZ: 0, 50, 100, 250, 500, 1000 or 2000 µg.mL⁻¹

^fAction = curative or protective action of TBZ

^gIsolate = sensitive or resistant isolate of *Penicillium digitatum*

Table 4.5.4.3. Non-linear regression equations indicating the effect of thiabendazole (TBZ) residues on curative control of green mould following inoculation of Clementine, navel and Valencia fruit with a sensitive isolate of *Penicillium digitatum*, with dip treatments at different TBZ concentrations (0 to 2000 µg.mL⁻¹) and incubation at 22°C for 4-5 days. Effective residue levels for 50% and 75% curative control were calculated from the equations.

Citrus type	Equation of the model	R ² value	Effective residue levels (µg.g-1)	
			TBZ-C ₅₀ ^b	TBZ-C ₇₅ ^c
Clementine	$C^a = 99.66*(1-Exp(-6.39*R^d))$	0.695	0.11	0.22
Navel	$C = 102.18*(1-Exp(-10.24*R))$	0.861	0.07	0.13
Valencia	$C = 96.82*(1-Exp(-23.54*R))$	0.965	0.03	0.06

^aC = percentage of control ^bC₅₀ = TBZ residue needed to obtain 50% curative control ^cC₇₅ = TBZ residue needed to obtain 75% curative control ^dR= Residue loaded onto fruit (µg.g⁻¹)

Table 4.5.4.4. Analysis of variance for TBZ residue data following curative and protective carnauba wax coating treatments on Clementine, Satsuma and Valencia fruit with treatments consisting of coating fruit at three different wax loads (0.6, 1.2 or 1.8 L.ton⁻¹) at a TBZ concentration of 0 or 4000 µg.mL⁻¹, or dipping fruit into an aqueous TBZ suspension of 1000 µg.mL⁻¹ (Valencia oranges) or 2000 µg.mL⁻¹ (Satsuma and Clementine mandarins) followed by non-amended wax coating treatment of 1.2 L.ton⁻¹.

Source	DF ^a	MS ^b	P ^c
Model	18	12.4952	< 0.0001
Citrus type	2	40.2179	< 0.0001
Treatment ^d	7	17.1503	< 0.0001
Citrus type*Treatment	9	2.7139	0.0009
Error	40	0.6676	
Corrected Total	58		

^aDF = Degrees of freedom ^bMS = Mean sum of squares ^cP = Probability ^dTreatment: Combination of TBZ concentration (0 or 4000 µg.mL⁻¹) and wax load loads (0.6, 1.2 or 1.8 L.ton⁻¹), or TBZ dips at 1000 µg.mL⁻¹ (Valencia oranges) or 2000 µg.mL⁻¹ (Satsuma and Clementine mandarins) followed by non-amended wax coating treatment of 1.2 L.ton⁻¹.

Table 4.5.4.5. Mean TBZ residues following curative and protective carnauba wax coating treatments on Clementine, Satsuma and Valencia fruit with treatments consisting of coating fruit at three different wax loads (0.6, 1.2 or 1.8 L.ton⁻¹) at a TBZ concentration of 0 or 4000 µg.mL⁻¹, or dipping fruit into an aqueous TBZ suspension of 1000 µg.mL⁻¹ (Valencia oranges) or 2000 µg.mL⁻¹ (Satsuma and Clementine mandarins) followed by non-amended wax coating treatment of 1.2 L/ton.

Treatment	TBZ residues (µg.g ⁻¹) ^a		
	Clementine	Satsuma	Valencia
Wax : 4000 µg.mL ⁻¹ TBZ at 0.6 L.ton ⁻¹	0.4f	0.4f	2.7cd
Wax : 4000 µg.mL ⁻¹ TBZ at 1.2 L.ton ⁻¹	1.3ef	1.3ef	5.7b
Wax : 4000 µg.mL ⁻¹ TBZ at 1.8 L.ton ⁻¹	3.1c	2.9cd	7.6a
Wax : 0 µg.mL ⁻¹ TBZ at 1.2 L.ton ⁻¹	0.0f	0.0f	0.0f
Dip : 1000 µg.mL ⁻¹ TBZ	n/a	n/a	0.9ef
Dip : 2000 µg.mL ⁻¹ TBZ	1.8de	1.2ef	n/a
Dip (1000 µg.mL ⁻¹ TBZ) and Wax (1.2 L.ton ⁻¹)	n/a	n/a	0.7ef
Dip (2000 µg.mL ⁻¹ TBZ) and Wax (1.2 L.ton ⁻¹)	0.5f	0.7ef	n/a

^aMeans followed by the same letter do not differ significantly.

Table 4.5.4.6. Analysis of variance of sporulation incidence (%) data following curative and protective dip treatments of Clementine mandarins, navel and Valencia oranges at different TBZ concentrations (0 to 2000 $\mu\text{g.mL}^{-1}$) inoculated with either a sensitive or resistant isolate of *P. digitatum*. Fruit was incubated at 22°C for 10 - 11 days before sporulation was recorded and incidence (%) calculated.

Source	Clementine			Navel			Valencia		
	DF ^a	MS ^b	P ^c	DF ^a	MS ^b	P ^c	DF ^a	MS ^b	P ^c
Model	47	0.0354	< 0.0001	42	0.6873	< 0.0001	43	0.6734	< 0.0001
Trial^d	1	0.0222	0.1833	1	0.5621	0.0008	1	0.8191	0.0003
Treatment^e	5	0.0319	0.0268	6	0.9817	< 0.0001	6	2.0575	< 0.0001
Action^f	1	0.0035	0.5973	1	2.1816	< 0.0001	1	0.0798	0.2538
Isolate^g	1	0.0047	0.5395	1	0.2477	0.0263	1	0.7190	0.0006
Trial*Treatment	5	0.0755	< 0.0001	3	0.6463	< 0.0001	3	0.0469	0.5133
Trial*Action	1	0.0103	0.3647	1	0.0856	0.1916	1	0.0143	0.6292
Trial*Isolate	1	0.2216	< 0.0001	1	0.0875	0.1865	1	0.0004	0.9337
Treatment*Action	5	0.0038	0.9110	6	0.5707	< 0.0001	6	0.3188	< 0.0001
Treatment*Isolate	5	0.0769	< 0.0001	6	1.1880	< 0.0001	6	0.6463	< 0.0001
Action*Isolate	1	0.0004	0.8500	1	2.0549	< 0.0001	1	0.0808	0.2509
Trial*Treatment*Action	5	0.0109	0.5016	3	0.4646	< 0.0001	3	0.0170	0.8414
Trial*Treatment*Isolate	5	0.0382	0.0096	3	0.6168	< 0.0001	3	0.0259	0.7365
Trial*Action*Isolate	1	0.0323	0.1087	1	0.0215	0.5123	1	0.2487	0.0441
Treatment*Action*Isolate	5	0.0102	0.5398	6	0.2591	< 0.0001	6	0.2108	0.0022
Trial*Treatment*Action*Isolate	5	0.0074	0.7077	2	0.1307	0.0741	3	0.1102	0.1455
Error	2461	0.0130		1914	0.0501		1881	0.0610	
Corrected Total	2508			1956			1924		

^aDF = Degrees of freedom

^bMS = Mean sum of squares

^cP = Probability

^dTrial= Each experiment was repeated twice

^eTreatment = concentrations of TBZ: 0, 50, 100, 250, 500, 1000 or 2000 $\mu\text{g.mL}^{-1}$

^fAction = curative or protective action of TBZ

^gIsolate = sensitive or resistant isolate of *Penicillium digitatum*

Table 4.5.4.7. Analysis of variance for infection (%) and sporulation incidence (%) data on fruit following curative and protective carnauba wax coating treatments of Valencia, Clementine and Satsuma fruit and inoculation with a TBZ-sensitive or TBZ-resistant isolate of *Penicillium digitatum*. Treatments consisted of coating fruit at three different wax loads (0.6, 1.2 or 1.8 L.ton⁻¹) at a TBZ concentration of 0 or 4000 µg.mL⁻¹. Fruit was incubated at 22°C for 4-5 days before infection was recorded and infection (%) calculated. Fruit was rated for sporulation incidence after 10 – 11 days.

Source	% Infection			Sporulation incidence		
	DF ^a	MS ^b	P ^c	DF ^a	MS ^b	P ^c
Model	71	35910.6	< 0.0001	71	7.207	< 0.0001
Citrus Type	2	196749	< 0.0001	2	5.813	< 0.0001
Treatment ^d	6	50078.9	< 0.0001	6	38.966	< 0.0001
Action ^e	1	7012.22	0.001	1	2.36	< 0.0001
Isolate ^f	1	33195	< 0.0001	1	108.683	< 0.0001
Citrus Type*Treatment	12	47529.8	< 0.0001	12	1.199	< 0.0001
Citrus Type*Action	2	21997.4	< 0.0001	2	0.161	0.051
Citrus Type*Isolate	2	159579	< 0.0001	2	0.701	< 0.0001
Treatment*Action	6	3459.78	< 0.0001	6	0.359	< 0.0001
Treatment*Isolate	6	68944.9	< 0.0001	6	28.171	< 0.0001
Action*Isolate	1	4306.92	0.012	1	0.04	0.388
Citrus Type*Treatment*Action	12	2599.79	< 0.0001	12	0.425	< 0.0001
Citrus Type*Treatment*Isolate	6	23931	< 0.0001	6	0.693	< 0.0001
Citrus Type*Action*Isolate	2	4643.78	0.001	2	2.937	< 0.0001
Treatment*Action*Isolate	6	6114.24	< 0.0001	6	0.508	< 0.0001
Type*Treatment*Action*Isolate	6	4727.28	< 0.0001	6	0.348	< 0.0001
Error	6840	684.102		5969	0.054	
Corrected Total	6911			6040		

^aDF = Degrees of freedom ^bMS = Mean sum of squares ^cP = Probability ^dTreatment = combination of three different wax loads (0.6, 1.2 or 1.8 L.ton⁻¹) at a TBZ concentration of 0 or 4000 µg.mL⁻¹ ^eAction = curative or protective action of TBZ ^fIsolate = sensitive or resistant isolate of *Penicillium digitatum*

Table 4.5.4.8. Mean percentage infection (%) on fruit following curative and protective carnauba wax coating treatments of Valencia, Clementine and Satsuma fruit and inoculation with a TBZ-sensitive or TBZ-resistant isolate of *Penicillium digitatum*. Treatments consisted of coating fruit at three different wax loads (0.6, 1.2 or 1.8 L.ton⁻¹) at a TBZ concentration of 0 or 4000 µg.mL⁻¹. Fruit was incubated at 22°C for 4-5 days before infection was recorded and infection (%) calculated. Fruit was rated for sporulation incidence after 10 – 11 days.

Treatment	Percentage infection (%)*					
	Satsuma		Clementine		Valencia	
	Curative	Protective	Curative	Protective	Curative	Protective
Sensitive isolate						
Control	98.2g	91.1abcde	90.1defg	90.9efg	82.8fghijkl	89.6klmn
Wax 0.6 L.ton ⁻¹	94.8cdefg	92.2bcdef	84.1cde	85.4cde	73.7de	93.8mn
Wax 1.2 L.ton ⁻¹	96.6fg	96.6fg	95.8g	91.1efg	79.9efghi	91.1lmn
Wax 1.8 L.ton ⁻¹	92.4cdef	96.4efg	92.7fg	83.3cd	90.4lmn	94.8n
TBZ wax 0.6 L.t ⁻¹	95.3defg	90.1abcd	89.8defg	87.8cdef	40.4c	14.1a
TBZ wax 1.2 L.t ⁻¹	92.2bcdef	86.7a	90.4defg	67.2b	27.1b	19.8ab
TBZ wax 1.8 L.t ⁻¹	94.0cdefg	87.0ab	81.5c	58.9a	78.1efgh	19.5ab
Resistant isolate						
Control	97.4fg	96.6fg	93.8fg	94.5fg	79.4efgh	89.3jklmn
Wax 0.6 L.ton ⁻¹	n/a	n/a	n/a	n/a	80.5efghi	86.7hijklmn
Wax 1.2 L.ton ⁻¹	n/a	n/a	n/a	n/a	80.7efghij	88.5ijklmn
Wax 1.8 L.ton ⁻¹	n/a	n/a	n/a	n/a	85.4ghijklm	81.2efghijk
TBZ wax 0.6 L.t ⁻¹	93.7cdefg	95.1cdefg	94.8fg	81.8c	68.5d	85.2ghijklm
TBZ wax 1.2 L.t ⁻¹	94.8cdefg	95.1cdefg	93.2fg	81.3c	75.0def	76.8defg
TBZ wax 1.8 L.t ⁻¹	89.8abc	95.6efg	87.8cdef	84.1cde	78.1efgh	86.7hijklmn

*Means with the same letter do not differ significantly.

Table 4.5.4.9. Mean sporulation incidence (%) following wax treatments on Valencia, Satsuma and Clementine fruit that were inoculated with either a sensitive or resistant isolate of *Penicillium digitatum* and treated curatively or protectively with a wax coating amended with 0 or 4000 µg.mL⁻¹ TBZ applied at 0.6, 1.2 or 1.8 L.ton⁻¹ and fruit was incubated at 22°C for 10 days before sporulation was rated.

Citrus type Treatment	Sporulation incidence (%)*					
	Satsuma		Clementine		Valencia	
	Curative	Protective	Curative	Protective	Curative	Protective
Sensitive isolate						
Control	99.0m	99.0m	99.0m	97.9lm	97.6lm	100.0m
Wax 0.6 L.ton ⁻¹	100.0m	100.0m	100.0m	96.8lm	96.8lm	75.0ghi
Wax 1.2 L.ton ⁻¹	99.0m	99.0m	100.0m	100.0m	100.0m	100.0m
Wax 1.8 L.ton ⁻¹	100.0m	99.0m	97.6lm	94.7lm	100.0m	100.0m
TBZ wax L.ton ⁻¹	81.9ij	95.7lm	98.9m	100.0m	61.1fa	43.6e
TBZ wax 1.2 L.ton ⁻¹	22.8c	54.8f	41.7e	73.7gh	30.5d	3.4ab
TBZ wax 1.8 L.ton ⁻¹	0.0a	8.7b	0.0a	12.6b	9.2b	8.3ab
Resistant isolate						
Control	99.0m	100.0m	97.9lm	100.0m	82.1ij	100.0m
Wax 0.6 L.ton ⁻¹	n/a	n/a	n/a	n/a	85.4jk	100.0m
Wax 1.2 L.ton ⁻¹	n/a	n/a	n/a	n/a	77.1hi	97.8lm
Wax 1.8 L.ton ⁻¹	n/a	n/a	n/a	n/a	82.1ij	100.0m
TBZ wax L.ton ⁻¹	100.0m	100.0m	100.0m	98.9m	69.5g	100.0m
TBZ wax 1.2 L.ton ⁻¹	100.0m	100.0m	100.0m	95.7lm	81.2ij	80.0hij
TBZ wax 1.8 L.ton ⁻¹	100.0m	100.0m	100.0m	98.9m	91.7kl	100.0m

*Means with the same letter do not differ significantly.

Table 4.5.4.10. Analysis of variance for chilling injury index on Valencia, Satsuma and Clementine fruit that were treated with a wax coating amended with 0 or 4000 $\mu\text{g.mL}^{-1}$ TBZ applied at 0.6, 1.2 or 1.8 L.ton^{-1} , or dipped in a 1000 (Valencia) or 2000 (Satsuma and Clementine) $\mu\text{g.mL}^{-1}$ TBZ suspension and then waxed with non-amended wax at 1.2 L.ton^{-1} . Fruit was incubated at -0.5°C for 40 days and then at 22°C for 4 days before being rated. Two trials were done on each citrus type.

Source	DF ^a	MS ^b	P ^c
Model	29	25336.4352	< 0.0001
Citrus Type	2	200415.1155	< 0.0001
Treatment^d	9	14902.6042	< 0.0001
Citrus Type*Treatment	18	13112.1126	< 0.0001
Error	2538	1805.8018	
Corrected Total	2567		

^aDF = degrees of freedom ^bMS = mean squares ^cP = Probability ^dTreatment = combination of three different wax loads (0.6, 1.2 or 1.8 L.ton^{-1}) at a TBZ concentration of 0 or 4000 $\mu\text{g.mL}^{-1}$

Table 4.5.4.11. Mean chilling injury incidence on Valencia, Clementine and Satsuma fruit that were treated with a wax coating amended with 0 or 4000 $\mu\text{g.mL}^{-1}$ TBZ applied at 0.6, 1.2 or 1.8 L.ton^{-1} , or dipped in a 1000 (Valencia) or 2000 (Clementine and Satsuma) $\mu\text{g.mL}^{-1}$ TBZ suspension and then waxed with non-amended wax at 1.2 L.ton^{-1} . Fruit was incubated at -0.5°C for 40 days and then at 22°C for 4 days before rated.

Treatment	CI incidence (%)***		
	Clementine	Satsuma	Valencia
Control	15.3hijkl	41.7bc	79.2a
Dip water	9.7kl	27.8defgh	64.6a
Dip TBZ*	8.3kl	37.5bcdef	47.2b
Dip TBZ, wax: 1.2 L.ton^{-1} **	4.2l	43.1b	18.1ghijk
Wax 0.6 L.ton^{-1}	28.1defgh	29.2cdefg	44.8b
Wax 1.2 L.ton^{-1}	14.6ijkl	47.9b	44.8b
Wax 1.8 L.ton^{-1}	4.2l	49.0b	38.5bcde
TBZ wax L.ton^{-1}	13.5jkl	29.2cdefg	27.1efgh
TBZ wax 1.2 L.ton^{-1}	14.6ijkl	26.0fghi	22.9ghij
TBZ wax 1.8 L.ton^{-1}	9.4kl	39.6bcd	18.8ghijk

*Valencia: 1000 $\mu\text{g.mL}^{-1}$ TBZ, Clementine and Satsuma: 2000 $\mu\text{g.mL}^{-1}$ TBZ.

**Valencia: 1000 $\mu\text{g.mL}^{-1}$ TBZ, Clementine and Satsuma: 2000 $\mu\text{g.mL}^{-1}$ TBZ, waxed at 1.2 L.ton^{-1} with non-amended wax.

***Means with the same letter do not differ significantly.

Table 4.5.4.12. Analysis of variance for green button incidence (%) on Valencia, Satsuma and Clementine fruit that were treated with a wax coating amended with 0 or 4000 $\mu\text{g.mL}^{-1}$ TBZ applied at 0.6, 1.2 or 1.8 L.ton^{-1} , or dipped in a 1000 (Valencia) or 2000 (Satsuma and Clementine) $\mu\text{g.mL}^{-1}$ TBZ suspension and then waxed with non-amended wax at 1.2 L.ton^{-1} . Fruit was incubated at -0.5°C for 40 days and then at 22°C for 4 days before being rated. Two trials were done on each citrus type.

Source	DF ^a	MS ^b	P ^c
Model	29	8.3369	< 0.0001
Citrus Type	2	104.5222	< 0.0001
Treatment ^d	9	1.5927	< 0.0001
Citrus Type*Treatment	18	0.7877	< 0.0001
Error	2538	0.1404	
Corrected Total	2567		

^aDF = degrees of freedom ^bMS = mean squares ^cP = Probability ^dTreatment = combination of three different wax loads (0.6, 1.2 or 1.8 L.ton^{-1}) at a TBZ concentration of 0 or 4000 $\mu\text{g.mL}^{-1}$

Table 4.5.4.13. Mean incidence of green buttons (%) ($R^2 = 0.404$) on Valencia, Satsuma and Clementine fruit that were treated with a wax coating amended with 0 or 4000 $\mu\text{g.mL}^{-1}$ TBZ applied at 0.6, 1.2 or 1.8 L.ton^{-1} , or dipped in a 1000 (Valencia) or 2000 (Satsuma and Clementine) $\mu\text{g.mL}^{-1}$ TBZ suspension and then waxed with non-amended wax at 1.2 L.ton^{-1} . Fruit was incubated at -0.5°C for 40 days and then at 22°C for 4 days before being rated. Two trials were done on each citrus type.

Treatment	Incidence of green buttons (%)***		
	Satsuma	Clementine	Valencia
Control	8.3j	77.8de	83.3bcde
Dip: Water	11.7ij	79.2de	58.3f
Dip: TBZ*	16.7ij	84.7bcd	47.2fg
Dip and wax**	13.9ij	93.1abc	56.9f
Wax 0.6 L.ton^{-1}	36.5gh	76.7de	79.4de
Wax 1.2 L.ton^{-1}	35.7h	96.9a	81.3de
Wax 1.8 L.ton^{-1}	37.5gh	96.9a	86.1bcd
TBZ wax 0.6 L.ton^{-1}	15.0ij	84.5bcd	85.4bcd
TBZ wax 1.2 L.ton^{-1}	20.4i	93.8ab	72.9e
TBZ wax 1.8 L.ton^{-1}	12.1ij	92.7abc	82.3cde

*Valencia: 1000 $\mu\text{g.mL}^{-1}$ TBZ, Clementine and Satsuma: 2000 $\mu\text{g.mL}^{-1}$ TBZ.

**Valencia: 1000 $\mu\text{g.mL}^{-1}$ TBZ, Clementine and Satsuma: 2000 $\mu\text{g.mL}^{-1}$ TBZ, waxed at 1.2 L/ton with non-amended wax.

***Means with the same letter do not differ significantly.

Table 4.5.4.14. Analysis of variance for TBZ residue data on navel oranges following drench treatment. Treatments consisted of a combination of TBZ concentration (1000 or 2000 $\mu\text{g.mL}^{-1}$) and exposure time (30, 60 or 90s).

Source	DF ^a	MS ^b	P ^c
Model	5	5.0604	0.0439
TBZ concentration	1	21.9864	0.0023
Exposure time	2	1.0851	0.5498
TBZ concentration*Exposure time	2	0.5726	0.7257
Error	18	1.7544	
Corrected Total	23		

Table 4.5.4.15. Analysis of variance for TBZ residue data on Clementine fruit following drench treatment. Treatments consisted of a combination of 1000 $\mu\text{g.mL}^{-1}$ TBZ with exposure times 30, 60 or 90 s, or 2000 TBZ $\mu\text{g.mL}^{-1}$ with an exposure time of 45 s.

Source	DF ^a	MS ^b	P ^c
Model	3	0.4935	0.1933
Treatment ^d	3	0.4935	0.1933
Error	9	0.2540	
Corrected Total	12		

^aDF = Degrees of freedom ^bMS = Mean squares ^cP = Probability ^dTreatment = combination of four different exposure times (30, 45, 60 and 90 s) at a TBZ concentration of 1000 or 2000 $\mu\text{g.mL}^{-1}$

Table 4.5.4.16. Analysis of variance for control (%) data obtained on navel orange fruit following drench treatment with TBZ concentrations of 1000 or 2000 $\mu\text{g}\cdot\text{mL}^{-1}$ and exposure times of 30, 60 or 90 s at different orientations (fruit facing up or down). Fruit were treated curatively (inoculated 24 or 6 hours before treatment) or protectively.

Source	DF ^a	MS ^b	P ^c
Model	35	28619.2108	< 0.0001
TBZ concentration	1	5051.0321	0.0158
Exposure time	2	7503.4985	0.0002
Action^d	2	425564.2163	< 0.0001
Orientation	1	88678.3428	< 0.0001
TBZ concentration*Exposure time	2	312.3804	0.6968
TBZ concentration*Action	2	4907.9107	0.0035
TBZ concentration*Orientation	1	94.8675	0.7405
Exposure time*Action	4	972.6187	0.3430
Exposure time*Orientation	2	634.5709	0.4802
Action*Orientation	2	9146.4927	< 0.0001
TBZ concentration*Exposure time*Action	4	843.7297	0.4195
TBZ concentration*Exposure time*Orientation	2	97.3500	0.8935
TBZ concentration*Action*Orientation	2	109.3344	0.8812
Exposure time*Action*Orientation	4	925.1562	0.3698
TBZ concentration*Exposure time*Action*Orientation	4	82.6523	0.9839
Error	1692	864.5787	
Corrected Total	1727		

Table 4.5.4.17. Analysis of variance for control (%) data obtained on Clementine mandarins following drench treatment with TBZ concentrations of 1000 or 2000 $\mu\text{g.mL}^{-1}$ and exposure times of 30, 45, 60 or 90 s. Fruit were treated curatively (inoculated 24 or 6 hours before treatment) or protectively, and placed at different orientations (top or bottom facing upwards) in the crate.

Source	DF ^a	MS ^b	P ^c
Model	23	26653.9742	< 0.0001
Treatment	3	146.8169	0.9075
Action^d	2	239427.7672	< 0.0001
Orientation	1	45984.7512	< 0.0001
Treatment*Action	6	3026.0947	0.0010
Treatment*Orientation	3	2013.7765	0.0568
Action*Orientation	2	3215.5699	0.0183
Treatment*Action*Orientation	6	812.6891	0.4129
Error	840	799.3563	
Corrected Total	863		

Table 4.5.4.18. Analysis of variance for sporulation incidence on navel fruit following drench treatment, which consisted of a combination of TBZ concentration (1000 or 2000 $\mu\text{g}\cdot\text{mL}^{-1}$) and exposure time (30, 60 or 90 s). Fruit were treated curatively (inoculated 6 or 24 hours before treatment) or protectively. Fruit were placed at different orientations in the drench crate, either facing button-up or button-down.

Source	DF ^a	MS ^b	P ^c
Model	35	0.8292	< 0.0001
TBZ concentration	1	5.4450	< 0.0001
Exposure time	2	5.3686	< 0.0001
Action^d	2	0.7523	0.0342
Orientation	1	0.4867	0.1392
TBZ concentration*Exposure time	2	0.7367	0.0366
TBZ concentration*Action	2	0.6204	0.0617
TBZ concentration*Orientation	1	0.0469	0.6462
Exposure time*Action	4	0.7402	0.0100
Exposure time*Orientation	2	0.1742	0.4570
Action*Orientation	2	0.4537	0.1303
TBZ concentration*Exposure time*Action	4	0.1620	0.5723
TBZ concentration*Exposure time*Orientation	2	0.6094	0.0648
TBZ concentration*Action*Orientation	2	0.0903	0.6664
Exposure time*Action*Orientation	4	0.0214	0.9837
TBZ concentration*Exposure time*Action*Orientation	4	0.4340	0.0994
Error	1692	0.2224	
Corrected Total	1727		

Table 4.5.4.19. Analysis of variance for sporulation incidence on fruit following drench treatment on Clementine mandarins. Treatments consisted of a combination of TBZ concentration (1000 or 2000 $\mu\text{g.mL}^{-1}$) and exposure time (30, 45, 60 or 90s). Fruit were treated curatively (inoculated 24 or 6 hours before treatment) or protectively, and placed at different orientations (top or bottom facing upwards) in the crate.

Source	DF ^a	MS ^b	P ^c
Model	23	1.1210	< 0.0001
Treatment	3	3.4367	< 0.0001
Action ^d	2	2.0410	< 0.0001
Orientation	1	0.0002	0.9777
Treatment*Action	6	0.8878	0.0003
Treatment*Orientation	3	0.1455	0.5520
Action*Orientation	2	0.0138	0.9356
Treatment*Action*Orientation	6	0.1847	0.5021
Error	791	0.2077	
Corrected Total	814		

Addendum B

Evaluation of green mould control following pyrimethanil application in wax coatings and drench applications

1. Introduction

South Africa accounts for 96% of the total citrus production and 64% of citrus fruit gets exported and only 9% is left for domestic consumption (National agricultural marketing council, 2013). But economic losses of fruit that occur due to postharvest decay developing from postharvest handling and on transit is estimated to be as high as 90% (Smoot et al., 1971). The major impacting factor on South African citrus is long distances equating to long transit times which subsequently lead to losses when fruit reaches the destination.

One of the major causes of postharvest losses is green mould. The losses occur during harvesting, marketing and export (Smilanick, 2011). It is caused by *Penicillium digitatum* (Pers.:Fr) Sacc. *Penicillium* species are filamentous fungi and grouped in the order Eurotiales. They are often referred to as Deuteromycetes, or Fungi Imperfecti. Their conidiophores branch into several branches at the tip, each carrying a cluster of spore bearing cells (phialides), giving it a brush-like appearance. Hence the name, *Penicillium* from the Latin word 'penicillus', meaning brush. The large ellipsoidal to cylindrical, unicellular spores of *P. digitatum* contain a green pigment that gives the colony its distinctive olive-green colour. Taking into account the micro- and macro-morphological, as well as physiological and extrolite (secondary metabolites) characteristics, *P. digitatum* has a unique combination of features, making it the only representative species in its section/series, *Digitata*. There is variation in their conidiophore and conidia structures and is comparatively large for *Penicillium*. Conidiophores are biverticillate and phialides are more cylindrical and longer. They produce tryptoquialanines as an extrolite that is unique to *P. digitatum*, except for one other *Penicillium* species, and need additional nitrogen sources to grow; unlike other species in the subgenus *Penicillium* that can grow on nitrate as the only N-source. Also, low water activities and high temperatures result in poor growth (Frisvad and Samson 2004).

In citrus producing areas, *P. digitatum* is commonly found on soil debris in citrus orchards from where it will infect damaged, fallen fruit under cooler temperatures. Large amounts of spores produced on the surfaces of these fruit will successively be carried to fruit surfaces of unharvested fruit by wind currents and rain splashes. Where rind or physiological induced injuries are present the spores will germinate and infect the fruit. Eckert et al. (1994) showed that volatiles, more specifically limonene, acetaldehyde, ethanol and carbon dioxide, surrounding wounded citrus fruit induced germination of *P. digitatum* spores. Colonisation of the fruit and disease symptoms will rapidly follow infection. A water soaked lesion becomes visible, after which the growth of white mycelium will cover the surface. Spores start forming from the centre of the lesion until the fruit is completely covered. Spores will then again be dispersed and the disease cycle repeated. *P. digitatum* spore is also abundantly in citrus packhouses and storage facilities and the disease cycle can be repeated many times within a citrus packing season (Brown, 2003).

To control green mould, various techniques are employed. However, fungicide application is the most commonly used and effective method. Fungicides that have been used primarily for over 25 years are thiabendazole and imazalil, with the latter being the most effective fungicide against green mould and blue mould due to its curative, protective and anti-sporulant ability (Brown et al., 1983; Zhang, 2007). However, prolonged use of these fungicides without alternative mixtures and poor sanitation process led to the efficacy diminishment due to the development of resistant strains which results in continuous selection pressure on the pathogen population (Holmes and Eckert, 1999). This situation in countries like California is aggravated by the susceptible tissues that are available all year round as in packhouse operations fruit get processed and stored continuously (Kanetis et al., 2010). Due to development of resistance, the development of new fungicides with different modes of action was imperative (Bus et al., 1991). Recently, three new fungicides with different modes of actions were introduced and registered against postharvest decay, including azoxystrobin, fludioxinil and pyrimethanil (Kinnetis et al., 2007).

The current study focuses on pyrimethanil as a newly registered fungicide. Pyrimethanil belongs to a class of fungicides called anilinopyrimidine and it has been registered and used on most trees and vegetable crops worldwide. It has an ability to inhibit germ tube elongation and mycelia growth (Kanetis et al., 2007). Moreover, pyrimethanil has been reported to show systemic penetration in the plant tissue subsequently providing curative and protective benefits. For pathogens like *Botrytis cinerea* in stone fruit, the guaranteed anilinopyrimidine are often recommended for protective use in order to provide good decay control and avoid the development of resistance (Rosslénbroich and Stuebler, 2000). Therefore, the objective of the study was

to evaluate green mould control, sporulation inhibition and residue loading following the single application of pyrimethanil in wax coating and also in the drench.

2. Materials and Methods

2.1 Fruit

Untreated Valencia and navel oranges (*Citrus sinensis* (L.) Osbeck) of export quality were collected from Citrusdal area in the Western Cape province of South Africa. Upon arrival at the laboratory, fruit meant for wax application trials was washed in a 1 mL L^{-1} didecyl dimethyl ammonium chloride solution (Sporekill, ICA International Chemicals, Stellenbosch, South Africa) and allowed to dry overnight at ambient temperature prior to commencement of the trials.

2.2. Pyrimethanil application in coating

Inoculation method described by Erasmus et al. (2011) was used in this study. In which two-week old cultures of IMZ sensitive (STE-U 6560) isolate of *P. digitatum* on potato dextrose agar (PDA) was used to prepare spore suspensions. Conidia was gently dislodged from cultures in 5 mL of sterile deionised water [containing 0.001 mL L^{-1} Tween 20 (Sigma-Aldrich, St. Louis, MO, USA)], which was then transferred to a 500-mL Scott's bottle to obtain a volume of 200 mL spore suspension. The spore concentration was adjusted to 1×10^6 spores mL^{-1} using a haemocytometer or spectrophotometer. Twelve fruit of similar size were wound inoculated through the flavedo into the top albedo layer using a wound inducer at four sites surrounding the stem end. Wound inducers consisted of three insect needles placed in a needle clamp to create three small wounds of 0.5 mm wide and 2 mm deep at a triangular distance of 1.5 mm apart. The inoculations were done curatively and protectively, *i.e.* prior to treatment and after treatment, respectively. For curative treatments, the fruit were incubated for 24 hours before treatment.

The fruit was treated using a custom built packline described previously (Njombolwana et al., 2013). Coating consisted of a medium solid (18%) carnauba-shellac based coating (875 High Shine, John Bean Technologies, Brackenfell, South Africa) pyrimethanil (Protector 400 SC, ICA International Chemicals, Stellenbosch, South Africa) at a concentration of $2000 \mu\text{g mL}^{-1}$ and $4000 \mu\text{g mL}^{-1}$. Pyrimethanil and the wax coating were kept agitated on a magnetic stirrer. The fruit exposure time on the coating applicator was 10 s, 20 s and 30 s, which affected a coating load of 0.6, 1.2 and 1.8 L ton^{-1} of fruit, respectively.

2.3. Pyrimethanil application in drench

Fruit was inoculated as described above. However, fruit was incubated for 6 and 24 hours (h) prior to treatment (curative) and also 24 hours (h) after treatment (protective). For treatment, a commercial bin-drenching system was simulated using plastic fruit crates with holes drilled at the bottom of the plastic crates with all four sides of the crate fully closed with a red polyethylene plastic bag. The system consisted of one crate at the top inserted with drilled pipes in order to release a shower of the fungicide mix through the holes into the bottom crate with the fruit. Pyrimethanil was applied at a recommended concentration of $1000 \mu\text{g mL}^{-1}$ at three different exposure times (30s, 60s and 90s) with three replications each. Approximately 15 min after each application, the experimental stack was disassembled and the fruit was left overnight to dry prior to packing.

2.4. Storage and evaluation

Twelve treated and inoculated fruit from each treatment combination were placed in lock back table grape boxes (APL cartons, Worcester, South Africa) on count SFT13 nectarine trays (Huhtamaki South Africa (Pty) Ltd., Atlantis, South Africa). Each box was covered with a transparent polyethylene bag and sealed. Fruit was incubated at 20°C and after 4 days the number of infected wounds were evaluated using a light source (UV-A at 365 nm, Labino Mid-light; www.labino.com). Under the light, the infected wound was visible in a form of yellow fluorescence on the surface of the fruit that was not visible with the naked eye at the period of evaluation. Sporulation was rated 10 to 12 days after treatment. A sporulation index of 0 to 6 was used, which described the percentage of the fruit surface covered with green spores, where 0 = no sign of disease, 1 = lesion but no sporulation, 2 = sporulating area covering a quarter of the lesion, 3 = sporulating area larger than a quarter but smaller than the half of the lesion, 4 = sporulating area larger than half but smaller than three quarters of the lesion, 5 = sporulating area larger than three quarters but smaller than the whole lesion, and 6 = sporulating area covering the whole fruit (Erasmus et al., 2011). Sporulation incidence (%) was determined from infected fruit with a sporulation index of 3 and higher.

2.5 Residue analysis

IMZ residues following the various treatments were determined from fruit sampled from 2 of the 3 replicates. From each treatment combination, 6 fruit were sampled and were frozen (20°C) until prepared for pyrimethanil residue analysis. Fruit were defrosted, measured and weighed and macerated to a fine pulp by

using a fruit blender (Salton Elite, Amalgamated Appliance Holdings Limited, Reuven, South Africa) and re-frozen. Sub-samples of the macerated fruit were submitted for IMZ (chloramizol) residue analyses by an accredited analytical laboratory (Hearshaw and Kinnes Analytical Laboratory, Westlake, Cape Town, South Africa). Samples were extracted by using acetonitrile followed by a matrix solid phase dispersion extraction. Analysis of the extracts was conducted in liquid chromatography mass spectrometry (LCMS/MS; Agilent 6410, Agilent Technologies Inc., Santa Clara, CA, USA).

2.6. Experimental layout and statistical analysis

The single application in coating trial was a 2 (curative and protective treatment) × 1 (sensitive isolate) × 3 (IMZ and the coating loads) factorial design with 12 fruit per treatment combination and 4 and 3 replications on Valencia and navel oranges, respectively; the trial was conducted 2 times on Valencia oranges and 2 times on navel oranges. The single drench application trial was a 3 (6h curative, 24h curative and 24h protective) × 1 (sensitive isolate) × 3 (exposure times) factorial design with 12 fruit per treatment combination and 3 replications. The trial was conducted twice on navel oranges and twice on Valencia oranges during the 2012 season. Fruit residue, wound infection, green mould control and sporulation incidence data were analysed using appropriate analysis of variance and Fisher's test was used to observe the significance differences among the treatments at a 95% confidence interval.

3. Results

3.1 Pyrimethanil application in coating

Analysis of variance of residue data on navel oranges showed no significant interaction between coating load × concentration ($P=0.211$). Residues increased with increasing coating load at 2000 $\mu\text{g}\cdot\text{ml}^{-1}$ (2.31 to 3.44 $\mu\text{g}\cdot\text{g}^{-1}$; Table 1), as well as at 4000 $\mu\text{g}\cdot\text{ml}^{-1}$ (3.18 to 5.46 $\mu\text{g}\cdot\text{g}^{-1}$), albeit as significantly higher levels. Analysis of variance of residue data on Valencia oranges showed a meaningful interaction between coating load × concentration ($P=0.057$). Similarly with navel oranges, Valencia oranges at 2000 $\mu\text{g}\cdot\text{ml}^{-1}$ showed increasing levels of residues with increasing coating load (2.21 to 3.56 $\mu\text{g}\cdot\text{g}^{-1}$), but with no significant difference between loads. At 4000 $\mu\text{g}\cdot\text{ml}^{-1}$, residues were significantly higher than those loaded at 2000 $\mu\text{g}\cdot\text{ml}^{-1}$, and residues increased with increasing coating load (4.22 to 8.39 $\mu\text{g}\cdot\text{g}^{-1}$; Table 1).

Analysis of variance of green mould control data on navel oranges showed no significant interactions and significant effects for concentration ($P=0.0001$) and coating load ($P=0.004$). Relatively poor green mould control was observed for curative (6.2%) as well as protective treatments (4.5%), showing no significance difference between these actions ($P=0.059$). Nonetheless, significant difference between the highest and lowest concentration was observed, as 4000 $\mu\text{g}\cdot\text{ml}^{-1}$ yielded 7.1% 2000 $\mu\text{g}\cdot\text{ml}^{-1}$ yielded 3.7% green mould control. Higher coating loads resulted in significantly higher level of control (7.5%) than 1.2 $\text{L}\cdot\text{ton}^{-1}$ (3.8%) and 0.6 $\text{L}\cdot\text{ton}^{-1}$ (4.8%).

Analysis of variance of sporulation incidence data showed a significant interaction between coating load × concentration × action ($P=0.0001$). Significant sporulation inhibition was only observed for the protective treatments at 4000 $\mu\text{g}\cdot\text{ml}^{-1}$ at 1.2 $\text{L}\cdot\text{ton}^{-1}$ (82%) and 0.6 $\text{L}\cdot\text{ton}^{-1}$ (86%) compared with 98.6-100% sporulation obtained in all other treatments.

Analysis of variance of green mould control data on Valencia oranges showed a significant interaction between coating load × concentration × action ($P=0.010$). Curative treatments at 2000 $\mu\text{g}\cdot\text{ml}^{-1}$ resulted in poor control but increased with increasing coating loads (10.5 to 21.3%; Table 2). Improved curative control compared was observed at 4000 $\mu\text{g}\cdot\text{ml}^{-1}$ at 0.6 $\text{L}\cdot\text{ton}^{-1}$ (22.2%) and 1.8 $\text{L}\cdot\text{ton}^{-1}$ (27.3%), although still relatively poor. Variable control was obtained in the protective treatments at 2000 $\mu\text{g}\cdot\text{ml}^{-1}$ at different coating loads, 0.6 $\text{L}\cdot\text{ton}^{-1}$ (32.6%), 1.2 $\text{L}\cdot\text{ton}^{-1}$ (28.8%) and 1.8 $\text{L}\cdot\text{ton}^{-1}$ (37.8%). Higher concentration resulted in significantly improved control with increasing coating load (41.0 to 53.7%).

Analysis of variance of sporulation incidence data on Valencia oranges showed a significant interaction between coating load × concentration × action ($P=0.022$). Curative treatments at 2000 $\mu\text{g}\cdot\text{ml}^{-1}$ at higher coating loads resulted in significantly reduced sporulation (72%) compared with 0.6 $\text{L}\cdot\text{ton}^{-1}$ coating loads (99%), while 4000 $\mu\text{g}\cdot\text{ml}^{-1}$ resulted in reduced sporulation at all coating loads ($\approx 61\%$). In the protective treatments, 2000 $\mu\text{g}\cdot\text{ml}^{-1}$ resulted in reducing levels of sporulation with increasing coating load (79.2 to 61.3%); at 4000 $\mu\text{g}\cdot\text{ml}^{-1}$ a similar trend was observed, albeit at lower sporulation incidence levels (68.4 to 51.1%; Table 2).

3.2 Pyrimethanil application in drench

Analysis of variance of residue data on navel oranges showed a significant effect of fruit batch ($P<0.0001$). The first batch of navel oranges resulted in significantly higher residue levels (6.04 $\mu\text{g}\cdot\text{g}^{-1}$) than the second

batch ($2.50 \mu\text{g.g}^{-1}$). Exposure time showed no significant effect with residue levels increasing marginally with increasing exposure time (3.97 to $4.80 \mu\text{g.g}^{-1}$). Analysis of variance of residue data on Valencia oranges showed a significant effect of fruit batch ($P=0.008$). Similar to navel oranges, there was a significant difference between the two Valencia orange batches, the first batch resulted in significantly higher ($3.66 \mu\text{g.g}^{-1}$) residue loading than the second batch ($2.61 \mu\text{g.g}^{-1}$). On Valencia, no difference was observed between exposure times (3.13 to $3.14 \mu\text{g.g}^{-1}$).

Analysis of variance of green mould control data on navel oranges showed a significant effect of treatment ($P<0.0001$). Fruit that was incubated for 24 h before curative treatment resulted in poorer percentage of green mould control (1%) than the fruit that was incubated for 6 h before treatment (6.8%). Protective treatments also resulted in poor control (4.6%). Analysis of variance of sporulation data showed a significant interaction between treatment \times exposure time ($P=0.024$). Curative treatments at 24 h incubation period resulted in higher levels of sporulation incidence of $\approx 95\%$ for all three exposure times (Table 3). At 6 h incubation period, the sporulation incidence decreased with increasing exposure time (87.9% to 68.8%). Similarly to 6 h incubation, protective treatments also resulted in decrease in sporulation with increasing exposure time (100 to 82.9%).

Analysis of variance of green mould control data on Valencia oranges showed significant effects of exposure time ($P=0.001$) and treatment ($P<0.0001$). Longer exposure time at 60 and 90 s resulted in significantly better control of 34.4-36.3% than 30 s exposure time (26.7%). In the curative treatments, 6 h incubation period resulted in significantly better green mould control (65.5%) than 24 h incubation period (10.8%). Protective treatments resulted in poor control (21.2%). Analysis of variance of sporulation data showed a significant interaction between treatment \times exposure time ($P=0.003$). Curative treatments at 24 h incubation period resulted in 100% sporulation incidence for all three exposure times (Table 3). However, at 6 h incubation time, there was significantly less sporulation incidence, which decreased with increasing exposure time (90.5 to 73.9%). Similar to the 24 h incubation period in the curative treatments, protective treatments resulted in high levels of sporulation incidence (97.2 to 100%).

Discussion

This study investigated the efficacy of pyrimethanil when applied in the drench and in wax coating. The results indicated that the efficacy of pyrimethanil is dependent on the method of application and timing on which the treatment is applied. Previous studies found that combination of pyrimethanil and wax coating resulted in limited green mould control and sporulation inhibition (Smilanick et al., 2006; Kanetis et al., 2007). This corresponded with previous reports done on other postharvest fungicides incorporated with wax coating, which found that the efficacy of the fungicide is reduced and pre-application in an aqueous solution prior to wax coating is necessary (Eckert and Eaks, 1989; Smilanick et al., 1997; Njombolwana et al., 2013). However, higher concentration provided better residue loading which was still below the MRL of $10 \mu\text{g.g}^{-1}$ (www.mrlatabase.com). Furthermore, this study provides a better understanding in the application of pyrimethanil in the drench, in terms of exposure time, curative or protective green mould control and the infection incubation time and residue loading. This is the first possible postharvest pyrimethanil treatment, therefore treatment parameters such as exposure time had to be optimised in this study.

The combination of pyrimethanil and wax coating resulted in lower residue loading at lower concentration than at higher concentration, however, the residues were still below the MRL of $10 \mu\text{g.g}^{-1}$ (www.mrlatabase.com). The residues in both concentrations increased with increasing coating loads from 0.6 to 1.8 L.ton^{-1} . Similar results were obtained in single application of imazalil in wax coating (Njombolwana et al., 2013). In terms of green mould control and sporulation inhibition, the single application of pyrimethanil in wax coating in navel oranges showed poor performance in curative and protective treatments when lower and higher concentrations were applied. However, in Valencia oranges, higher concentration showed improved green mould control and reduced sporulation in the protective treatments than lower concentration and both concentration showed poor performance in the curative treatments. These results are in agreement with previous reports with regards to reduced efficacy of the fungicides when applied in wax coatings (Brown, 1984; Kanetis et al., 2007; Njombolwana et al., 2013). It was also interesting to observe the differences in level of green mould control in navel oranges and Valencia oranges, which was attributed to the difference in levels of susceptibility of citrus fruit to green mould. The ability of the mixture to reduce sporulation in the protective treatments in Valencia oranges was a good benefit due to the fact that spores on the surface of the infected fruit result in a cosmetic defect called soilage to the uninfected healthy neighbour fruit (Eckert and Brown, 1986). According to Kanetis et al. (2007), fruit sprayed with pyrimethanil in wax coating following 13 to 15 hours of incubation resulted in 2.2% decay compared with 82.9% of the control treatment; in this experiment, pyrimethanil performed better than imazalil (7.8%).

Fungicide drench application is a popular method in many commercial packhouses in South Africa and there is a number of fungicides registered for drench use including pyrimethanil. The results obtained in single drench application of pyrimethanil in this study showed that residue loading remains similar irrespective of fruit exposure time. However, longer exposure time showed improved green mould control and sporulation inhibition in Valencia oranges. The South African citrus industry recommendation for drench exposure time is 1 to 3 min. Incubation period played a significant role in green mould control in the drench. At 6 hour incubation time, Valencia oranges showed improved green mould and sporulation inhibition control but at 24 hours and in the protective treatments, there was poor green mould control. These results were similar to the single application of thiabendazole in the drench; however, for thiabendazole green mould control was also improved at 24 hour incubation time (M. Kellerman, unpublished results). Other citrus producing countries e.g. California are active in the use of recirculating in-line drench application system and have shown to reduce decay incidence significantly (Forster et al., 2007). Although preliminary, the results from this study indicate that drench application is not an effective method for postharvest disease control. However, a drench application is common prior to degreening of the fruit. Degreening of fruit using ethylene in the early season after harvest is often essential, but this process favours the development of *Penicillium* decay (Brown and Miller, 1999).

Single application of pyrimethanil in wax coating and in the drench presented non-satisfactory results in this study and whether the method of inoculation or the inoculum load is the underlying problem, warrants further investigations.

Table 1. Imazalil residues loaded on navel and Valencia orange fruit, treated with pyrimethanil and coating at 2000 µg mL⁻¹ and 4000 µg mL⁻¹ using carnauba coating on brushes at 0.6, 1.2 and 1.8 L ton⁻¹.

Fruit type/coating load	Residue loading (µg.g ⁻¹)	
	2000 µg. mL ⁻¹	4000 µg. mL ⁻¹
Navel*		
Coating load 0.6 L ton ⁻¹	2.31d	3.18cd
Coating load 1.2 L ton ⁻¹	2.70cd	4.15b
Coating load 1.8 L ton ⁻¹	3.44bc	5.46a
Valencia*		
Coating load 0.6 L ton ⁻¹	2.21d	4.22bc
Coating load 1.2 L ton ⁻¹	2.57d	5.53b
Coating load 1.8 L ton ⁻¹	3.56d	8.39a

*For each fruit type, means followed by the same letter do not differ significantly ($P>0.05$).

Table 2. Mean percentage green mould control and sporulation incidence on Valencia oranges that were wound inoculated with a sensitive *P. digitatum* isolate and curatively or protectively treated with pyrimethanil at a concentration of 2000 µg mL⁻¹ and 4000 µg mL⁻¹ in carnauba coating on brushes and a coating load of 0.6, 1.2, and 1.8 L ton⁻¹ incubated for 4 days at ambient (20°C).

Treatment	Green mould control (%) [*]	Sporulation incidence (%) [*]
Curative		
2000 µg. mL ⁻¹		
Coating load 0.6 L ton ⁻¹	10.5j	99.0a
Coating load 1.2 L ton ⁻¹	15.4hij	71.9bc
Coating load 1.8 L ton ⁻¹	21.3ghi	71.4bc
4000 µg. mL ⁻¹		
Coating load 0.6 L ton ⁻¹	22.2fgh	58.5cd
Coating load 1.2 L ton ⁻¹	14.3ij	62.1cd
Coating load 1.8 L ton ⁻¹	27.3efg	60.7cd
Protective		
2000 µg. mL ⁻¹		
Coating load 0.6 L ton ⁻¹	32.6de	79.2b
Coating load 1.2 L ton ⁻¹	28.8ef	67.9bc
Coating load 1.8 L ton ⁻¹	37.8cd	61.3cd
4000 µg. mL ⁻¹		
Coating load 0.6 L ton ⁻¹	41.0bc	68.4bc
Coating load 1.2 L ton ⁻¹	46.7ab	53.1d
Coating load 1.8 L ton ⁻¹	53.7a	51.1d

*For green mould control and sporulation incidence separately, means followed by the same letter do not differ significantly ($P>0.05$)

Table 3. Mean percentage sporulation incidence of fruit infected with green mould on navel and Valencia oranges caused by sensitive isolate of *P. digitatum* after 10 days' incubation at 20°C, following curative 6 h and 24 h treatment and protective treatment with pyrimethanil drench at 1000 µg mL⁻¹ at a 30, 60 and 90 s exposure time.

Treatment / exposure time	Sporulation incidence (%)	
	Navel oranges [*]	Valencia oranges [*]
6-hour curative treatment		
30 s drench	87.9bcd	90.5bc
60 s drench	84.8cd	86.5c
90 s drench	68.8e	73.9d
24-hour curative treatment		
30 s drench	94.4abc	100.0a
60 s drench	97.2ab	100.0a
90 s drench	94.4abc	100.0a
Protective treatment		
30 s drench	100.0a	98.6a
60 s drench	87.5bcd	97.2ab

90 s drench	82.9d	100.0a
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*For navel and Valencia oranges separately, means followed by the same letter do not differ significantly ($P>0.05$)

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4.5.5 **PROGRESS REPORT: The JBT heated flooder as an alternative application method for fungicides in citrus packhouses**

Project 1050 (April 2012 - March 2013) by Arno Erasmus (CRI-Nelspruit), Paul Fourie (CRI-USPP), Wilma du Plooy (JBT South Africa) and Charlene Jewell (JBT California)

Summary

The majority of South African packhouses use a dip tank to apply imazalil (IMZ) for the control of green mould (caused by *Penicillium digitatum*). John Bean Technologies' (JBT) division in California developed an alternative to the dip tank that has been in use for the past decade. The JBT heated flooder (JHF) applies fungicide in an aqueous solution by means of a number of weirs that creates a seamless laminar flow that falls onto the fruit over rotating brushes. This type of application gives more consistency in terms of residue loading and disease control. JBT's division in South Africa build and installed an experimental flooder unit at CRI in Nelspruit to compare this new technology to current technology in use (dip application). Due to technical problems the unit was only installed during July 2012 in the CRI Nelspruit postharvest laboratory. Results on work conducted so far are presented in this report. The dip was compared to the flooder application and the effect of flooder solution temperature on IMZ residue loading and green mould control was investigated. Results obtained so far are presented in this report. Higher application temperatures lead to higher residual loading. This mean the applied dosage should be halved to prevent overload. The interpretation of the 2012 data should be done in the context of the 2013 data. More trials are planned on different citrus types for the 2013 season. Work during the 2013 season should confirm the JHF as an alternative to dip application and therefore no new work are planned for 2014 under this topic. Further work with the JHF will be incorporated with new projects as needed.

Opsomming

Die meerderheid pakhuse in die Suid-Afrikaanse sitrusbedryf gebruik 'n dompelbad vir die aanwending van imazalil (IMZ) om groenskimmel te beheer. Die Kaliforniese afdeling van John Bean Technologies (JBT) het 'n alternatief vir die dompelbad ontwikkel wat reeds die afgelope dekade gebruik word. Die JBT verhitte vloed-toediener wend swamdoders in water oplossings op vrugte aan bo-oor roterende borsels deur middel van vloeders wat 'n soomlose laminêre waterval veroorsaak. Hierdie tipe toediener bied beter stabiliteit in terme van residu-lading en siektebeheer. Die Suid-Afrikaanse afdeling van JBT het 'n eksperimentele vloed-toediener gebou en by CRI in Nelspruit geïnstalleer sodat hierdie nuwe tegnologie met die huidige tegnologie in gebruik (dompelbad) vergelyk kan word. Weens tegniese probleme kon die eenheid eers in Julie 2012 in die CRI Nelspruit naoes laboratorium installeer word. Resultate ingewin sover word in hierdie verslag voorgelê. Die dompelbad is met die vloed-toediener vergelyk and die effek van temperatuur in die vloed toediener-oplossing op residu lading en groenskimmel beheer is ondersoek. Resultate tot dusver ingewin word in hierdie verslag voorgelê. Uit die eerste rondte se data was bevind dat die hoër temperature tot hoër residu lading lei. Daarvolgens kon die gebruiksdosis halveer word om oorlading te voorkom. Die interpretasie van die 2012 data moet in konteks met die 2013 data gedoen word. Meer proewe op verskillende sitrus soorte word vir die 2013 seisoen beplan. Werk gedurende die 2013 seisoen behoort the vloed-toediener as 'n alternatief vir dompel toediening te bevestig en daarom word geen nuwe werk onder hierdie onderwerp vir 2014 beplan nie. Verdere werk met die vloed-toediener sal met nuwe projekte geïnkorporeer word soos nodig.

4.5.6 **PROGRESS REPORT: Practical impact of fungicide resistance on control of postharvest citrus green and blue mould**

Project 1034 (June 2011 - March 2013) by Arno Erasmus (CRI-Nelspruit) and Paul Fourie (CRI-USPP)

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 analysis for the benchmark values is in progress. Sensitive PD isolated 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 in 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).

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 geïnfekteer 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 pakhuse en ook om die minimum residu beperking vir siekte beheer beter te verstaan teenoor die maksimum residu beperking (MRL)

4.6 PROGRAMME: CITRUS BLACK SPOT Programme coordinator: G.C. Schutte (CRI)

4.6.1 Programme summary

Technical problems were experienced with weather stations and two spore traps experienced in 2011-2012 season during the critical periods when ascospore releases took place. The weather station at Hermitage was also stolen and two new Adcon weather stations were installed on 17 February 2012 at Summerville and Kirkwood. With data to our disposal, ascospore releases took place during 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. Rainfall occurred in mid-October 2012 before the growers could apply their first spray round. Conditions may have been favourable for infection, but extremely favourable conditions did occur during March 2013. It seems as if pycnidiospore infection did take place from twigs onto lower hanging fruit (4.6.2).

Genomic sequence data of *Guignardia citricarpa* was generated with the Ion Torrent Personal Genome Machine and assembled into 123 contigs, which was used to mine for microsatellites and for primer design. In total 437.14 Mb sequence information was obtained. Eight of the 17 primers designed were shown to be polymorphic. Four South African populations that were genotyped were shown to be similar in genetic structure. Although the markers were not highly polymorphic in the South African population, loci that were monomorphic in these populations were polymorphic in a population representing different countries around the world. A further 15 primer pairs were designed of which five were shown to amplify polymorphic loci (4.6.3).

Of the 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, both BAS70004F and BAS70301F tested at rates of 10 and 20 ml/hl water in tank mixtures with mancozeb and mineral spray oil gave excellent control of citrus black spot and can be recommended for registration. Problems with runoff with Organo Silica were experienced as the 2x rate resulted in poor CBS control. New spray programmes where mancozeb were altered with RB1 and RB2, as well as two applications consisting of tank mixtures of benomyl (Spoton-B) plus RB2 and BreakThru as well as benomyl (Spoton-B) + RBA + mancozeb + mineral spray oil also resulted in good control of C+BS. If registered, these treatments will save the growers two spray rounds (4.6.4).

Two phosphonates were tested on their own and in combinations with two adjuvants (A and B) using fluorometry to determine the retention and quantitative deposition of these phosphonates in laboratory- and field trials. Laboratory trials showed that they had good retention if sprayed on their own. Adjuvant A performed the worst of the two adjuvants tested. It seems that phosphonates do not need adjuvants to improve their deposition on leaves (4.6.5).

Citrus Black Spot is the most important citrus disease in South Africa, especially given its impact on market access and *ad hoc* research which is conducted on an ongoing basis to focus on developing and improving a

model for *Guignardia* pseudothecium maturation and ascospore dispersal based on meso-climatic weather data. A collaborating project funded by the Florida citrus industry in USA is aiming to develop a quantitative pest risk assessment of *Guignardia citricarpa*, with special emphasis on the fresh fruit pathway. Research gaps in the model were identified to be addressed (4.6.6).

Two Valencia orchards were sprayed with mancozeb and copper hydroxide SC and WP formulations in tank mixtures with SARDI yellow fluorescent pigment. Fruit and leaf samples from both trial sites were taken and photographed and sent to SU for digital image analyses. The %FPC values on leaves for both copper formulations stayed stable during both experimental periods from the day 0 to day 35. The copper residues decreased by half from after 7 days. On leaves, %FPC values for both mancozeb formulations decreased significantly but increased during the second experimental period. The %FPC for the SC formulation was significant more than that of the WP formulation showing that the adjuvants added to SC resulted in good spreading of the residues on the fruit surface (4.6.7).

Programopsomming

Tegniese probleme is met weerstasies en twee spoorvangers gedurende die 2011-2012 seisoen 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. Met die data tot ons beskikking is bepaal dat daar twee askosporvystellings einde November en einde Desember 2011 by Kirkwood plaasgevind het en het ooreengestem met die vorige seisoen. Dit beklemtoon dat daar wel askosporvystelling in mid-somer plaasgevind het en dat die spuitprogramme wat voorgestel en gespuit is, korrek was. Gedurende die 2012-2013 seisoen het daar heelwat reën in mid-Oktober geval alvorens die kwekers hulle eerste spuitronde kon toedien. Toestande was gunstig vir askosporvystelling gedurende hierdie periode, met uiters gunstige toestande in Maart 2013. Vrugte wat ondersoek is toon moontlike piknidiospor-infeksie vanaf besmette takke na laaghangende vrugte (4.6.2).

Data van genoomvolgordes van *Guignardia citricarpa* is gegeneer met die "Ion Torrent Personal Genome" Masjien en in 123 contigs saamgevoeg, wat gebruik is om vir mikro-satelite en vir "primer" ontwikkeling te myn. 'n Totaal van 437.14 Mb volgorde data is vir *G. citricarpa* verkry. Agt uit 17 primers was polimorfies. Vier Suid-Afrikaanse populasies wat genotipeer is, was van dieselfde genetiese struktuur. Alhoewel die merkers nie hoogs polimorfies in die Suid-Afrikaanse populasies was nie, was loki wat monomorfies in hierdie populasies was, polimorfies in 'n populasie wat ander lande van die wêreld verteenwoordig. Nog 15 primer-pare is ontwikkel waarvan vyf getoon het om polimorfiese loki te amplifiseer (4.6.3).

Uit verskeie nuwe sistemiese- en kontakswamdoders asook adjuvante in kombinasies met geregistreerde swamdoders wat op 'Valencia' lemoene vir die beheer van swartvlek beproef is, het beide BAS70004F en BAS70301F teen dosisse van 10 en 20 ml/hl water in tankmengels met mancozeb en minerale spuit-olie uitstekende beheer van swartvlek gegee. Probleme met afloop is met die 2x konsentrasie van Organo Silica ondervind wat tot swak beheer van swartvlek gelei het. Nuwe spuitprogramme waar mancozeb met RB1 en RB2, asook twee toedienings van tankmengels bestaande uit benomyl (Spoton-B) plus RB2 en BreakThru asook benomyl (Spoton-B) + RBA + mancozeb + minerale spuit-olie het goeie beheer van swartvlek gegee (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 gevaar in laboratorium- en veldproewe. Bymiddels blyk nie nodig te wees om fosfonate beter op blare te laat kleef nie (4.6.5).

Sitruswartvlek is die belangrikste sitrus-siekte in Suid-Afrika, veral gegewe sy impak op marktoegang word geformaliseer deur *ad hoc* navorsing wat fokus op die ontwikkeling en verbetering van 'n model vir *Guignardia pseudotesium* rypwording en spoorvystelling gebaseer op meso- en mikroklimate data. 'n Samewerkingsprojek wat deur die Florida sitrusbedryf in VSA befonds word, beoog om 'n kwantitatiewe pesrisiko-analise-model vir *Guignardia citricarpa*, met spesifieke fokus op vars vrugte as verspreidingsweg, te ontwikkel. Gapings is in die model geïdentifiseer wat aangespreek word (4.6.6).

Twee Valencia boorde is met mancozeb en koperhidroksied SK en BP formulasies in 'n tankmengsel met SARDI se geel fluorisensiepigment gespuit. Vrug- en blaarmonsters is van beide proefboorde getrek en gefotografeer waarna hulle na US gestuur is digitale foto analise. Die %FPC (persentasie fluoriserende pigment bedekking) waardes is op blare en vrugte vir beide koper- en mancozebformulasies bepaal oor twee

seisoene. Die %FBC op vrugte en blare het stabiel gebly oor beide eksperimentele periodes van dag 0 tot dag 35. Die koperresidue het ook met die helfte verminder na 7 dae. Op blare het die %FPC waardes vir beide mancozeb formulasies betekenisvol afgeneem, maar het toegeneem tydens die tweede eksperimentele periode. Die %FPC vir die SK formulasie was betekenisvol meer as die BP formulasie wat toon dat die olie wat as bymiddel in die SK formulasie toegevoeg is, beter bedekking op die vrugoppervlaktes tot gevolg gehad het (4.6.7).

4.6.2 **PROGRESS REPORT: Monitoring ascospore releases in the Eastern Cape to determine the critical period for CBS infection**

Project 919 (September 2008 – June 2012) 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. 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 should have protected the fruit in those critical periods against ascospore infection. Rainfall occurred from 3 to 14 October 2012 before the growers could apply their first spray round. Conditions may have been favourable for infection, but extremely favourable conditions did occur during March 2013. It seems as if pycnidiospore infection did take place from twigs onto lower hanging fruit.

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 gedurende die 2011-2012 seisoen ondervind in die kritiese tye toe askospoorvrystellings 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. Met die data tot ons beskikking kon ons aflei dat daar twee askospoorvrystellings 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 askospoorvrystelling in mid-somer plaasgevind het en dat die spuitprogramme, wat voorgestel en gespuit is, korrek was en behoort vrugte in daardie kritiese periodes teen askospoorinfeksie beskerm het. Gedurende die 2012-2013 seisoen het daar heelwat reën vroeg in Oktober geval alvorens die kwekers hulle eerste spuitronde kon toedien. Toestande was moontlik gunstig vir askospoorvrystelling gedurende hierdie periode, maar uiters gunstige toestande het eers in Maar 2013 voorgekom. Dit blyk asof pycnidiospore infeksie vanaf takke op laag-hangende vrugte kon plaasgevind het.

4.6.3 **PROGRESS REPORT: The global population structure and reproductive biology of the fungal pathogen, *Guignardia citricarpa* Kiely**

Project 977 (2010/11 – 2014/15) by E. Carstens (CRI)

Summary

Microsatellites (simple sequence repeats (SSRs)) are amongst the most widely used population genetic markers, since they show high levels of allelic variation. Development of SSR loci for such studies traditionally was difficult, expensive and time consuming. However, with the increased availability of next generation sequencing techniques, rapid and affordable identification of multiple SSR loci is possible. Genomic sequence data of *Guignardia citricarpa* was generated with the Ion Torrent Personal Genome Machine and assembled into 123 contigs, which was used to mine for microsatellites and for primer design. The genome size of *G. citricarpa* is not known, but in total 437.14 Mb sequence information was obtained. Of the 17 primers designed, eight were shown to be polymorphic. Four South African populations that were genotyped were shown to be similar in genetic structure. Although the markers were not highly polymorphic in the South African population, loci that were monomorphic in these populations were polymorphic in a population representing different countries around the world. A further 15 primer pairs were designed of which five were shown to amplify polymorphic loci. A total of four national populations ($n = 116$) and seven international populations ($n = 253$) has been collected, and will be genotyped with the 13 SSR markers.

Opsomming

Mikrosateliete ('simple sequence repeats' (SSRs)) is van die mees gebruikte populasie genetiese merkers omdat hulle hoë vlakke van alleliese variasie toon. Ontwikkeling van SSR loci vir sulke studies was tradisioneel moeilik, duur en tydrowend. Die toenemende beskikbaarheid van "next generation sequencing" tegnieke maak egter vinnige en bekostigbare identifisering van veelvuldige SSR loci moontlik. Data van genomvolgordes van *Guignardia citricarpa* is gegenereer met die "Ion Torrent Personal Genome" Masjien en in 123 contigs saamgevoeg, wat gebruik is om vir mikro-sateliete en vir "primer" ontwikkeling te myn. Die genomgrootte van *G. citricarpa* is nie bekend nie maar 'n totaal van 437.14 Mb volgorde data is verkry. Van die 17 primers wat ontwikkel is, het agt geblyk om polimorfies te wees. Vier Suid-Afrikaanse populasies wat genotipeer is, het getoon om van dieselfde genetiese struktuur te wees. Alhoewel die merkers nie hoogs polimorfies in die Suid-Afrikaanse populasies was nie, was loci wat monomorfies in hierdie populasies was, polimorfies in 'n populasie wat ander lande van die wêreld verteenwoordig. 'n Verdere 15 primer pare is ontwikkel waarvan vyf getoon het om polimorfiese loci te amplifiseer. 'n Totaal van vier nasionale populasies (n = 116) en sewe internasionale populasies (n = 253) is versamel en sal met hierdie 13 SSR merkers genotipeer word.

4.6.4 PROGRESS REPORT: Development of new spray programmes for the control of citrus black spot

Project 970 (April 2009 – June 2014) 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. Of the fungicides tested, both BAS70004F and BAS70301F tested at rates of 10 and 20 ml/hl water in tank mixtures with mancozeb and mineral spray oil gave excellent control of citrus black spot and can be recommended for registration. Although some problems with runoff was experienced with the 2x rate of Organo Silica, which resulted in poor CBS control, the 1x rate performed well and resulted in good control of CBS. Kannar 202 also performed well, but phytotoxicity problems were experienced. New spray programmes where mancozeb were altered with RB1 and RB2, as well as two applications consisting of tank mixtures of benomyl (Spoton-B) plus RB2 and BreakThru as well as benomyl (Spoton-B) + RBA + mancozeb + mineral spray oil also resulted in good control of CBS. If registered, these treatments will save the growers two spray rounds.

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. Van die swamdoders wat getoets is, het beide BAS70004F en BAS70301F teen dosisse van 10 en 20 ml/hl water in tankmengels met mancozeb en minerale spuit-olie uitstekende beheer van swartvlek gegee en kan aanbeveel word vir registrasie. Alhoewel probleme met afloop is met die 2x konsentrasie van Organo Silica ondervind is wat tot swak beheer van swartvlek gelei het, het die 1x konsentrasie goeie beheer van die swartvlek gegee. Kannar 202 het ook goed gewerk teen swartvlek, maar fitotoksiese probleme is ondervind. Nuwe spuitprogramme waar mancozeb met RB1 en RB2, asook twee toedienings van tenkmengsels bestaande uit benomyl (Spoton-B) plus RB2 en BreakThru asook benomyl (Spoton-B) + RBA + mancozeb + minerale spuit-olie het ook goeie beheer van swartvlek tot gevolg gehad. Laasgenoemde bespuitings sal kwekers twee spuitronde spaar as dit so registreer kan word.

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

Two Valencia orange orchards with a history of CBS were selected. The one was at Croc Valley Citrus Co. and the other at the Lowveld Agricultural College. Rates and dates of applications are listed in Tables 1 to 6. 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

Kannar 202

Results from the trial site at Crocodile Valley Citrus Co. showed that there were no significant differences ($P < 0.05$) between the standard registered mancozeb and copper oxychloride treatments and both rates of Kannar 202 that were evaluated against citrus black spot. Kannar 202 at rates of 250 and 500 ml/hl water resulted in 99.8 and 99.2% clean exportable fruit respectively. Both rates of Kannar 202 that were evaluated against citrus black spot at rates of 250 and 500 ml/hl water resulted in 95.0% and 83.2% clean exportable fruit respectively. Although Kannar 202 at a rate of 250ml/100L water resulted in 2.2% fruit with 1-3 CBS lesions, it was however not significant different from all the other treatments. The same scenario was experienced with four and more CBS lesions where both Kannar 202 treatments resulted in 2.8% and 11.2% fruit with four and more CBS lesions (Table 1). All these treatments were significantly different from the control. Although the Kannar 202 controlled CBS effectively, serious phytotoxicity was observed at harvest (Fig.4.6.4.1).

Similar results were obtained from the trial site at Lowveld Agricultural College. No significant differences ($P < 0.05$) were observed between the standard registered mancozeb and copper oxychloride treatments which resulted in 92.4% and 95.2% clean exportable fruit respectively. The standard mancozeb and copper

oxychloride applications resulted in 99.8 and 99.2% clean exportable fruit respectively. Although Kannar 202 at a rate of 250ml/hl water resulted in 2.8% fruit with 1-3 CBS lesions, it was however not significantly different from all the other treatments. The same scenario was experienced with four and more CBS lesions where both Kannar 202 treatments resulted in 4.8% and 4.6% fruit with four and more CBS lesions. All these treatments were however significantly different from the control (Table 4.6.4.2).

Disease pressure in both trial sites was high as the untreated control resulted in only 46.6% and 54.2% clean exportable fruit. In the criterion fruit with 1-3 CBS lesions and 4 and more lesions per fruit, all the treatments were also significantly different from the control.

BASF

Results from the field trial conducted at Crocodile Valley Citrus Co. (Table 4.6.4.3) shows that there were no significant differences ($P < 0.05$) between the criterion clean exportable fruit used for evaluation and the standard registered spray programmes of 4 application of mancozeb (97.4%) and mancozeb / Cabrio+mancozeb+oil / Cabrio+mancozeb+oil / mancozeb (4 applications) (99.2%) and the experimental spray programmes consisting of either or mancozeb / BAS70004F+mancozeb+oil / BAS70004F +mancozeb+oil / mancozeb (4 applications) at 10 and 20 ml/hl water resulting in 99.4 and 91.8% clean exportable fruit with no CBS.

However, the treatment where mancozeb was excluded from the tank mixture with BAS70004F viz. mancozeb/ BAS70004F+oil / BAS70004F +oil /mancozeb (4 applications) at 10 ml/hl water, was significant different the other two BAS70004F treatments and the standard mancozeb and Cabrio tank mixture with mancozeb and mineral spray oil, resulting only in 80.4% clean exportable fruit. This was also the only treatment that resulted in 12.6% fruit with 4 and more CBS lesions per fruit which was significant different from all other spray programmes except for the untreated control.

The experimental spray programmes consisting of either or mancozeb/ BAS70301F + mancozeb + oil / BAS70301F + mancozeb + oil /mancozeb (4 applications) at 10 and 20 ml/hl water in both cases resulted in 99.6 % clean exportable fruit with no CBS.

Furthermore, the treatment where mancozeb was excluded from the tank mixture with BAS70301F viz., mancozeb/ BAS70301F + oil / BAS70301F + oil /mancozeb (4 applications) at 10 ml/hl water, was not significantly different the other two BAS70301F treatments and the standard mancozeb and Cabrio tank mixture with mancozeb and mineral spray oil, resulting in 93.8% clean exportable fruit.

Organo Silica

Field trial results from the trial site at the Lowveld Agricultural College showed that Organo Silica evaluated at a rate of 200 ml/hl water resulted in 97.2% clean exportable fruit. This was significant higher than ($P > 0.05$) than Organo Silica evaluated at rates of 100ml and 400 ml/hl water. In the duplicate trial at Crocodile Valley Citrus Co., the same treatments were no significant different from each other ($P < 0.05$) but the highest rate of Organo Silica tested at a rate of 400 ml/hl water, had between 4.8 and 34% less clean exportable fruit. This rate also had the highest rate of fruit with 4 and more fruit with CBS lesions. This may be due to the adjuvants that are included in the product that caused excessive run-off of the product (Table 4.6.4.3. x 3).

Mancozeb altered with F, RB1 and RB2

Results from two field trial sites where spray programmes consisting of mancozeb was altered with either F, RB1 or RB2, showed there were no significant differences ($P < 0.05$) between products and rates of each product that were evaluated with all criteria used for evaluation. All the spray programmes performed very well and can be registered and evaluated on a commercial scale (Table 4.6.4.4.x 4).

Benomyl + RB2 + BreakThru

Although benzimidazole resistance is common in the Nelspruit region, two applications of Benomyl + RB2 + BreakThru a tank mixture of gave good control of CBS. The higher rate of Benomyl (50 g/hl water) + RB2 resulted in 94% clean exportable fruit. Benomyl sprayed at the lower rate (25 g/hl water) at Crocodile Valley Citrus Co. was the only Benomyl treatment that resulted in significant less CBS control (87.2%) while control of the same rate at the Lowveld Agricultural College resulted in 89.8% clean exportable fruit (Table 4.3.x.5).

Benomyl + RBA + mancozeb + oil

In a similar trial (as the previous one) where benomyl was mixed with RBA, mancozeb and mineral spray oil and sprayed twice (November and January) resulted in 96.6% and 98.8% clean exportable fruit. These treatments were not significantly different from the four copper oxychloride and mancozeb treatments but can save growers two spray rounds.

Conclusion

Both BAS70004F and BAS70301F tested at rates of 10 and 20 ml/h_l water in tank mixtures with mancozeb and mineral spray oil gave excellent control of citrus black spot and can be recommended for registration. New spray programmes where mancozeb was altered with RB1 and RB2, as well as two applications consisting of tank mixtures of Benomyl (Spoton-B) plus RB2 and BreakThru as well as Benomyl + RBA + mancozeb + mineral spray oil, also performed well. These treatments will save the growers two spray rounds.

Future research

The trials 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 sprayed alone or in tank mixtures with new or existing registered 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 2014.

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Table 4.6.4.1. Evaluation of Kannar 202 applied during the susceptible period from October to January for citrus black spot (CBS) control on Valencia oranges at Lowveld Agricultural College and Crocodile Valley Citrus Co. during 2011 and 2012.

Treatments ^z	Rate per 100 ℓ water	Lowveld Agricultural College			Crocodile Valley Citrus Co.		
		% lesions/fruit ^y					
		0	1-3	>4	0	1-3	>4
Kannar 202	250 ml	92.4 a	2.8 a	4.8 a	95.0 a	2.2 a	2.8 a
Kannar 202	500 ml	95.2 a	0.2 a	4.6 a	83.2 a	5.6 ab	11.2 a
Copper oxychloride	200 g	99.2 a	0.6 a	0.2 a	99.6 a	0.4 a	0.0 a
Mancozeb	200 g	97.4 a	1.2 a	1.4 a	99.8 a	0.2 a	0.0 a
Untreated control	-	54.2 b	9.0 b	46.6 b	46.6 b	10.6 b	44.2 b

^y Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.

^z Spray dates were 11 October 2011, 8 November 2011, 6 December 2011 and 3 January 2012.

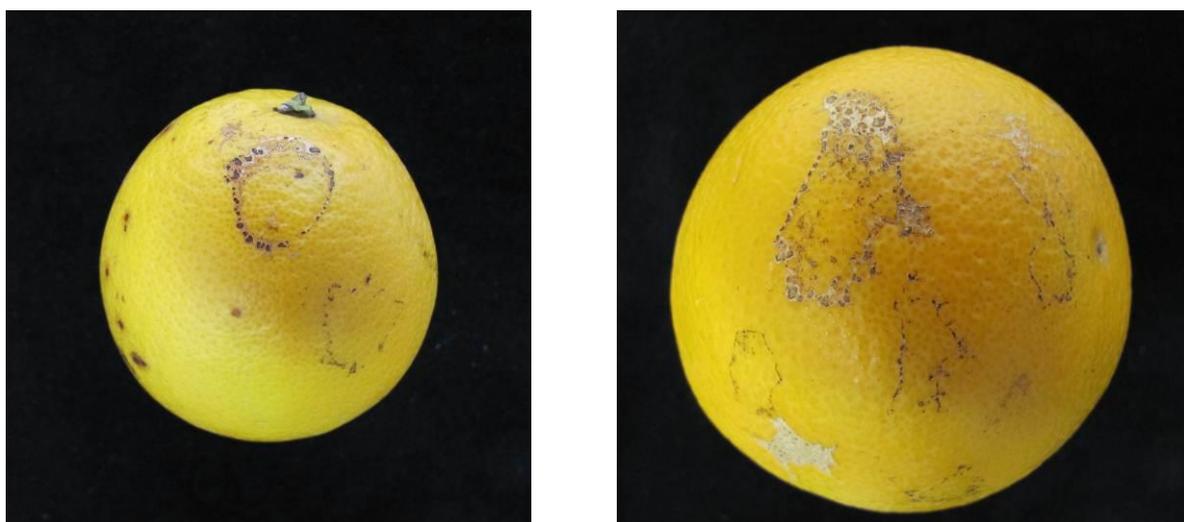


Fig. 4.6.4.1. Concentric rings at the bottom of Valencia oranges after 4 foliar applications of Kannar 202 at a rate of 500ml/100L water.

Table 4.6.4.2. Evaluation of BAS 70004F and BAS70301F applied from October and January during 2011 and 2012 for the control of *Guignardia citricarpa* on 'Valencia' oranges at Lowveld Agricultural College, Nelspruit, South Africa.

Treatments		% lesions/fruit ^w		
		0	1-3	≥4
1	Control	54.2 c	9.0 c	36.8 c
2	Mz/BAS70004F+Mz+oil/ BAS70004F+Mz+oil/Mz ^y 1x	99.4 a	0.4 a	0.2 a
3	Mz/BAS70004F+Mz+ oil/ BAS70004+Mz+oil/Mz ^y 2x	91.8 a	4.8 ab	3.4 a
4	Mz/BAS70004F+oil/ BAS70004F+oil/Mz ^y	80.8 b	6.6 c	12.6 b
5	Mz/ BAS70301F+Mz+oil 1x BAS70301F+Mz+oil/Mz ^y	99.6 a	0.2 a	0.2 a
6	Mz/ BAS70301F+Mz+oil 2x BAS70301F+Mz+oil/Mz ^y	99.6 a	0.2 a	0.2 a
7	Mz/BAS70301F+ oil 1x BAS70301F + oil/Mz ^y	93.8 a	3.2 b	3.0 a
8	Mz/Cabrio+Mz+oil/ Cabrio+Mz+oil/Mz ^y	99.2 a	0.6 ab	0.2 a
9	Mancozeb ^x	97.4 a	1.2 ab	1.4 a

^w Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.

^x Spray dates were 11 October 2011, 8 November 2011, 6 December 2011, 3 January 2012.

^y Spray dates were 11 October 2011, 8 November 201, 20 December 2011 and 31 January 2012

Mz = mancozeb

Table 4.6.4.3. Evaluation of Organo Silica applied during the susceptible period from October to January for citrus black spot (CBS) control on Valencia oranges at Lowveld Agricultural College and Crocodile Valley Citrus Co. during 2011 and 2012.

Treatments ^z	Rate per 100 ℓ water	Lowveld Agricultural College			Crocodile Valley Citrus Co.		
		% lesions/fruit ^y					
		0	1-3	>4	0	1-3	>4
Organo Silica	100 ml	82.8 b	3.6 ab	13.6 ab	94.0 a	2.8 a	3.2 a
Organo Silica	200 ml	97.2 a	0.6 a	2.2 a	93.0 a	2.0 a	5.0 a
Organo Silica	400 ml	62.8 c	7.0 b	30.2 b	88.2 a	0.6 a	11.2 ab
Copper oxychloride	200 g	99.2 a	0.6 a	0.0 a	99.6 a	0.4 a	0.0 a
Mancozeb	200 g	97.4 a	1.2 a	1.4 a	99.8 a	0.2 a	0.0 a
Untreated control	-	54.2 c	9.0 b	36.8 b	46.6 b	10.6 b	44.2 b

^y Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.

^z Spray dates were 11 October 2011, 8 November 2011, 6 December 2011 and 3 January 2012.

Table 4.6.4.4 Evaluation of alternating spray programmes consisting of mancozeb and RB1 or F applied during the susceptible period from October to January for citrus black spot (CBS) control on Valencia oranges at Lowveld Agricultural College and Crocodile Valley Citrus Co. during 2011 and 2012.

Treatments ^z	Rate per 100 ℓ water	Lowveld Agricultural College			Crocodile Valley Citrus Co.		
		% lesions/fruit ^y					
		0	1-3	>4	0	1-3	>4
Mancozeb/RB1/ mancozeb/RB1	200g/100mℓ/ 200g/100mℓ	92.8 ab	3.2 a	4.0 a	100 a	0 a	0 a
Mancozeb/RB1/ mancozeb/RB1	200g/200mℓ/ 200g/200mℓ	91.4 ab	2.8 a	5.8 a	98.8 a	0.8 a	0.4 a
Mancozeb/RB2/ mancozeb/RB2	200g/100mℓ/ 200g/100mℓ	89.8 b	3.2 a	7.0 a	97.6 a	0.8 a	1.6 a
Mancozeb/RB2/ mancozeb/RB2	200g/100mℓ/ 200g/200mℓ	98.6 ab	0.2 a	1.2 a	99.6 a	0.2 a	0.2 a
Mancozeb/F/ mancozeb/F	200g/570mℓ/ 200g/570mℓ	99.6 a	0.4 a	0 a	95.8 a	0.2 a	4.0 a
Copper oxychloride	200 g	99.2 a	0.6 a	0.2 a	99.6 a	0.4 a	0.0 a
Mancozeb	200 g	97.4 ab	1.2 a	1.4 a	99.8 a	0.2 a	0.0 a
Untreated control	-	54.2 b	9.0 b	46.6 b	46.6 b	10.6 b	44.2 b

^y Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.

^z Spray dates were 11 October 2011, 8 November 2011, 6 December 2011 and 3 January 2012.

Table 4.6.4.5. Evaluation of spray programmes consisting of benomyl + RB2 + BreakThru applied during the susceptible period from October to January for citrus black spot (CBS) control on Valencia oranges at Lowveld Agricultural College and Crocodile Valley Citrus Co. during 2011 and 2012.

Treatments ^z	Rate per 100 ℓ water	Lowveld Agricultural College			Crocodile Valley Citrus Co.		
		% lesions/fruit ^y					
		0	1-3	>4	0	1-3	>4
Benomyl +RB2 + BreakThru	25g + 500 ml	87.2 b	3.4 a	9.4 a	87.2 a	3.4 a	9.4 a
Benomyl +RB2 + BreakThru	50g + 1000ml	95.0 ab	2.8 a	2.2 a	95.0 a	2.0 a	3.0 a
Copper oxychloride	200 g	99.8 a	0.2 a	0.0 a	99.6 a	0.4 a	0.0 a
Mancozeb	200 g	97.4 ab	1.2 a	1.4 a	99.8 a	0.2 a	0.0 a
Untreated control	-	54.2 c	9.0 b	36.8 b	46.6 b	10.6 b	44.2 b

^y Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.

^z Spray dates were 11 October 2011, 8 November 2011, 6 December 2011 and 3 January 2012.

Table 4.6.4.6. Evaluation of spray programmes consisting of benomyl + RBA + mancozeb + oil applied during the susceptible period from October to January for citrus black spot (CBS) control on Valencia oranges at Lowveld Agricultural College and Crocodile Valley Citrus Co. during 2011 and 2012.

Treatments ^z	Rate per 100 ℓ water	Lowveld Agricultural College			Crocodile Valley Citrus Co.		
		% lesions/fruit ^y					
		0	1-3	>4	0	1-3	>4
Benomyl + RBA + mancozeb + oil	25g + 500 ml	96.6 a	0.8 a	2.6 a	96.8 a	1.4 a	1.8 a

Benomyl + RBA + mancozeb + oil	50g + 1000ml	98.8 a	1.0 a	0.2 a	98.0 a	0.4 a	1.6 a
Copper oxychloride	200 g	99.8 a	0.2 a	0.0 a	99.6 a	0.4 a	0.0 a
Mancozeb	200 g	97.4 ab	1.2 a	1.4 a	99.8 a	0.2 a	0.0 a
Untreated control	-	54.2 c	9.0 b	36.8 b	46.6 b	10.6 b	44.2 b

^y Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.

^z Spray dates were 11 October 2011, 8 November 2011, 6 December 2011 and 3 January 2012.

4.6.5 PROGRESS REPORT: Improving the retention of suspension liquid phosphonate fungicides on citrus fruit and leaves

Project 1012 (April 2011- March 2014) by G.C. Schutte, C. Kotze, M.C. Pretorius (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. More than 0.5 ml spray application per side per leaf or 1 ml per whole leaf is required if at all possible any coverage of Valencia leaves with phosphonates. 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 significantly 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 was 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. Meer as 0.5 ml spuitstof per kant per blaar of 1 ml per hele blaar word benodig om enigsins bedekking van Valencia blare te kry met fosfonate. Bymiddel B teen geregistreerde dosisse het 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 PROGRESS REPORT: Epidemiology and pest risk assessment of *Guignardia citricarpa*
Project 1026 (April 2011 - March 2015) 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 published 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. One workshop was held in Florida and further progress was made by developing 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 for the past period included data collection to improve the models and to distinguish between the CBS pathogen and endophytic *Guignardia* sp., new modelling approaches and surveys and experiments to determine efficacy of CBS orchard and packhouse control measures.

Opsomming

Sitruswartvlek 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 gebaseer op meso- en mikroklimaat data. Op hierdie onderwerp is aanvanklike modelering afgehandel en 'n artikel in 'n toonaangewende wetenskaplike joernaal gepubliseer. 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. Een werkwinkel is gehou, en noemenswaardige vordering is gemaak, spesifiek deur die verskillende stappe in die model verder te ontwikkel en 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 swartvlekpatogene en 'n endofitiese *Guignardia* sp. te onderskei, nuwe modellering-strategie te beproef, asook ondersoeke om die sukses van swartvlekbeheer in boorde en pakhuisse te kwantifiseer.

4.6.7 FINAL REPORT: Retention of suspension concentrate fungicides versus wettable powder copper hydroxide and mancozeb formulations on citrus fruit and leaves as determined by fluorescent pigment deposition analyses

Project 1044 (2012 - 2013) by G.C. Schutte, C. Kotze, J.G. van Zyl and P.H. Fourie (CRI)

Summary

Two Valencia orchards were sprayed with mancozeb and copper hydroxide SC and WP formulations in tank mixtures with SARDI yellow fluorescent pigment. Fruit and leaf samples from both trial sites were taken and photographed and sent to SU for digital image analyses. The %FPC values on leaves for both copper formulations stayed stable during both experimental periods from the day 0 to day 35. The copper residues decreased by half from day 1 to day 7 and then decrease steadily to day 35. On fruit, the %FPC measured was significant higher on day 1 and also decreased to lower levels on day 35. Copper residue levels on fruit followed a similar trend and decreased by 50% from day one to day 35. On leaves, %FPC values for both mancozeb formulations decreased significantly by 60% from the day of application to day 28 during 2010 but increased during the second experimental period. The %FPC for the SC formulation was significant more than that of the WP formulation showing that the mineral oil as adjuvants added to SC resulted in good spreading of the residues on the fruit surface. On fruit, %FPC values for the 2011 season for both mancozeb formulations increased significantly from the day of application to day 28 while the residues stayed stable. This is in contrast with the previous season when there was a steady decrease in the %FPC and residues for both formulations. The increase in fruit surface area had little to no effect on wash-off of copper residue of the Copstar and Kocide 2000 treatments while rainfall had a limited effect on the reduction of copper residues on both fruit and leaves. The increase in fruit surface area had an effect on mancozeb residue of the Mancozeb while accumulative rainfall had a limited effect on the reduction of CS² residues on fruit. On the other hand, the increase in fruit surface area had no effect on CS² residue of the Pennfluid treatment.

Comparing Mancozeb with Pennfluid showed that Pennfluid was marginally more affected by rainfall than Mancozeb.

Opsomming

Twee Valencia boorde is gespuit met mancozeb en koperhidroksied SK en BP formulasies in 'n tenkmengsel met SARDI se geel fluorisensiepigment. Vrug- en blaarmonsters is van beide proefboorde getrek en gefotografeer waarna hulle US gestuur is digitale foto analise. Die %FPC (persentasie fluoriserende pigment bedekking) waardes op blare het vir beide koperformulasies stabiel gebly oor beide eksperimentele periodes van dag 0 tot dag 35. Die koperresidue het ook met die helfte verminder van dag 1 tot dag 7 en toe geleidelik afgeneem oor 35 dae. Op vrugte was die %FPC ook betekenisvol hoër op dag 1 en het ook afgeneem na laer vlakke na 35 dae. Koperresidu vlakke op vrugte het 'n soortgelyke tendens gevolg en het met 50% afgeneem van dag 1 tot dag 35. Op blare het die %FPC waardes vir beide mancozeb formulasies betekenisvol met 60% afgeneem van die dag van toediening tot dag 28 gedurende die eerste eksperimentele periode, maar het toegeneem tydens die tweede eksperimentele periode. Die %FPC vir die SK formulasie was betekenisvol meer as die BP formulasie wat toon dat die minerale spuitolie as bymiddel in die SK formulasie beter bedekking op die vrugoppervlaktes tot gevolg gehad het. Op vrugte het die %FPC waardes vir die 2011 seisoen vir beide mancozeb formulasies van die dag van toediening tot dag 28 betekenisvol toegeneem terwyl die residue stabiele gebly het. Dit is in kontras met die vorige eksperimentele periode van 2010 toe daar 'n geleidelike afname in die %FPC en vir residue was vir beide formulasies. Die toename in vrugoppervlek het min tot geen effek op die afwas van koperresidue gehad van beide Copstar and Kocide 2000, terwyl reënval 'n beperkte effek op die afname van koper-residue gehad het op beide vrugte en blare. Die toename in vrugoppervlakte het 'n effek of die afname van mancozeb residue van Mancozeb gehad terwyl akkumulatiewe reënval 'n beperkte effek op die afname van CS² residue op vrugte gehad het. Daarenteen het die toename in vrugoppervlak geen effek op CS² residue afname van Pennfluid gehad nie. Indien die twee met mekaar vergelyk word, was Pennfluid marginaal deur reënval geaffekteer.

Introduction

Copper and dithiocarbamate fungicides are still registered for the control of CBS since the 1960's and various new SC formulations of both groups were recently registered for CBS control, viz. Copstar (copper hydroxide) and Pennfluid (mancozeb (Mz)). The active ingredient (a.i.) in both the SC formulations is 45% in comparison with the 80% WP formulation of Tridex (mancozeb) and 54% WG formulation for Kocide 2000 (copper hydroxide). The spray intervals for both the copper hydroxide formulations are 35 days and 28 days for both of the mancozeb WP and SC formulations. How is this possible? Will the fungicides with less a.i. (in this case the SC formulations) not degrade faster than the fungicides with a higher a.i. resulting in unprotected areas on the leaves and fruit? Apart from field trials using different rates with different spray intervals to establish effective spray programmes, no one has ever looked scientifically at the target areas (leaves and fruit) and how fungicides adhere to the surfaces of citrus leaves and fruit.

By using fluorescent pigment deposition analyses and residue analyses of the same samples, one can determine the degradation of both these fungicides over time under natural conditions. If the SC formulations do not degrade faster and perform the same as the WP, then it will benefit the citrus growers as it will result in less fungicidal residues on the exported fruit. Recently, experiments were performed in Spain to evaluate the protective effect of reduced copper concentrations on fruits of the Mandarins as sprayed for the control of *Alternaria* brown spot. All coppers evaluated protected the trees effectively for 28 days under optimal conditions for infection and reducing the concentration of copper in the spray tank from 1 to 0.5 g/L metallic copper had no negative effect on persistence.

In order to study the optimisation of spray application on citrus, researchers at Stellenbosch University's Plant Pathology department (USPP) have developed a spray assessment protocol using fluorometry, photomacrography and digital image analyses (van Zyl *et al.*, 2013). This protocol will be used to determine the persistence of copper hydroxide and mancozeb under natural conditions. The average increase of fruit surfaces can be calculated over time and correlated with the dilution effect caused by rain over the experimental period. Concomitantly, the copper and mancozeb residue-analysis will also be correlated with the latter two. The efficacy of extended spray intervals for the control of CBS will be determined when the fruit ripens.

Objectives

To compare the retention of mancozeb and copper hydroxide SC and WP formulations as foliar sprays on Valencia orange fruit and leaves.

Materials and methods

a) Field applications of copper hydroxide and mancozeb (MZ) for Cu and MZ-residue analysis as well as to determine Cu and MZ-persistence

Two Valencia orange orchards were selected at Croc Valley Citrus Co. in the Nelspruit region to do the evaluations. A randomised block design with 3 single-tree plots per treatment was used. Fungicides were applied once in November with a trailer-mounted, high-volume, high-pressure (2,500-3,000 kPa) sprayer with two hand-held spray guns. Spray volumes varied according to the size and canopy density of the tree but all trees were sprayed to the point of run-off. Trees were also selected for uniformity in canopy density and tree size. The fungicides to be tested at registered rates were copper hydroxide (Kocide 2000, 77% WG and Copstar, 12% SC) and mancozeb (Tridex, 80% WP and Pennfluid, 42% SC). Fruit and leaves were exposed to normal rainy conditions. Leaves and fruit were drawn on a weekly basis for 5 weeks for both copper treatments and every two weeks for mancozeb. The samples were sent to SU for fluorometry analyses. The same fruit and leaf samples were couriered to Absolute Science in Pretoria for copper residue analysis and the mancozeb residues were analysed by the SABS in Pretoria.

b) Determine persistence of mancozeb and copper hydroxide on Valencia fruit and leaves using fluorometry

Yellow Fluorescent Pigment® (400 g/L, EC; South Australian Research and Development Institute) at 100 ml L/100 L were added to each application and samples were taken on days, 0, 7, 14, 21, 28 and 35. Leaves (upper and lower leaf surface) and fruit (stylar – and blossom-ends) were illuminated under black light and photos were taken with a digital camera and image analysis of photos done with Image-Pro Plus software to determine the total area of deposited pigment on leaves and fruit. The percentage area covered by fluorescent pigment was subsequently calculated.

c) Copper and mancozeb residue analysis

Each replicate, consisting of 20 leaves per sample, was individually dried in paper bags in an oven at 70°C for 12 h, bulked and submitted for copper residue analysis (Absolute Science, Silverton, Pretoria, South Africa) and mancozeb residue analysis (SABS, Muckleneuk, Pretoria, South Africa). There the leaves were grinded and sieved through a 1 mm sieve where after 1 g was cremated at 500°C for 4 hours. The ashed leaf samples were digested with 5 ml 6 N HCl and 6 N HNO₃ mixture and the volume was made up to 100 ml with distilled water, heated to boiling point, filtered and analyzed, using 25 ml of each sample which was injected into a ICP spectrometer (Perkin-Elmer A-Analyst 400). Total copper dissolved in the acid extract, expressed as parts per million (ppm), were determined with ICP spectrometer.

The same fruit used for deposition assessment and fruit size measurements were sent directly by courier to Absolute Science for copper residue analysis. The outer fruit tissue comprising the flavedo, cuticle and cuticular wax layers (1-2 mm) were removed by using a grater. A portion (1 g) of each fruit sample was digested in 5 ml 6 N HCl and 6 N HNO₃ mixture and the volume was made up to 100 ml with distilled water and copper residue determined as described above.

d) Statistical analyses

Deposition, residue and fruit size data were subjected to analyses of variance (ANOVA), Student's T-test for least significant difference ($P = 0.05$) and Pearson's correlations using XLSTAT 2013.2.01. Deposition and residue data will furthermore be subjected to logarithmic regression analyses to compare trends between treatments.

e) Determining fruit growth rate

Valencia orange fruit was marked and measured using a calliper during this period using the formula: $4\pi r^2$. The average increase in fruit surface area was correlated with the distribution pattern of copper residues on the fruit from the fluorometry results.

Results and discussion

2010 season

Copper hydroxide SC and WG formulations

On leaves, %FPC values for both copper formulations stayed stable during the experimental period from the day of application to day 35. On the other hand, the mean copper residues of Valencia orange leaves on day 1 of the WG formulation was similar to that of a previous study (Schutte *et al.*, 2012). Results showed that the copper residues decreased by half from day 1 to day 7. Thereafter it showed a steady decrease to day 35. Likewise, the copper hydroxide SC formulation showed a similar trend on the leaves. The copper residue levels of both copper treatments were, however, significantly more than that of the untreated control during the experimental period (Table 4.6.7.1).

On Valencia orange fruit, the %FPC measured was significant higher on day 1 and decrease to lower levels on day 35. Concomitantly, the copper residue levels measured followed a similar trend and decrease by 50% from day one to day 35. The copper residue levels of both copper formulations were significant higher than that of the untreated control and were also much lower than a previous study (Schutte *et al.*, 2012).

Mancozeb SC and WP formulations

On leaves, %FPC values for both mancozeb formulations decreased significantly by 60% from the day of application to day 28. The %FPC for the SC (Pennfluid) formulation was significantly more than that of the WP formulation showing that the adjuvants added to SC resulted in good spreading of the residues on the fruit surface. The three mancozeb replicates of both SC and WP treatments for all the evaluation dates had to be bulked before they were sent to the SABS for CS₂ analyses due to high costs involved. Residual results of both mancozeb formulations showed that the WP formulation had 60% more CS₂ residues than the SC formulation which corresponds with the active ingredient in the type of formulation (Tridex, 80% WP and Pennfluid, 42% SC). CS₂ residues of both formulations decreased to 2.1 - 2.4 ppm on day 28 (Table 4.6.7.2).

2011 season

Copper hydroxide SC and WP formulations

Although lower than the previous year, the %FPC values for both copper formulations stayed stable on Valencia orange leaves during the experimental period from the day of application to day 35. In both cases, the rainfall was lower from the day of application until day 21 when there was a huge increase in rainfall. This however had no effect in the decrease in the %FPC over 35 days. On the other hand, the %FPC measured for the SC formulation on Valencia orange fruit was significant higher on day 1 to 14 and then decrease to significant lower levels on day 35. Concomitantly, the %FPC values for the WG copper formulation stayed stable on Valencia orange fruit. Similar results were obtained where the copper residue levels were measured on the fruit. The SC formulation had 60% more residues on the fruit in 2011 than in 2010 while the WG formulation had a similar residual level for both years. However, none of the copper formulations resulted in a decrease of copper residues on Valencia orange fruit over the same experimental period as during the previous year. The copper residue levels of both copper treatments were however significant more than that of the untreated control during the same experimental period (Table 4.6.7.1).

Mancozeb SC and WP formulations

On fruit, %FPC values for both mancozeb formulations increased significantly from the day of application to day 28. This is in contrast with the previous season when there was a steady decrease in the %FPC for both formulations. The fruit surface area of the WP formulation increased with 33% with as well as more rain during the 2010 season. The same trend was observed with the residue results of both mancozeb formulations. The residue analyses on Valencia orange fruit for the WP formulation was also lower during 2011 if compared with the 2010 season. Handling of treated fruit prior to analyses may result in less residues to be measured (Table 4.6.7.4).

Influence of rainfall and increase of fruit surface area on copper residues for both seasons

According to Pearson's correlation matrix, the increase in fruit surface area had little to no effect on copper residue of the Copstar treatment for both seasons ($R^2 = 0.048$ and 0.050 for year 1 and year 2 respectively).

Rainfall had a limited effect on the reduction of Copstar's copper residues on both fruit ($R^2 = 0.565$) and leaves ($R^2 = 0.610$).

For Kocide 2000, the Pearson's correlation matrix showed that the increase in fruit surface area had an effect on copper residue for the first seasons ($R^2 = 0.769$) and in contrast to that in the second year, the increase in fruit area no effect on copper residue reduction ($R^2 = 0.137$). Rainfall had a marginal effect on the Kocide 2000's copper residue reduction on both fruit and leaves for season one and two, but more so for copper residues on leaves for the first season ($R^2 = 0.285$).

Influence of rainfall and increase of fruit surface area on mancozeb residues for both seasons

According to Pearson's correlation matrix, the increase in fruit surface area had an effect on mancozeb residue of the Mancozeb treatment for both seasons ($R^2 = 0.803$) and the accumulative rainfall had a limited effect on the reduction CS² residues on fruit ($R^2 = 0.281$). Concomitantly, the increase in fruit surface area had no effect on mancozeb residue of the Pennfluid treatment for both seasons ($R^2 = 0.386$). Comparing Mancozeb with Pennfluid showed that Pennfluid was marginally more affected by rainfall than Mancozeb ($R^2 = 0.503$ vs. 0.281).

Task table

Objective / Milestone	Achievement
Apr-Jun Literature survey.	Done
Jul-Sep Preparations for orchard applications	Done
Oct-Dec Conduct orchard spray trials to characterise spray deposition with current spray application methods Leaf sampling for Cu and Mz residue analysis and fluorometry. Measure fruit size on a weekly basis to calculate fruit surface area ($4\pi r^2$)	Two field applications were completed and samples taken for photographic analyses The same samples were sent away for residue analyses Fruit sizes were also taken.
Jan-Mar Computer analyses at SU Statistical analyses Report writing	JG van Zyl did the computer analyses PH Fourie did the stats on 25 March 2013. A publication will be drafted

Conclusion to date

From residue analyses it seems that both SC and WP mancozeb and copper hydroxide formulations have enough residues left to protect citrus fruit and leaves at registered spray intervals. The increase in fruit surface area had little to no effect on copper residue of the Copstar treatment for both seasons while rainfall had a limited effect on the reduction of Copstar's copper residues on both fruit and leaves. Rainfall had a marginal effect to no effect on the Kocide 2000's copper residue reduction on both fruit and leaves over a period of two seasons, but more so for copper residues on leaves for the first season.

The increase in fruit surface area had an effect on mancozeb residue of the Mancozeb treatment for both seasons while accumulative rainfall had a limited effect on the reduction of CS² residues on fruit. On the other hand, the increase in fruit surface area had no effect on mancozeb residue of the Pennfluid treatment for both seasons. Comparing Mancozeb with Pennfluid showed that Pennfluid was marginally more affected by rainfall than Mancozeb.

Technology transfer

Talks or presentations: Oral presentation at 7th CRI Symposium 2012.

Title for SAFJ article: Retention of copper hydroxide and mancozeb SC and WP formulations as sprayed on 'Valencia' oranges for the control of citrus black spot

Title for refereed paper: Retention of copper hydroxide and mancozeb SC and WP formulations as sprayed on 'Valencia' oranges for the control of citrus black spot.

Further objectives and work plan

This project will terminate after the current financial year.

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Table 4.6.7.1. Mean fluorescent pigment deposition (%FPC) and copper residue (ppm) measured from Valencia orange leaves and fruit as determined over a period of 35 days after spray application with copper hydroxide SC and WG formulations each with SARDI Yellow Fluorescent Pigment.

Treatment	Rate per 100ℓ	Days after treatment	2010 season ^x				2011 season ^x			
			Leaf		Fruit		Leaf		Fruit	
			% FPC	Cu (ppm)	% FPC	Cu (ppm)	% FPC	Cu (ppm)	% FPC	Cu (ppm)
Copper hydroxide SC (Copstar)	350 ml	0	10.22de	191.42d	16.21de	4.23c-e	4.92a	324.55fg	6.93cd	13.22fg
		7	9.08cd	94.12b	15.32d	4.49de	10.08g	308.99e-g	5.98bc	11.94e-g
		14	12.61f	107.76bc	15.57de	3.26b-d	7.35de	332.04g	8.54e	15.42g
		21	10.27de	104.2bc	12.28c	2.84bc	5.89b	203.35c	5.11b	10.22c-f
		28	9.01cd	104.86bc	10.73b	2.84bc	5.33ab	182.12c	5.28b	9.45b-e
		35	10.10de	68.09b	5.23a	2.21b	6.08bc	124.29b	3.48a	11.32d-f
Copper hydroxide WG (Kocide 2000)	200 g	0	6.39a	419.47e	16.36e	9.46h	6.89cd	433.08i	7.59de	10.76c-f
		7	10.35e	184.44d	11.90bc	7.1g	8.79f	389.52h	11.90g	11.48d-f
		14	10.79e	174.93d	16.66e	6.41fg	7.54de	442.64i	10.23f	8.03b-d
		21	12.41f	154.59cd	15.90de	5.12ef	8.97f	263.88d	11.38g	6.12b
		28	7.57ab	146.60cd	11.37bc	4.64de	7.45de	275.24de	10.84fg	8.70b-e
		35	8.19bc	118.72bc	4.50a	4.89e	7.86e	281.84d-f	6.12bc	7.36bc
Control		0	—	3.03a	—	0.13a	—	3.66a	—	0.31a
		7	—	3.47a	—	0.33a	—	3.99a	—	0.20a
		14	—	3.09a	—	0.37a	—	3.57a	—	0.05a
		21	—	3.28a	—	0.32a	—	3.30a	—	0.24a
		28	—	2.39a	—	0.36a	—	2.32a	—	0.17a
		35	—	1.09a	—	0.27a	—	2.15a	—	0.15a

^x Means in a column (based on 3 replicates; n =20) followed by the same letter do not differ significantly ($P \geq 0.05$) according to the Student's T-test.

Table 4.6.7.2. Mean fluorescent pigment deposition (%FPC) and mancozeb residue (CS₂; ppm) measured from Valencia orange fruit as determined over a period of 28 days after spray application with mancozeb SC and WP formulations each with SARDI Yellow Fluorescent Pigment during 2010 and 2011 growing seasons.

Treatment	Rate per 100ℓ	Days after treatment	2010 season ^x		2011 season ^x	
			% FPC	CS ₂ (ppm)	% FPC	CS ₂ (ppm)
Mancozeb (WP)	200 g	0	13.62c	10.00	8.10a	4.70
		14	10.20b	3.30	11.2bc	3.45
		28	4.39a	2.45	10.63bc	4.35
Pennfluid (SC)	200 ml	0	15.85d	3.50	7.53a	2.45
		14	15.61d	2.90	11.64c	2.70
		28	5.32a	2.15	10.50b	2.30

^x Means in a column (based on 3 replicates with 20 fruit each) followed by the same letter do not differ significantly ($P \geq 0.05$) according to the Student's T-test.

Table 4.6.7.3. Mean increase in Valencia orange fruit surface area (mm²) over a period of 35 days after spray applications of copper hydroxide SC and WG formulations during 2010 and 2011 growing seasons.

Treatment	Days after treatment	Fruit area (mm ²) ^x	
		2010 season	2011 season
Copper hydroxide SC (Copstar)	0	6395.35a	7328.23a
	7	6828.44bc	7283.24a
	14	7622.61fg	8715.87b-d
	21	7213.98c-e	8650.54bc
	28	7684.93f-h	8696.45bc
	35	7934.89g-h	9123.28d-f
Copper hydroxide WG (Kocide)	0	6357.31a	9216.30ef
	7	6481.79ab	8470.30b
	14	7521.65ef	8934.27c-e
	21	7337.94d-f	9452.06f
	28	8222.87i	9212.41ef
	35	8070.79hi	9458.98f
Control	0	7184.82c-e	8512.32b
	7	7643.29fg	8779.17b-d
	14	7067.19cd	9222.62ef
	21	7485.36ef	10270.38g
	28	7716.36f-h	9235.23ef
	35	7057.09cd	8835.49b-e

^xMeans in a column (based on 3 replicates with 20 fruit each) followed by the same letter do not differ significantly ($P \geq 0.05$) according to the Student's T-test.

Table 4.6.7.4. Mean increase in Valencia orange fruit surface area (mm²) over a period of 35 days after spray applications of mancozeb SC and WP formulations during 2010 and 2011 growing seasons.

Treatment	Days after treatment	Fruit area (mm ²)	
		2010 season	2011 season
Mancozeb WP	0	6052.15a	8821.80ab
	14	8166.15c	8930.53ab
	28	9107.85c (33%)	9270.83bc (5%)
Pennfluid SC	0	5808.08a	8623.95a
	14	6923.38b	9132.55abc
	28	7022.47b (17%)	9573.54c (10%)

*Means in a column (based on 3 replicates) followed by the same letter do not differ significantly ($P \geq 0.05$) according to the Student's T-test.

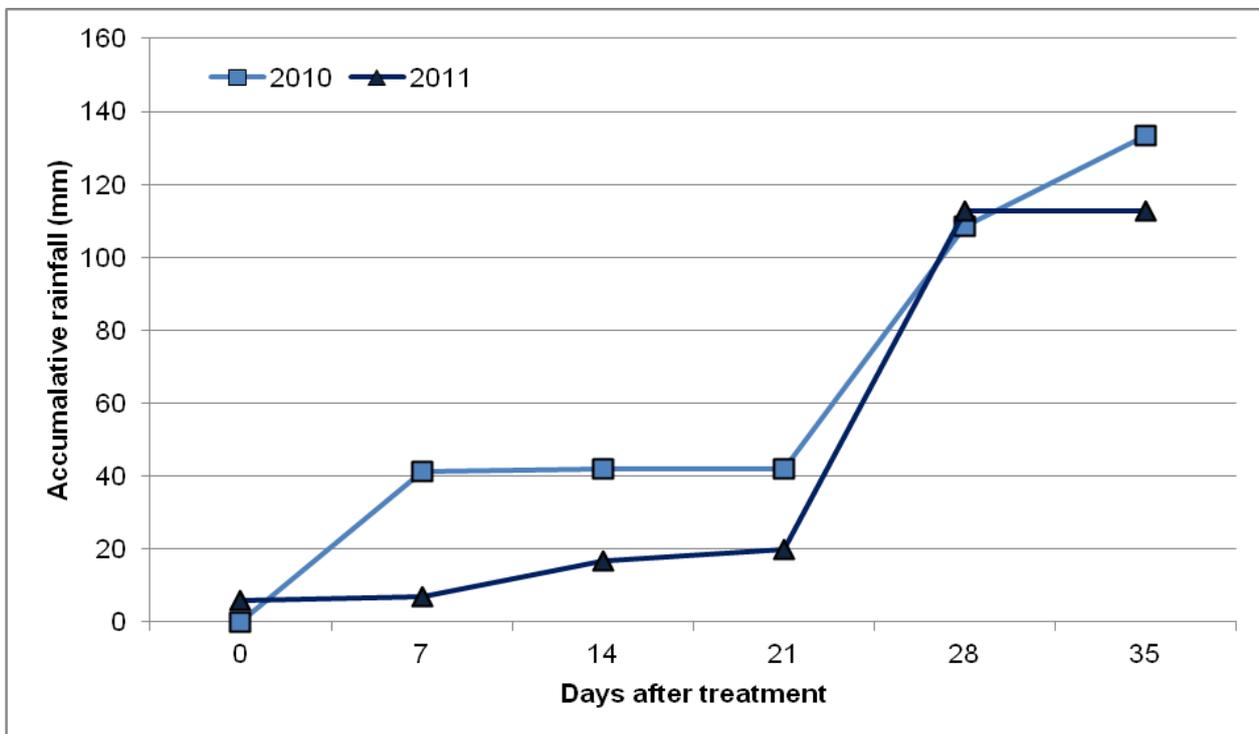


Fig. 4.6.7.1. Accumulative rainfall at Crocodile Valley Citrus Co. during the two experimental periods from 31 January 2010 to 7 March 2010 and 22 January 2011 to 26 February 2011.

4.7 **CRI DIAGNOSTIC CENTRE** (Elaine Basson, M.C. Pretorius, Vongani Rikhotsi and Timothy Zulu - CRI)

Analysis	Citrus nurseries	Commercial samples	Other crops	Research samples	River Bioscience
Nematode:Roots	9	1019	26	698	0
Nematode:Soil	2	25	11	852	0
<i>Phytophthora</i>	3193	1208	52	407	0
Water spore trap	205	15	2	0	0
Black spot identification (PCR)	0	16	0	614	0
Black spot benzimidazole resistance	0	0	0	0	0
Citrus greening (PCR)	0	7	0	1	0
Fungal Isolation (other)	5	0	4	0	0
Fruit & Foliar identification	0	7	0	0	0
Soil dilution plating	0	116	11	0	0
Quality control	0	0	0	0	54
TOTAL	3414	2413	106	2572	54

Citrus Accredited Nurseries

It is compulsory for all citrus nurseries participating in the Citrus Improvement Scheme to send samples for *Phytophthora* analysis on a quarterly basis. The irrigation water must also be tested for *Phytophthora* by making use of the spore trap method. In total, 3193 nursery samples were received by the diagnostic centre for *Phytophthora* analyses. Of these samples, 12.06% 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, 0% 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 six percent of the 1019 samples analysed for citrus nematode had counts above the threshold value of 1000 females per 10g of roots, and nematicide treatments were recommended. Fifty five percent of the 1208 samples analysed for *Phytophthora* tested positive.

Other crops

Nematode counts were done on soil or root samples of apple, banana, macadamia, maize, pomegranate, potatoes, tobacco and wheat. Nematodes found present on these crops included: *Criconema*, *Hemicycliophora*, *Meloidogyne*, *Paratylenchus*, *Pratylenchus*, *Rotelynychulus*, *Scutellonema*, and *Tylenchus*. *Phytophthora* and *Pythium* analyses were done on apple, avocado, curcubits, macadamia, maize, pine, pomegranate, potato and tobacco. The diagnostic centre analysed five soil samples from macadamia nurseries and nine from avocado nurseries for the presence of *Phytophthora cinnamomi*.

Research samples

Nematode and *Phytophthora* analysis were done on 1957 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, 54 samples were analysed and results reported to River Bioscience.

4.7 **CRI DIAGNOSTIESE SENTRUM** (Elaine Basson, M.C. Pretorius, Vongani Rikhotsi and Timothy Zulu - CRI)

Ontleding	Sitrus kwekerye	Kommersiële monsters	Ander gewasse	Navorsings-monsters	River Bioscience
Aalwurms: Wortels	9	1019	26	698	0
Aalwurms: Grond	2	25	11	852	0
<i>Phytophthora</i>	3193	1208	52	407	0
Water	205	15	2	0	0
Swartvlek (PKR)	0	16	0	614	0
Swartvlek benzimidazole bestandheid	0	0	0	0	0
Swamisolasies	0	7	0	1	0
Sitrusvergroeningsiekte (PKR)	5	0	4	0	0
Grondverdunningsplate	0	7	0	0	0
Vrug en blaarsiektes	0	116	11	0	0
Kwaliteitsbeheer	0	0	0	0	54
TOTAAL	3414	2413	106	2572	54

Sitrus Geakkrediteerde Kwekerye

Dit is verpligtend vir al die sitruskwekerye wat aan die Sitrus Verbeteringskema deelneem om kwartaalliks monsters vir *Phytophthora* te laat ontlead. Die besproeiingswater moet ook deur middel van die spoorlokval metode vir *Phytophthora* getoets word. In totaal 3193 monsters is deur die diagnostiese sentrum vir *Phytophthora* ontleding ontvang, waarvan 12.06% positief getoets het. Benewens die water en grondmonsters, moet kwekerye een keer per jaar 'n wortelmonster instuur om vir die teenwoordigheid van *Tylenchulus semipenetrans* te toets. Van die 9 wortelmonsters wat ontvang is, het 0.0% positief vir die teenwoordigheid van *T. penetrans* getoets en van die 2 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*, ontlead. Ses-en-veertig persent van die 1019 aalwurmmonsters wat ontlead is, het tellings hoër as die drempelwaarde van 1000 wifies per 10g wortels gehad. Aalwurmdoderbehandelings is aanbeveel. Vyf-en-vyftig persent van die 1208 monsters wat vir *Phytophthora* ontlead is het positief getoets.

Ander Gewasse

Aalwurmtellings is op grond- of wortelmonsters van aartappel, appel, granaat, koring, macadamia, mielies, piesang, en tabak. Aalwurms teenwoordig gevind op hierdie gewasse sluit in: *Criconema*, *Hemicycliophora*, *Meloidogyne*, *Paratylenchus*, *Pratylenchus*, *Rotelynychulus*, *Scutellonema*, en *Tylenchus*. Makadamia, avokado, granaat, denneboom, mielies, appel, aartappel, 'curcurbit' en tabak monsters is vir *Phytophthora* en *Pythium* ontlead. Die diagnostiese sentrum het vyf monsters vanaf macadamia kwekerye en nege monsters vanaf avokado kwekerye vir *Phytophthora cinnamomi* ontlead.

Navorsingsmonsters

Aalwurm en *Phytophthora* ontledings is op 1957 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 54 monsters ontlead en resultate aan River Bioscience gestuur.

5 PORTFOLIO: HORTICULTURE

5.1 PORTFOLIO SUMMARY

By Tim G Grout (Manager: Research & Technical)

The value of horticultural research usually becomes evident when something goes wrong and there are enormous losses. With pests and diseases there is often an opportunity to intervene and minimise losses but in horticulture this is not always the case, especially if the reason for the losses is not fully understood. After years of investigation, good progress has now been made with peteca spot of lemons and sprays of Ethephon before harvest have been found to reduce this rind condition. Reducing dehydration between harvest and packing has also been found to reduce the incidence of pitting. More has been learnt about preharvest conditions that can increase the likelihood of chilling injury and the non-destructive use of near infra-red spectroscopy shows promise for identifying factors involved in various rind disorders. Much of the preharvest research focuses on ways of increasing production per hectare while minimizing input costs. More efficient use of water, better uptake of foliar micronutrients, more natural and improved soil nutrition and possible increased resistance to plant pathogens through silicon applications, are all under investigation. Some of these projects are extremely complex because they involve many variables but occasionally a simple solution is found that may be of great value and that is the case with the use of a 2,4-D spray to reduce fruit splitting in some easy-peelers. Research on how to cool fruit down as fast as possible in the cold chain has become more important with the need to slow the development of citrus black spot and kill false codling moth. Disposable kits to change the airflow in containers and accelerate cooling are giving promising results. Changes in the personnel working on cultivars provided the opportunity to place an evaluator in the Western Cape so that the needs in that area and the Northern Cape can be addressed more effectively. Access to private cultivars for evaluation purposes has improved so the value of these evaluations for growers has increased with more of the available cultivars being covered. Unfortunately we have been unable to fill the vacant preharvest horticulturist position due to a lack of appropriately qualified applicants, but this remains a priority.

PORTEFEULJEOPSOMMING

Die waarde van tuinboukundige navorsing is gewoonlik eers duidelik wanneer iets verkeerd gaan en daar enorme verliese is. Met plaë en siektes is daar gewoonlik 'n geleentheid om in te gryp en verliese te minimaliseer, maar in hortologie is dit nie altyd die geval nie, veral as die rede vir verliese nie ten volle verstaan word nie. Na jare van navorsing, is daar nou goeie vordering met peteka-kol van suurlemoene gemaak en die bespuiting van Ethephon voór oes is gevind om hierdie skiltoestand te verminder. Die vermindering van dehidrasie tussen oes en verpakking is ook gevind om die voorkoms van gepokte skil te verminder. Meer is geleer oor voór-oes toestande wat die waarskynlikheid van koueskade verhoog, en die nie-vernietigende gebruik van naby-infra-rooi spektroskopie toon belofte vir die identifisering van faktore wat by verskeie skilprobleme betrokke is. Baie van die voór-oes navorsing fokus op maniere om produksie per hektaar te verhoog terwyl die insetkoste geminimaliseer word. Meer effektiewe gebruik van water, beter opname van blaar-mikrovoedingstowwe, meer natuurlike en verbeterde grondvoeding en moontlike verhoogde weerstand teen plantpatogene deur middel van silikontoedienings, word almal ondersoek. Sommige van hierdie projekte is uiters kompleks omdat verskeie veranderlikes 'n rol speel, maar soms word 'n eenvoudige oplossing gevind wat van groot waarde kan wees, soos die geval is met die gebruik van 'n 2,4-D bespuiting om vrugsplit in sommige easy-peelers te verminder. Navorsing op hoe om vrugte so vinnig as moontlik in die koueketting af te koel het meer belangrik geraak, met die noodigheid om die ontwikkeling van sitrus swartvlek te vertraag en valskodlingmot te dood. Wegdoenbare toerusting om die lugvloei in houers te verander en verkoeling te versnel gee belowende resultate. Veranderinge in die personeel wat met die kultivars werk het die geleentheid verskaf om 'n evalueerder in die Wes-Kaap te plaas sodat die behoeftes in daardie area en die Noord-Kaap meer effektief aangespreek kan word. Toegang tot privaat-kultivars vir evaluasie-doeleindes het verbeter, dus het die waarde van hierdie evaluasies vir produsente verhoog met meer van die beskikbare kultivars wat gedek kan word. Ongelukkig was ons nie suksesvol met die vulling van die vakante voór-oes hortoloogpos nie weens die gebrek aan geskikte, gekwalifiseerde applikante, maar dit bly steeds 'n prioriteit.

5.2 **PROGRAMME: RIND CONDITION** Programme coordinator: Paul Cronjé (CRI-SU)

5.2.1 **Programme summary**

In the rind condition project, disorders that occur in the various citrus cultivars and production areas were studied. These disorders include postharvest chilling injury, non-chilling rind pitting of Valencia and mandarin as well as peteca of lemon. This programme is designed to incorporate projects to determine the influence of preharvest factors that could predispose fruit to a disorder as well as postharvest handling practices which could result in the development of the disorder. Encouraging advances have been made in the understanding of the physiology of these disorders (5.2.5) in addition to identifying some management actions that could be taken to control the incidences (5.2.6). The involvement of the ethylene metabolism of the rind has been shown to influence the lemon fruit susceptibility to peteca (5.2.3). By applying a foliar spray of Ethephon before harvest a significant reduction in incidence was seen. These experimental results need to be tested on a larger scale, but could offer a strategy to be employed in order to reduce the impact of this disorder. Changes in vapour pressure deficit between harvest and packhouse treatments (resulting in dehydration of the rind) resulted in higher pitting incidence (5.2.4). These results concur with reports from Florida and Spain which show the negative impact of rind moisture loss after harvest. Strategies to control the incidence of chilling injury, a constant threat in all cold-sterilization markets, should incorporate preharvest and postharvest aspects (5.2.2). It is reported that fruit from colder parts of an area (i.e. frost prone orchards) could be more susceptible, as is smaller fruit. In addition, postharvest application of TBZ and wax reduce the development of chilling injury symptoms and application should be optimised in the packline. One of the long term goals of this project viz. to non-destructively identify fruit susceptibility, received attention. The use of NIR (near infra-red) spectroscopy to quantify the rind according to its biochemical make-up (sugars, organic acids etc.), seems to be a viable option and will be further tested (5.2.7). In the following season research will be undertaken to further elucidate the factors involved in the various rind disorders.

Programopsomming

Verskillende skil-defekte was bestudeer in die skilkondisie program gedurende die afgelope seisoen, wat koueskade van Nawel, gepokteskil van lemoene en mandaryne en peteka van suurlemoen ingesluit het. Die program staan ten doel om die voor-oes asook na-oes faktore wat die voorkoms van skildefekte beïnvloed te bestudeer. Gedurende die tydperk was positiewe vordering gemaak in die ontrafeling van die fisiologiese onderbou van die defekte (5.2.5) asook in die identifisering van produksiepraktyke wat die voorkoms kan beïnvloed van skildeefekte (5.2.6). In die peteka van suurlemoene projek is daar getoon dat die skil se etileenmetabolisme 'n rol speel in die vasstel van vatbaarheid vir die fisiologiese defek (5.2.3). Deur die etileenmetabolisme te manipuleer d.m.v. 'n Ethephon blaarbespuiting 1 week voor oes, word die peteka voorkoms betekenisvol verlaag. Deur die dampdruk verskil, en sodoende die vog verlies van die skil, te verhoog na pluk word die waarskynlikheid van gepokteskil vergroot in mandaryne en lemoene (5.2.4). Die resultate stem ooreen met informasie van Florida en Spanje wat daarop dui dat te veel vogverlies uit die skil die voorkoms van fisiologiese defekte aanhelp. Uit die navorsing is dit duidelik dat die beheer van koueskade 'n strategie vereis wat voor-oes en na-oes aspekte insluit (5.2.2). Daar was gevind dat vrugte uit die kouer gedeeltes in 'n produksie area meer vatbaar is, en so ook kleiner vrugte. Die na-oes aanwending van TBZ en waks is egter belangrik om die simptome ontwikkeling van koueskade te verminder. Die nie-destruktiewe sortering van vrugte in verskillende klasse t.o.v. sensitiwiteit tot die ontwikkeling van skildefekte is 'n langtermyn doelwit in die program. NIR (near infra red) spektroskopie blyk na die tegnologie wat potensieel die beste kans bied om die doel te haal. Die data dui daarop dat die skil se vlakke van spesifieke biochemiese stowwe bv. Suikers en organiese sure en bepaal deur die NIR, gebruik kan word om 'n vrug met 'n hoe waarskynlikheid van 'n lae waarskynlikheid te skei (5.2.7). In die daaropvolgende seisoen sal uitgebou word op elk van die onderskei defekte en faktore betrokke in die voorkoms van fisiologiese skildefekte.

5.2.2 **PROGRESS REPORT: Development of postharvest treatments to prevent chilling injury in various citrus species**

Project 832 (2005/6-2014/15) by P.J.R. Cronje (CRI) and J. Hordijk (SU)

Summary

Citrus fruit exported from South Africa to markets such as the USA and China undergo a mandatory 24 day exposure of -0.6 °C during shipment to kill any insect larvae in the fruit, however, this protocol causes chilling injury (CI). The aim of this study was firstly to determine the influence of various preharvest factors on chilling sensitivity. In addition, Near-Infrared (NIR) spectroscopy was tested as a potential management tool to identify variation in CI susceptibility of fruit and lastly the efficacy of thiabendazole (TBZ) applied in the

packline to reduce CI was determined. Various factors influence the susceptibility of a navel orange fruit to CI including cultivar, micro-climate, harvest date, fruit size and rind colour. In this study it was found that 'Washington' was more susceptible to CI compared to 'navelina' navel orange. Fruit from the coldest part of Citrusdal (Tharakama) had the highest incidence of CI, which concurred with literature. The incidence of CI was overall less when fruit were harvested in the middle of the commercial harvest window; however, the internal maturity at harvest does not appear to be related to the sensitivity of orange fruit to CI. Near infrared (NIR) spectroscopy was tested as a potential tool to predict fruit quality parameters in relation to CI. Analysing the NIR data with principal components analysis (PCA), score plots were obtained that separate fruit in clusters from the inside and outside of the canopy positions as well as different sizes and rind colours (green vs. orange). However, analysing data with partial least square regression (PLS) using fruit quality parameters (firmness, rind colour and mass), the NIR spectra obtained with the integrated sphere did not provide a good prediction model for CI index. Thiabendazole (TBZ) is reported to reduce the incidence of CI of citrus fruit and this fungicide was applied in a semi-commercial packline in the wax as well as the drench. The results of the application of different fungicides from the TBZ chemical group indicated that the TBZ dip treatments had the highest efficacy in reducing both the incidence and severity of CI and in addition were more effective when applied in warm (40°C) than cold water (10°C). Applications at the commercial recommended rate (20mL.L⁻¹) and half of the commercial recommended rate were both effective in reducing the incidence of CI. Wax application was effective in reducing the incidence of CI, however, the application of TBZ in the wax reduced the incidence of CI even more. For the successful reduction of CI incidence in commercial shipments of citrus fruit the focus should not be on a single factor but rather a strategy that encompasses pre-harvest factors that would influence rind quality as well as specific postharvest technologies known to decrease the impact of CI. Such research is continuing.

The full thesis on research conducted to date is available on the University of Stellenbosch web site:
<http://hdl.handle.net/10019.1/80254>

Opsomming

Sitrus vrugte ondergaan 'n verpligte 24 dae blootstelling aan -0,6°C om moontlike insek-larwes te dood gedurende die uitvoer na markte soos die VSA en China, maar hierdie protokol veroorsaak koueskade. Die doel van hierdie studie was eerstens om die invloed van verskillende voor-oes faktore op koueskade-sensitiwiteit van nawel lemoene te bepaal. Daarbenewens is naby-infrarooi (NIR) spektroskopie as 'n potensiële tegniek getoets om variasie in koueskade-sensitiwiteit van nawel lemoene te identifiseer, en laastens is die effektiwiteit van thiabendazole (TBZ) toediening in die verpakkings lyn, om koueskade te verminder, ondersoek. Verskillende faktore soos kultivar, mikroklimaat, oesdatum, vruggrootheid en skilkleur beïnvloed die koueskade-sensitiwiteit van sitrus. Hierdie studie het bevind dat die 'Washington' meer sensitief is vir koueskade as die 'navelina' nawels. Vrugte afkomstig uit die koudste deel van Citrusdal (Tharakama) het die hoogste voorkoms van koueskade. In die algemeen was vrugte ge-oes in die middel van die kommersiële-venster die minste koueskade-sensitief, maar interne rypheid hou nie verband met koueskade-sensitiwiteit nie. Naby-Infrarooi (NIR) spektroskopie is getoets as 'n potensiële instrument om vrugkwaliteit parameters te voorspel met betrekking tot koueskade. Deur ontleding van die NIR data met behulp van 'PCA' kon vrugte groepeer word volgens posisie (binne vs. buite blaredak), groottes en skilkleur. Deur 'PLS Regression' verdere data ontleding en met inagneming van vrugkwaliteit parameters (fermheid, skil kleur en massa), kon die NIR spektra wat verkry was egter nie 'n goeie voorspelling model vir koueskade verskaf nie. TBZ verminder die voorkoms van koueskade van sitrusvrugte na dit toegedien was in 'n semi-kommersiële verpakkingslyn in die waks, 'drench' of baddens. Die toediening van verskillende swamdoders van die TBZ chemiese groep in baddens, het aangedui dat die TBZ doop behandeling effektief was om die voorkoms van koueskade te verminder. Daarbenewens was TBZ meer effektief in verlaging van koueskade as dit toegedien word in warm (40°C) as koue (10°C) water, asook teen die volle (20 mL.L⁻¹) en die helfte van die aanbevole kommersiële dosis. Wakstoediening was effektief in die vermindering van die voorkoms van koueskade en byvoeging van TBZ in die waks het die effektiwiteit verhoog. Die suksesvolle vermindering van koueskade tydens kommersiële verskeping van sitrusvrugte moet egter nie fokus op 'n enkele faktor nie, maar op 'n strategie wat bestaan uit voor-oes faktore wat die vrugskil kwaliteit beïnvloed, sowel as spesifieke na-oes tegnologieë en hanteringsprotokolle wat bekend is vir die vermindering van koueskade. Soortgelyke navorsing word vir komende seisoen beplan.

Die volledige tesis is beskikbaar op die Univ. Stellenbosch se biblioteek webwerf:
<http://hdl.handle.net/10019.1/80254>

5.2.3 **PROGRESS REPORT: Effect of different chemical applications on development of Peteca spot in lemons**

Project 833 (2006/7-2014/5) by P.J.R. Cronje (CRI)

Summary

Peteca of lemon is a postharvest physiological disorder resulting in the collapse of the oil glands. Subsequently, the oil leaks into the adjacent tissue and causes a darkened depression. The occurrence can be severe, without any specific pre- or postharvest practices to employ to reduce the incidence. Over several seasons the incidence of peteca 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 within an orchard in a season, with the early fruit being highly susceptible. In experiments to identify postharvest factors which influence the peteca incidence, 3 ppm ethylene and 5% CO₂ were applied in a continuous flow-through system (20°C for 3 d). The CO₂ treatment resulted in significantly higher incidence compared to the rest. 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 peteca. In the subsequent season, the same treatments were applied in an orchard one week before harvest and a similar reduction in peteca was recorded. Ethylene production from these fruit after harvest was measured and showed a transient spike in fruit from the Ethephon and AVG treatments. The results collected could indicate a protective action of ethylene in reducing rind sensitivity to peteca. It is hypothesised that if the internal ethylene synthesis is increased prior to harvest in sensitive fruit, i.e. immature fruit, a reduction in peteca can occur.

Opsomming

Peteka van suurlemoene is 'n na-oes fisiologiese defek wat lei tot die verval van die olieklier, uitlek van die olie en die beskadiging van die omliggende skil en die vorming van 'n donker insinking. Die voorkoms van peteka kan tot baie hoë vlakke van kwaliteitsverliese lei en daar bestaan tans geen voor of na-oes praktyk wat die kan beperk of voorkom nie. Tydens opeenvolgende seisoene is daar proewe gedoen hieroor en die eerste waarneming was dat hoë vlakke van CO₂ (5%) toegedien in 'n deurvloei sisteem die voorkoms betekenis verhoog. Daarteenoor het 3 ppm etileen die voorkoms verlaag. In die daaropvolgende seisoene is daar gepoog om deur voor-oes toediening van Ethephon (2-Chloroethyl phosphorig suur) (200 mg/L and 400 mg/L) en AVG (aminoethoxy-vinylglycine) (400 mg/L and 800 mg/L) die voorkoms te verlaag deur die etileenmetabolisme te manipuleer. Die resultate wat oor verskei seisoene ingesamel was het getoon dat beide die middels die vlakke verminder maar Ethephon meer so. Die Ethephon en AVG toediening het gelei tot 'n tydelike toename in etileen produksie wat kan dui op 'n moontlike verhaasde veroudering in die skil wat lei tot 'n verlaagde sensitiwiteit vir peteka.

5.2.4 **PROGRESS REPORT: Studies on aspects concerning rind pitting/staining citrus fruit**

Project 958 (2009/10 – 2014/5) by P.J.R. Cronje (CRI)

Summary

Postharvest physiological rind disorders, such as staining and pitting, affects 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 have been shown to play a part in inducing rind disorders. By altering the VPD (vapour pressure deficit) between harvest and wax application of Valencia and mandarin fruit a higher incidence of pitting was recorded. This supports the previous season's results which indicated the negative impact on rind condition of high moisture loss. In contrast the application of various foliar treatments (Gibberellic acid and Vapor Gard[®]) did not result in any reduction of rind pitting of Valencia orange. Wax application was shown not to cause pitting in Valencia orange during this season. The exposure of Nadorcott mandarin to variation in VPD did induce pitting and staining, in results similar to those recorded in 2011. The results of this season's experiments indicate that the water balance of the rind, and specifically in the period from harvest to wax application, does have a significant influence on the incidence and will be further investigated. The project will continue and will aim to determine the impact of preharvest water content in the rind on postharvest pitting.

Opsomming

Volgens verkeie bronne asook voorafgaande navorsing van CRI 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. Deur die dampdruk verskil in die periode na pluk en voor waks aanwending te verander, en sodoende die vogverlies uit die skil te verhoog, was daar getoon dat die voorkoms van gepokte

skil verhoog word in Valencia lemoen en Nadorcott mandaryne. Hierdie bevinding strook met vorige seisoene se resultate wat daarop dui dat 'n drastiese vogverlies uit die skil kan lei tot die ontwikkeling van fisiologiese skildefekte. Gedurende die 2013 seisoen is gevind dat die voor-oes aanwending van gibberellien suur (Januarie) en Vapor Gard[®] ('n anti-transpirant middel) nie enige positiewe verlaging van gepokteskil in Valencia teweeg gebring het nie. Daar is ook gesien dat waks aanwending nie die oorsaak van gepokteskil in Valencia lemoen is nie. Die data ingesamel in 2012 wys daarop dat die periode van pluk tot die vrug gewaks word baie belangrik is in die beheer van gepokteskil, en daar moet gepoog word om so min vog as moontlik uit die skil te laat verloor. In die volgende seisoen sal verder in die projek gekyk word na die rol wat voor-oes vobalans in die boom op die na-oes skilvobalans speel t.o.v. die voorkoms van gepokteskil.

5.2.5 FINAL REPORT: Development of laboratory based biochemical methods to determine the physiological condition of the citrus fruit flavedo

Project 962 (2009/10-2011/12) by P.J.R. Cronje (CRI)

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. During 2009/10 four methods *viz.* ascorbic acid content (for the spectrophotometer and HPLC), FRAP [Ferric Reducing Ability of Plasma (or Plants)] assay and the Lipid peroxidation (TBARS assay) were developed. During 2010/11 two methods *viz.* a spectrophotometric and HPLC analysis for lycopene and β -carotene content was developed. During 2011/12 a protocol was tested and adjusted to quantify for low concentrations of ACC in citrus in order to determine the levels of this ethylene precursor in conjunction with ethylene measurements on a GC. All the full methods are available from the researcher.

Opsomming

In die navorsingprojekte 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 2009/10 is vier laboratorium-metodes, bekend vir hulle beskrywende eienskappe van die plantsel-fisiologie, was gekies en aangepas om die sitruskil suksesvol te ekstraheer en analiseer. Die metodes is askorbiensuur inhoud (HLPC en spektrofotometer metodes), FRAP [Ferric Reducing Ability van Plasma (of Plante)] analise asook die Lipid peroksidase analise (TBARS analise). Gedurende 2010/11 is twee metode verfyn nl. 'n spektrofotometer, asook 'n HPLC protokol om likopeen en β -karoteen te ekstraheer en analiseer gedurende 2011/12 is 'n metode om etileen en ACC vlakke te bepaal op 'n gas chromatograaf suksesvol ontwikkel en getoets op suurlemoen vrugte. Al die metode is in detail uitgewerk in beskikbaar in van die verantwoordelik navorser.

Introduction

Detailed biochemical studies of the citrus rind are necessary to understand the impact of environmental and management constraints on rind condition. Therefore the development of detailed biochemical-based methods to quantify cellular changes is necessary to describe the condition of the flavedo. These descriptive methods would be used in various experiments in the rind condition projects especially those on chilling injury. During the research period 4 methods were developed which could be used to determine various aspects of the citrus rind. The most problematic aspect of the citrus rind, and which necessitate adjustment of published methodology, is the essential oil content of the rind. For biochemical analysis of specific cell components e.g. lipid content, it is therefore necessary to purify each sample by removing the essential oil.

Stated objectives

- Develop the research methodology of four analyses to measure the antioxidant capacity and condition of membrane lipids in the flavedo.
- Develop spectrophotometric as well as HPLC protocols to analyse lycopene content in citrus fruit and rind.
- Develop method in order to adjust for low concentrations of ACC in citrus in order to quantify the levels of this ethylene precursor.

Techniques developed

Ascorbic acid (spectrophotometer)

Ascorbic acid, Vitamin C, is an important compound in citrus. This spectrophotometer method is a rapid method to determine the ascorbic acid concentration in a specific sample. The absorbance, at 265 nm, is read before and after addition of the enzyme ascorbate oxidase. An L-Ascorbic acid standard curve is prepared against which the concentration (μM) in the sample can be calculated. The specific amount ($\mu\text{g/ml}$) can also be calculated. Seasonal changes as well as the influence of storage temperature can be observed from the data.

This method is much more rapid than the HPLC analysis, but less accurate (Teklemariam and Sparks, 2004).

The FRAP [ferric reducing ability of plasma (or plants)] assay

The ferric reducing ability of plasma (FRAP) assay has been described as a novel method for measuring “antioxidant power”. Biological antioxidants have been defined as “any substance that, when present at low concentrations compared to those of an oxidisable substrate, significantly delays or prevents oxidation of that substrate”. This definition covers every member of the antioxidant defence team.

The FRAP assay is the only assay that directly measures antioxidants or reductants in a sample. At a low pH, the ferric reducing ability of the sample is measured when a ferric-tripyridyltriazine (Fe^{3+} -TPTZ) complex is reduced to the ferrous (Fe^{2+}) form. This results in the formation of an intense blue colour with an absorption maximum at 593 nm, indicating that a reductant (antioxidant) is present. In this assay, the reducing ability of the sample is thus the rate-limiting factor. The FRAP assay is recommended for researchers interested in oxidative stress and its effects.

The FRAP assay is inexpensive, the procedure is straightforward and speedy to perform, and reagents are also simple to prepare. An aqueous solution of known Fe^{2+} ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) concentration is used for calibration. Results from the FRAP assay are highly reproducible and linearly related to the molar concentration of the antioxidant(s) present (Iris and Strain, 1999; Bente *et al.*, 2002)

Ascorbic acid & glutathione analysis (HPLC)

Ascorbic acid is a well-known antioxidant, especially in citrus. Temperature increases result in a decrease in stability of the ascorbic acid. Oxidation is also a serious problem causing a loss in quantity of the ascorbic acid content. Another antioxidant, glutathione, protects cells from toxins such as free radicals, and exists in its reduced (GSH) and oxidized (GSSG) states. It is found almost exclusively in its reduced form since glutathione reductase, the enzyme reverting glutathione from its oxidized form, is constitutively active and inducible upon oxidative stress. An increased GSSG-to-GSH ratio is considered as an indication of oxidative stress.

High-performance liquid chromatography (HPLC) is a form of column chromatography in which compounds are separated, identified, and quantified. In this specific method ascorbic acid (AA) and glutathione (GSH) is measured. The total amount (reduced) ascorbic acid as well as glutathione is also measured, in order to determine the oxidized amount (difference) ascorbic acid (DHA) and glutathione (GSSG). Preparation of samples for the HPLC is simple and relatively rapid. A standard is also run on the HPLC, and results can be interpreted directly after the run of a sample has been completed. From the results, the level of these two antioxidants during the season, as well as the influence of storage temperature can be observed.

Lipid peroxidation (TBARS-assay)

Lipid peroxidation is the process whereby electrons are “stolen” from the **lipids in cell membranes** by free radicals, which results in **cell damage and an increased production of free radicals**. A useful indication of general lipid peroxidation is the occurrence of a secondary end product of the oxidation of polyunsaturated fatty acids, namely malondialdehyde (MDA). MDA can be measured using the thiobarbituric acid-reactive-substances (TBARS) assay, in which MDA reacts with two molecules of thiobarbituric acid (TBA) via an acid-catalyzed nucleophilic-addition reaction, resulting in a pinkish chromagen with an absorbance maximum at 532 nm.

The TBARS assay is acknowledged as a reliable estimator of lipid peroxidation in nearly all plant species. Lipid peroxidation levels in tissues normally containing anthocyanins and other interfering compounds which would absorb at 532 nm, as well as in tissues which may up regulate them through exposure to **environmental stress**, can be estimated. The assay is also very popular due to its simplicity, lack of expense, and rapidity with which large numbers of samples can be processed (Hodges *et al.*, 1999)

ACC and ethylene determination in citrus fruit

Test and develop a robust and low cost method to measure 1-amino-cyclopropane-1-carboxylic acid (ACC) (Hoffman et al., 1980; Dong et al., 1983; Li et al., 2005) on the gas chromatograph. ACC synthesis and its conversion to ethylene is the critical step in the ethylene metabolism and its concentration at different stages during fruit development and after exposure to climatic cues could give indications of the rind's stage of maturation and sensitivity to pete spot. Both these methods are very valuable in understanding ethylene synthesis and reaction of the fruit on external applied chemicals. In addition, these two methods give an indication of fruit stress e.g. chilling injury during cold storage. The reason for their method development relative to methods used on other fruit is that citrus produce very low levels of ethylene compared to horticultural crops. These methods were tested and found to be able to detect low concentrations of ethylene and ACC. In addition both techniques are very stable and robust and can now be used on any citrus cultivar.

Lycopene analysis (HPLC and spectrophotometric)

Lycopene synthesis in the fruit rind of not only 'Star Ruby' grapefruit have been identified as being exceptional antioxidants leading to a reduced susceptibility to chilling injury. Therefore lycopene mutations could become a very important segment of the citrus industry due to its better rind condition. Two methods were developed during the 2010/11 season, viz. a spectrophotometric and as well as an extraction and HPLC analysis method for lycopene and β -carotene content in the flavedo. The first method was improved from earlier work done by Prof Bower on lycopene content in the fruit pulp, at Outspan Citrus Centre. The second method was developed during a research visit to IATA, Valencia in the laboratory of Drs. Zacarias and Rodrigo, 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. Both these methods are available in full from the researcher.

Future research

These methods have already been used in on-going research and could form part of new projects.

Technology transfer

Not applicable.

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5.2.6 **FINAL REPORT: Increasing Ca and Mg content in the flavedo using novel techniques** Project 978 (2010/1-2011/2) by P.J.R. Cronje (CRI)

Summary

In previous studies it was determined that the content of the two important mineral nutrients, namely Ca and Mg, could be sub-optimal in the flavedo of 'hules 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. During 2010/11 season it was shown that application of Maxim and Corasil P could effect a change in some mineral nutrients' accumulation patterns in the fruit rind viz. increases in Ca and decreases in K were seen. During 2011/12 application of Regalis reduced the vegetative growth of 'nadorcott mandarin' and it is thought that these chemicals could influence the fruit sink positively resulting in a higher nutrient allocation.

Opsomming

In 'n afgehandelde uitgebreide projek, waarop gefokus was op skilafbraak van 'hules 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 'hules 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 t.o.v. minerale akkumulاسie.

Introduction

By manipulating and improving the sink strength of the citrus 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. Furthermore, high levels of endogenous gibberellins are thought to lead to Ca-deficiency related disorders such as blossom-end rot of tomato and bitter pit of apple. This relationship is due to the higher allocation of nutrients to vegetative growth, as well as the sequestering of Ca in the vacuole, resulting in a reduced Ca content in the apoplast. Therefore, by reducing endogenous gibberellin levels, Ca levels may be elevated. Subsequently it was shown that by double application of Regalis on the January shoot flush, an anti-gibberellic agent, that the rind mineral nutrient could be altered (CRI annual report 2011). Furthermore auxin is known to be involved in determining sink strength. It could therefore be reasoned that augmenting fruit auxin could influence the vegetative/reproductive balance in favour of the fruit (reproductive).

Objective

The objective of this experiment was to improve the mineral nutrient content in the rind by testing novel experimental techniques during fruit development. By sifting the allocation of nutrients and carbohydrate from the vegetative to reproductive plant organs an improvement of fruit quality could possibly be realised.

Materials and methods

During the 2011/12 season two vegetative growth retardants i.e. Sunny (Uniconazole, 5 ml/L water) and Regalis® (Prohexadione-calcium, 8 g/L water) were applied to 10 year old Nadorcott mandarin trees planted in Porterville (Sunny as soil and Regalis as foliar spray). Regalis was applied twice (Dec and Jan) whereas Sunny only once (Jan) before elongation of the summer flush. Shoot length was measured in the Regalis treatments at the end of January and March of 10 shoots per replicate tree (10 replications). In addition the number of nodes was counted. At harvest leaves and fruit from both treatments were harvested and mineral nutrient analysis was done of the leaves and the rind.

Results and discussion

The results indicate that Regalis offers the most promise to change vegetative growth in citrus trees. It reduced the shoot length of Nadorcott trees but not the amount of nodes. In addition, its application increased the amount of Ca in the rind even though it did not result in a reduction in leaf Ca content. Sunny did not change the amount of any nutrient significantly; however, Ca was also numerically increased in the rind of these treatments (Tables 5.2.6.1-3).

Table 5.2.6.1. The effect of two Regalis foliar sprays (December and January) on Nadorcott mandarin shoot growth and nodes per shoot (Ten shoots were used per treatments and replicated 10 times).

Treatment	Shoot length-Jan (cm)	Shoot length-Mar (cm)	Total growth (cm)	Avg. nodes per shoot
Control	34.4 ns	342.7 a	309.7 a	15.2 ns
Regalis	28.8	271.2 b	244.4 b	15.2
<i>p-value</i>	<i>0.057</i>	<i>0.007</i>	<i>0.007</i>	<i>0.992</i>

Table 5.2.6.2. The influence of Regalis foliar applications (Dec. and Jan.) on mineral nutrient content of 'nadorcott' mandarin rind and leaves at harvest (n=10, $P \geq 0.05$).

Plant material	Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
Leaf	Control	2.2 ^{ns}	0.14 ^{ns}	1.4 ^{ns}	2.6 ^{ns}	0.4 ^{ns}
	Regalis	2.1	0.14	1.4	2.8	0.5
	<i>p-value</i>	<i>0.365</i>	<i>0.711</i>	<i>0.683</i>	<i>0.383</i>	<i>0.091</i>
Rind	Control	1.07a	0.11 ^{ns}	1.1 ^{ns}	0.75b	0.18 ^{ns}
	Regalis	0.94b	0.1	1	0.87a	0.18
	<i>p-value</i>	<i>0.047</i>	<i>0.459</i>	<i>0.639</i>	<i>0.0128</i>	<i>0.923</i>

Table 5.2.6.3. The influence of Sunny soil application on mineral nutrient content of 'nadorcott' mandarin rind and leaves at harvest (n=10, $P \geq 0.05$).

Plant material	Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
Leaf	Control	2 ^{ns}	0.16 ^{ns}	1.2 ^{ns}	3.5 ^{ns}	0.42 ^{ns}
	Sunny	2.2	0.18	1.3	3.1	0.38
	<i>p value</i>	<i>0.2974</i>	<i>0.3703</i>	<i>0.5545</i>	<i>0.1497</i>	<i>0.5154</i>
Rind	Control	1 ^{ns}	0.1 ^{ns}	0.91 ^{ns}	0.74 ^{ns}	0.14 ^{ns}
	Sunny	1.1	0.12	0.91	0.84	0.12
	<i>p-value</i>	<i>0.1255</i>	<i>0.0564</i>	<i>0.9703</i>	<i>0.2953</i>	<i>0.5615</i>

Conclusion

Rind condition is the result of various preharvest physiological processes acting simultaneously during the development of the citrus fruit rind, resulting in a fruit with high or low susceptibility to develop a physiological disorder. In previous reports the importance of exposure to direct sunlight during stage I and II of fruit development was shown. Outside fruit receiving high PAR levels had higher Mg and Ca and lower K as well as higher carbohydrates compared to the inside fruit, which had a high propensity to rind breakdown (Cronje, 2009).

In this project I first tried to influence the allocation of nutrients to the rind via decreasing shoot elongation in stage II and III (CRI annual report 2011). This proved to be successful and increased the nutrient content of the rind. In the 2011 season, application of auxin resulted in a higher Ca and Mg content and lower K compared to the control (CRI annual report 2012). The 2011 data were confirmed in 2012 and this is the first indication of a possible direct impact on rind condition of this agrochemical (Regalis). Novel usage of these plant growth regulators to increase rind mineral nutrient content could improve rind condition and should form the basis of an in-depth study on various cultivars that are prone to rind disorders.

Technology transfer

The technology will be communicated to producers, to do semi-commercial experiments in 2013/4 season to confirm if the effects seen in the experiment are commercially effective. However, these data are an indication that the use of these new agro-chemicals could result in increased in fruit quality and the need of an in-depth study would be critical to determine optimal application rate and timing.

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5.2.7 PROGRESS REPORT: Non-destructive monitoring and prediction of postharvest rind quality of 'Nules Clementine' Mandarin and Valencia orange fruit

Project 1031 (2010/11-2013/14) by P.J.R. Cronje (CRI), Lembe Magwasa (SU), and Linus Opara (SU), L Terry (Cranfield University), and Bart Nicolai (KUL)

Summary

Diffuse reflectance near infrared (NIR) spectroscopy was explored as a non-destructive method to detect external and internal quality of 'Valencia' oranges. The study compared three different Fourier transform NIR acquisition methods, namely, a fibre-optic probe for solid samples (SP), an integrating sphere (IS) and an emission head (EH). Fruit quality attributes measured included mass, colour index, total soluble solids (TSS), titratable acidity (TA), maturity index expressed as TSS:TA ratio and vitamin C. Partial least squares regression was applied to spectral data to develop prediction models for each quality attribute and by randomly dividing the data into calibration and independent validation sets. To test robustness, a set of fruit harvested from another location was used for external validation. Fruit mass, colour index, TSS and vitamin C were predicted with significant accuracy showing RPD-values of 3.53, 1.99, 1.87 and 1.33, respectively. The spectral acquisition method had a significant influence on the calibration regression statistics and accuracy of prediction. The models developed using the EH gave the best prediction statistics for mass ($R = 0.96$, RMSEP = 10.45 g), colour index ($R = 0.83$, RMSEP = 0.82) and vitamin C ($R = 0.66$, 8.01 mg/100 mL), while the IS gave the best prediction for TSS ($R = 0.83$ and RMSEP = 0.58). The model parameters remained fairly constant when the models were validated using fruit from another location, indicating high level of model robustness. Good prediction statistics demonstrated by EH demonstrated the potential of this spectrometer as a non-destructive tool to holistically evaluate external and internal quality parameters.

Opsomming

Diffusie refleksie van "Near Infra Red" spektroskopie was getoets om die wesenlikheid daarvan te bepaal om eksterne en interne kwaliteit aspekte van "Valencia" lemoene te bepaal. In hierdie studie was daar drie verskillende maniere om Fourier getransformeerde NIR data op te neem gebruik nl., 'n soliede optiese vesel (SP), 'n geïntegreerde speer (IS) en 'n emissie kop (EH). Die kwaliteit aspekte wat gemeet was sluit in: kleur, massa, totale opgeloste stowe en sure asook vitamien C. Die spektrale data is dmv PLS regressie is gebruik om voorspellings modelle vir elke aspek te ontwikkel deur die data ewekansig te verdeel in 'n kalibrasie en toets groep. Om die modelle se robuustheid te toets, was 'n addisionele stel vrugte van 'n ander area gebruik. Die vrugmassa, skilkleur, TSS en vitamien C was voorspel met betekenisvolle akkuraatheid met RPD-waardes van onderskeidelik 3.53, 1.99, 1.87 en 1.33. Die metode waarmee die data ingesamel is, het ook 'n betekenisvolle invloed op kalibrasie en akkuraatheid van die voorspellings gehad. Die modelle wat dmv die EH ontwikkel is, het die beste voorspellings gelewe vir massa ($R = 0.96$, RMSEP = 10.45 g), skilkleur ($R = 0.83$, RMSEP = 0.82) en vitamien C ($R = 0.66$, 8.01 mg/100 mL), daarteen oor het die IS die beste voorspelling gegee vir TSS ($R = 0.83$, RMSEP = 0.58). Die modelparameters het grotendeels konstant gebly wanneer die model getoets wa met die vrugte van die tweede area wat dui op 'n hoe valk van robuustheid.

Die goeie voorspellings statistiese waardes van die EH, dui daarop dat hierdie tegnologie die beste potensiaal toon om as 'n nie-destruktiwe toerusting gebruik te word die eksterne en interne kwaliteit van 'n sitrusvrug te bepaal nie.

5.3 PROGRAMME: FRUIT PRODUCTION AND QUALITY

Programme coordinator (Acting): Tim G Grout (CRI)

5.3.1 Programme summary

With increasing costs for labour and transport it is essential for growers to ensure that the volume and quality of the fruit that they harvest per hectare is profitable, year after year. Some easy-peeling cultivars provide a bumper crop one year, then fall into an alternate bearing cycle and produce very few fruit the next year. This has a serious effect on cash flow and research has found that in 'nadorcott', leaf starch concentration In April can give an indication of bearing potential the following season (5.3.2). Research on the timing of chemical-thinning sprays to manage this problem is continuing. Water is becoming increasingly critical for citrus production and a large project with joint funding from the Water Research Commission and CRI will ensure that we know exactly how much water citrus trees require at different ages and in different climates (5.3.3). Too much water at the wrong time can result in fruit splitting and fruit with thin rinds are especially susceptible to this. In order to reduce the likelihood of fruit splitting in 'Marisol' Clementine and 'Mor' mandarin a foliar application of 2,4-D has been found to be effective when applied just after physiological fruit drop (5.3.4). Growers requested research on humic and fulvic acids a few years ago to see whether these products would benefit citrus production. Results so far have shown that they both increase the culturable fraction of soil microbes and their use with reduced amounts of fertiliser will be investigated further in a long-term study (5.3.5). Research on ways to maximise foliar uptake of micronutrients is also underway with the initial development of optimal quantification techniques having been completed (5.3.6). Earlier research on the possible benefits of silicon applications showed that the foliar sprays were of little benefit. Soil applications are now being evaluated to see whether they can reduce infections of brown spot, *Alternaria alternata*. This will conclude the work on silicon (5.3.7).

Programopsomming

Met toenemende kostes vir arbeid en vervoer is dit noodsaaklik vir produsente om te verseker dat die volume en gehalte van die vrugte wat hul per hektaar oes, jaar na jaar winsgewend is. Sommige easy-peeling kultivars verskaf 'n goeie oes een jaar, en verval dan in 'n alternatiewe dragsiklus en produseer baie min vrugte in die volgende jaar. Dit het 'n ernstige effek op kontantvloei, en navorsing het getoon dat in 'nadorcott', styselkonsentrasie in die blare in April 'n aanduiding van drag-potensiaal die volgende seisoen kan gee (5.3.2). Navorsing oor die tydsberekening van chemiese uitdunning om hierdie probleem te bestuur, gaan voort. Water word toenemend krities vir sitrusproduksie en 'n groot projek met gesamentlike befondsing vanaf die Waternavorsing-kommissie en CRI sal verseker dat ons presies sal weet hoeveel water sitrusbome op verskillende ouderdomme en in verskillende klimate benodig (5.3.3). Te veel water op die verkeerde tyd kan vrugsplitsing veroorsaak en vrugte met dun skille is veral vatbaar hiervoor. Om die waarskynlikheid van vrugsplitsing in 'Marisol' Clementine en 'Mor' mandaryn te verminder, is 'n blaartoediening van 2,4-D gevind om effektief te wees wanneer dit net ná fisiologiese vrugval toegedien is (5.3.4). Produsente het 'n paar jaar gelede versoek dat navorsing op humussuur en "fulvic" suur gedoen word om te sien of hierdie produkte sitrusproduksie kan bevoordeel. Resultate sover het getoon dat beide die bewerkbare fraksie van grond-mikrobes verhoog, en hul gebruik met verminderde hoeveelhede bemestingstowwe sal verder in 'n langtermyn studie ondersoek word (5.3.5). Navorsing op maniere om die blaar-opname van mikro-voedingstowwe te verhoog is ook aan die gang, met die aanvanklike ontwikkeling van optimale kwantifiseringstegnieke wat afgehandel is (5.3.6). Vroeëre navorsing oor die moontlike voordele van silikon-toedienings toon dat blaarbespuitings van min waarde was. Grondtoedienings word nou geëvalueer om te sien of dit infeksies van bruinvrot, *Alternaria alternata*, kan verminder. Dit sal die werk met silikon afsluit (5.3.7).

5.3.2 FINAL REPORT: Effect of leaf carbohydrate concentrations on flowering and fruit set of alternate bearing late mandarins

Project 981 (April 2010 - March 2013) by Paul Cronje (CRI), Karen Theron and Schalk van der Merwe (SU)

Summary

Alternate bearing is a common phenomenon in most commercial perennial fruit trees. In citrus, the “on” year consists of a heavy crop load with mostly small fruit, often followed by an “off” year with few, large and coarse fruit. Carbohydrates play an important role in affecting alternate bearing, especially during fruit set, but also flowering and fruit maturation, and are essential in maintaining a regular bearing habit. Changes in starch and total sugar accumulation in the leaves of the alternate bearing prone ‘nadorcott’ mandarin were followed over an entire season for both “on” and “off” trees to evaluate the possibility of using carbohydrate levels to predict bearing potential. Starch accumulation followed a distinct pattern with differences between “on” and “off” trees visible in April and May. Starch concentrations in April showed a moderate negative correlation with yield and a moderate positive correlation with return bloom. Rapid starch accumulation started prior to harvest with a peak at the beginning of flowering. Thereafter a sharp decrease in starch levels occurred until after full bloom followed by a steady decrease from physiological fruit drop towards fruit maturity. “On” trees bore 53% more fruit than “off” trees, but the return bloom of “off” trees was 140% more than “on” trees, thus illustrating the negative effect that a large crop has on the next season’s bloom. It was concluded that for ‘nadorcott’ mandarin, leaf starch concentration in April can be used as an indication of bearing potential the following season. Pruning is a well-established management tool to control alternate bearing. Summer pruned trees had more spring flush vegetative shoots, more nodes per shoot and also more growth per parent shoot overall, compared to unpruned, control trees. Control trees had higher light levels inside the tree compared to summer pruned trees. However, no differences in leaf starch or total sugar levels during April were measured between treatments. Production of new bearing sites should therefore be considered in this experiment. It was concluded that pruning during November followed by early regrowth management gave the best balance between light penetration and production of new bearing units. Pruning in November, rather than during winter, also allowed selective pruning of shoots with or without flowers, depending on whether it was an “on” or an “off” year. When fruit thinning chemicals are applied at the optimum time and concentration, it is an effective way of moderating an alternate bearing cycle. Unfortunately no significant differences were obtained in this experiment even though the thinning treatments did show slightly higher starch levels in April 2012, indicating that the demand for energy was lower in these trees. This response was most likely due to the slightly lower yield and fruit number of the thinning treatments compared to the control. The dichlorprop treatment also showed a higher fruit growth rate. Future research should focus on timing of chemical thinning sprays in late mandarin cultivars. *The full thesis is available on the University of Stellenbosch library website at: <http://hdl.handle.net/10019.1/7182>*

Opsomming

Alternerende drag is ’n algemene verskynsel by die meeste meerjarige kommersiële vrugtebome. In die “aan” jaar by sitrus word ’n swaar oeslading gedra wat hoofsaaklik uit klein vrugte bestaan gevolg deur ’n “af” jaar met minder, groter en growwer vrugte. Koolhidrate speel ’n belangrike rol, veral gedurende vrugset, maar ook tydens blomtyd en vrugrypwording, en is noodsaaklik om ’n reëlmatige drasiklus te verseker. Veranderinge in stysel- en totale suiker akkumulاسie in die blare van ‘nadorcott’ mandarynbome, is deur die loop van ’n volle seisoen gevolg op beide “aan” en “af” bome om die moontlikheid te ondersoek dat koolhidraatvlakke gebruik kan word om dragpotensiaal te bepaal. Verskille tussen “aan” en “af” bome was in April en Mei sigbaar. Styselvlakke in April het ’n matige negatiewe korrelasie met drag getoon en ’n matige positiewe korrelasie met die volgende seisoen se blom. Styselvlakke het voor oestyd begin toeneem en aan die begin van blomtyd ’n piek bereik waarna ’n skerp daling voorgekom het tot na volblom. Dit is gevolg deur ’n geleidelike afname vanaf fisiologiese vrugval totdat die vrugte ryp was. “Aan” bome het 53% meer vrugte gedra as “af” bome, maar die volgende seisoen se blom van “af” bome was 140% meer. Dit illustreer die negatiewe effek wat ’n groot oes op die volgende seisoen se blom het. Die gevolgtrekking is dat styselvlakke in blare gedurende April gebruik kan word as ’n aanduiding van die drag-potensiaal vir die komende seisoen vir ‘nadorcott’ mandarynbome. Snoei is ’n gevestigde manier om alternerende drag te beheer. Bome wat in die somer gesnoei is, het ’n groter aantal vegetatiewe lote in die lente, meer knoppe per loot en ook meer groei op ouer-lote gehad in vergelyking met die kontrole bome wat nie gesnoei is. Kontrole bome het hoër ligvlakke binne-in die boom gehad in vergelyking met die bome wat in die somer gesnoei is. Daar is egter in April geen verskille gemeet in die blare se stysel- en totale suikervlakke tussen behandelings nie. Produksie van nuwe dra-posisies moet dus vir hierdie eksperiment in ag geneem word. Die gevolgtrekking was dat, deur in November te snoei en vroeë bestuur van nuwe groei toe te pas, die beste boomvorm verkry is. Deur in November te snoei eerder as in die winter, kon daar ook selektief gesnoei word aan lote met of sonder blomme, afhangende of dit ’n “aan” of “af” jaar was. Korrekte chemiese vruguitdunning is een van die mees

effektiewe maniere om 'n alternerende drag-siklus te verminder. Ongelukkig is geen betekenisvolle verskille in hierdie eksperiment verkry nie, ten spyte van die feit dat die uitdunningsbehandelings wel ietwat hoër styselvlakke in April 2012 getoon het. Dit dui daarop dat die behoefte aan energie in hierdie bome laer was. Die reaksie was waarskynlik te wyte aan die effens laer oes en vruggetalle as gevolg van die uitdunningsbehandelings in vergelyking met die kontrole. Die dichlorprop-behandeling het ook 'n hoërvruggroeiempo gestimuleer. Navorsing in die toekoms behoort te fokus op die tydberekening waarvolgens die chemiese uitdunningsmiddels op laat mandarynkultivars toegedien word.

Die vollegde tesis is beskikbaar op die Universiteit Stellenbosch se biblioteek webwerf:

<http://hdl.handle.net/10019.1/7182>.

General introduction

The South African citrus industry consists of 60 355 ha with approximately two-thirds of all fruit produced, 95 million 15-kg equivalent cartons in 2011, being exported due to the high financial returns for export quality fruit (Citrus Growers' Association of Southern Africa, 2012). Mandarins are an important part of citrus production, especially in the colder production areas where most mandarins are planted. In recent years a few late mandarin cultivars, namely Nadorcott, Morri and Orri, have become available to producers in South Africa. These cultivars attain high prices in the export market compared to Navel and Valencia sweet orange cultivars due to high sugar levels and good acid to sugar ratios, and particularly the easy peeling characteristics of these cultivars. These cultivars have therefore become a very important component of the citrus industry in South Africa. These cultivars do, however, have some production problems, e.g. low yields in some cases and, more importantly, alternate bearing (Monselise and Goldschmidt, 1982). Alternate bearing impacts on consistent financial returns to producers, since crop load, fruit size and quality are often compromised.

This study focuses on 'Nadorcott' mandarin, where the main objective was to develop a technique to predict bearing potential of trees so that alternate bearing can be moderated in advance. It was also decided that carbohydrates should be used to determine bearing potential, especially leaf starch levels, since carbohydrates play an important role in alternate bearing (Goldschmidt, 1999) and is relatively easy to quantify. Another objective was to determine what cultural practices could be used to reduce alternate bearing once a citrus producer knows whether the fruit set should be reduced or increased. In addition the goal was to determine how the manipulations should be executed in the orchard.

A literature review was conducted to gain knowledge on how alternate bearing and carbohydrates are linked. The focus was on the factors that influence the starch or total sugar levels of citrus trees, since this information could help in planning the experiments. All relevant literature on the manipulations used in the study was also explored and discussed so that a better understanding of the underlying modes of action could be gained.

Predicting bearing potential would be a valuable tool to citrus producers to ensure a constant annual yield, but little research has been done on this aspect, and the only other study found of this nature was that of Okada (2004) on 'Aoshima' satsuma mandarin trees. This study attempted, therefore, to find a reliable indicator for bearing potential and also to ensure an easy method of prediction. The goal was, therefore, to conduct new research to see if predicting bearing potential was possible or practical. Thereafter different manipulations such as pruning, fruit thinning and time of harvest were studied to provide citrus producers with clear and concise information on how to manipulate alternate bearing orchards to ensure regular annual yields.

Previous research on how pruning can be used to reduce an alternate bearing cycle, did not focus on the effect that different pruning strategies have on carbohydrate levels (Moss, 1972; Procopiou 1972). Furthermore, 'Nadorcott' mandarin is known to have excessive vegetative regrowth when pruned. Research was therefore conducted to determine the best method of pruning for 'nadorcott' mandarin, focusing on regrowth management, and also to determine the effect of different pruning strategies on carbohydrate levels. Although research has been conducted on thinning of different citrus types with Corasil E[®], i.e. dichlorprop, it is dangerous to extrapolate results to new cultivars such as 'nadorcott' mandarin, without first conducting research on the effects of dichlorprop on 'Nadorcott' mandarin.

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Chapter 1: Changes in leaf carbohydrate levels over one season in alternate bearing 'nadorcott' mandarin trees and the relationship between leaf carbohydrate status, return bloom, set percentage and subsequent yield

Alternate bearing is a common phenomenon in most commercial perennial fruit trees. In Citrus, the "on" year consists of a heavy crop load with mostly small fruit followed by an "off" year with few and large fruit. Carbohydrates play an important role, especially during fruit set, but also in flowering and fruit maturation and are essential in maintaining a regular bearing habit. Late mandarin types such as 'Nadorcott' mandarin are prone to alternate bearing. The aim of this study was to follow the change in starch and total sugar accumulation in the leaves across an entire season for both "on" and "off" trees and to see if leaf carbohydrate levels can be used to predict bearing potential. Ten "on" and ten "off" trees were chosen in a randomized complete block design. Vegetative leaves from the previous spring flush were sampled on a monthly basis from April 2010 until March 2011 for starch and sugar analysis. Phenology was followed by determining the yield and counting the number of flowers in the return bloom. Fruit growth was also measured from after physiological fruit drop until harvest the following year. Starch accumulation followed a distinct pattern with differences between "on" and "off" trees visible in April and May (Fig. 1). Starch levels in April showed a moderate negative correlation with yield and a moderate positive correlation with return bloom. Rapid starch accumulation started prior to harvest with a peak at the beginning of flowering. Thereafter a sharp decrease in starch levels occurred until after full bloom and a steady decrease was observed from physiological fruit drop onwards as fruit matured. Total sugar levels were more constant, but the increase in yield overall in 2011 compared to 2010 was reflected in the leaf total sugar levels as the demand for carbohydrates was increased by the higher fruit load. "On" trees yielded 53% more than "off" trees, but the return bloom for "off" trees was 140% more than "on" trees, thus illustrating the negative effect that a large crop has on the next season's bloom. We conclude that leaf starch levels in April can be used as an indication for bearing potential the following season for 'Nadorcott' mandarin trees.

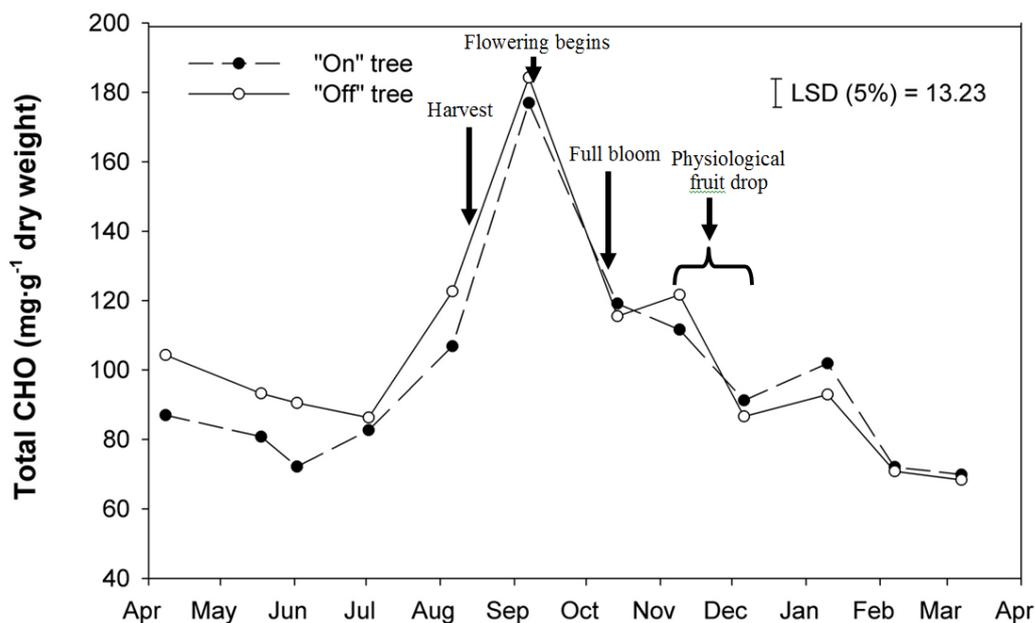


Figure 5.3.2.1. Changes in total leaf carbohydrates (Tot CHO) levels (mg·g⁻¹ dry weight) for alternating 'Nadorcott' mandarin trees for the 2010/2011 season. The bar represent least significant difference (LSD) between treatments as well as sampling dates of the same treatment (n=10).

Chapter 2: The effect of different pruning strategies on the phenology and carbohydrate status of alternate bearing 'nadorcott' mandarin trees.

Alternate bearing is a common phenomenon in citrus trees. The “on” year consists of a heavy crop load with mostly small fruit followed by an “off” year with few and large fruit. Mandarin cultivars such as Nadorcott are prone to alternate bearing and one method that has been researched in the past to control alternate bearing is pruning. In general, heavy pruning before an expected “on” year should reduce the yield and light pruning before an “off” year can ensure the highest yield for that year. Five treatments were used in a randomized complete block design with 10 single tree replications. All treatments except the summer pruning treatment were pruned on 3 November 2011 by removing old regrowth and three to five vigorous and complex shoots from the centre of the tree. The summer pruning treatment was exactly the same as the control except that trees were pruned on 26 January 2012 instead of 3 November 2011. The control and summer pruned trees were left as is for the rest of the experiment. For the early regrowth treatment, regrowth was thinned in late January 2012 so that shoots were spaced 15 cm apart in all directions. Vigorous shoots were pinched and new shoots that formed after the initial thinning were removed throughout the rest of the season. For the late regrowth treatment all regrowth was simply removed from the tree on 26 March. For the Uniconazole-P treatment, trees were treated twice, once when the summer flush had reached 5 cm in length and the same for the autumn flush. The number of spring flush vegetative shoots was determined on 26 September 2011 and the vegetative growth (in mm) was determined on 22 March 2012. PAR measurements were taken for the control and summer pruning treatments in late January 2012. Leaf total sugar and starch levels were determined during September 2011 and April 2012. Summer pruned trees had a higher number of spring flush vegetative shoots, more nodes per shoot and also more growth per parent shoot compared to control trees. Control trees had higher light levels inside the tree compared to summer pruned trees. No differences in leaf starch or total sugar levels during April were found between treatments. Favourable tree architecture should therefore be considered in this experiment. We conclude that pruning during November with early regrowth management will give the best balance between light penetration and production of good bearing units. Pruning in November, rather than during winter, also allows selective pruning of shoots with or without flowers, depending on whether it is an “on” or an “off” year, in an alternate bearing orchard.

General discussion and conclusions

Starch accumulation in leaves follows a distinct pattern throughout the season in 'Nadorcott' mandarin trees. Rapid starch accumulation started prior to harvest with a peak at the beginning of flowering. Thereafter a sharp decrease in starch levels occurred until after full bloom and a steady decrease was observed from physiological fruit drop onwards as fruit matured. The accumulation of starch starting prior to harvest is suggested to coincide with fruit reaching maximum TSS levels. It is possible that the sink strength of the fruit is reduced after maximum sugar accumulation in the pulp, allowing the tree to accumulate starch in the leaves. Initially it was hypothesized that “on” trees would reach a lower maximum starch level compared to “off” trees, but both “on” and “off” trees reached similar maximum starch levels at flowering. Differences in leaf starch levels for “on” and “off” trees did occur during April and May with no significant differences during any of the other months. The peak at flowering was followed by a sharp decrease as large amounts of starch needed to be mobilized to support the growth of floral parts and vegetative shoots. A steady decline was observed from then onwards as fruit matured. Whereas most previous studies only focused on certain growth stages or critical periods during a season, these data show the continuous accumulation and mobilization of starch and total sugars across an entire season, thereby giving the “bigger picture” of the carbohydrate economy of the tree during all phenological stages.

Correlations between leaf starch and total sugar levels for each month, and yield, return bloom and set showed that April has a moderate negative correlation ($r = -0.66$) with yield and a moderate positive correlation with return bloom ($r = 0.57$). This illustrates that April starch levels will be low when there is a large crop on the tree, due to the high demand for carbohydrates of the developing fruit, and this will result in a reduction in bloom the following year. The opposite will happen when there is a small crop. Correlations show that starch levels in April could give an indication of bearing potential for 'Nadorcott' mandarin and whether the current crop load is too heavy, adequate or too light. Identifying an “on” or “off” year at this stage is difficult since fruit are still small in size and have a dark green colour, especially in a regular bearing orchard where an “on” or “off” year was initiated. Therefore, if producers take leaf samples during the first two weeks of April and combine this with historical yield data, an alternate bearing orchard can easily be identified. Producers will already know by April that the next year will either be an “on” or an “off” year, depending on the leaf starch levels, and this will give them adequate time to manipulate trees into producing more or fewer fruit the next year, depending on the situation. Leaf starch levels in October and December (coinciding with the beginning and end of physiological fruit drop) showed moderate correlations with set percentage, confirming results from previous research that showed that starch has a direct relationship with fruit set. Future research should be done on other citrus cultivars to determine if leaf starch concentration

gives a good indication of bearing potential for these cultivars as well. It would be especially interesting to determine if other late maturing mandarins follow the same starch accumulation pattern and to compare it to early mandarin types such as 'hules' Clementine mandarin, which is harvested in May.

No significant differences in leaf starch levels were recorded in April for any of the pruning treatments. Little research has been done on the effect that different pruning techniques have on the carbohydrate levels of trees and the current research suggests that it has little or no effect and the focus of pruning research should remain on producing trees with many and good bearing units.

Unfortunately the thinning trial in this study did not yield any significant results. It did, however, give an insight into the potential that thinning (especially chemical thinning) has for managing alternate bearing of 'nadorcott' mandarin. Chemical thinning increased the fruit growth rate slightly as well as reduced the number of fruit and yield compared to the control treatment (albeit not significant differences). This illustrates that research should be done on optimizing the timing and rate of dichlorprop application for 'nadorcott' mandarin trees, as this will give producers a good tool for reducing the yield during "on" years.

Alternate bearing is a complex phenomenon with many different factors controlling the extent thereof. This study showed that trying to turn an alternate bearing orchard into a regular bearing orchard is difficult. A way to reduce alternate bearing in citrus is by better understanding all the factors involved (carbohydrates, hormones, fruit load and climate) and how they interact. Research should therefore be continued in order to better understand this complex phenomenon.

Technology transfer

Grower presentation

I.S. van Der Merwe, K.I. Theron J.S. Verreyne and P.J.R. Cronje. 2012. Phenology of Alternate Bearing 'Nadorcott' Mandarin Trees. CRI research symposium, Champagne Castle, KwaZulu-Natal 19-22 Aug 2012.

International symposium

I.S. van Der Merwe, K.I. Theron J.S. Verreyne and P.J.R. Cronje. 2012. Phenology of Alternate Bearing 'Nadorcott' Mandarin Trees. 2nd All Africa Horticultural Congress. 15-20 Jan, Skukuza, Mpumalanga, South Africa.

Grower advice

Pruning of 'nadorcott' mandarin trees can result in excessive regrowth, which has a negative effect on producing good bearing units. The early regrowth treatment where vigorous shoots were pinched and shoots were thinned to 15 cm apart in all directions showed great potential for producing good bearing units while maintaining a favourable tree size and this strategy is recommended to producers. Furthermore, it was observed that pruning in November instead of just after harvest (September) can be helpful when pruning alternate-bearing orchards. With the flowers already formed, shoots with no or little flower can be pruned during the "off" year. During the "on" year shoots with flowers can be removed and more shoots can be removed compared to a normal year, thereby effectively thinning the crop for that year. Future research should focus on long-term management of regrowth and how it should be pruned after two years from starting the treatment, as this information is currently lacking.

5.3.3 **PROGRESS REPORT: A novel approach to water and nutrient management in citrus**

Project 986 (August 2010 – Mar 2017) by 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. An experimental site was selected to monitor water use and nutrient concentration in the groundwater of a citrus orchard (Rustenburg Navels) in the Western Cape on the farm Patrysberg near Citrusdal. 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 were 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 are 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 nodige besproeiingswater aan te vul om droogtespanning of oor-besproeiing van die sitrusbome te voorkom. 'n Eksperiment om die water gebruik van sitrusbome (Rustenburg Nawels) te monitor, is op die plaas Patrysborg naby Citrusdal, Wes Kaap geïnisieer. Inligting oor stamomtrek, blaardak-dimensies, eienskappe van die hout, blaaroppervlakte, stomata-geleiding en blaarwaterpotensiaal word gereeld deur die seisoen bepaal. In Citrusdal is inligting oor die oes-opbrengs, vruggrootte, 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.

5.3.4 FINAL REPORT: Effect of 2,4-D on fruit splitting and fruit size of citrus

Project 1027 (November 2010 - March 2013) by Paul Cronje (CRI at SU), Jakkie Stander and Karen Theron (SU)

Summary

Fruit size and the integrity of the rind are key components that determine the value of a citrus fruit. The application of 2,4-dichlorophenoxy acetic acid (2,4-D) to reduce splitting, a *physiological disorder which entails cracking of the rind*, as well as to increase fruit size was conducted on three different split-susceptible mandarin and two split-susceptible orange cultivars. Treatments were applied directly after the physiological fruit drop period, as well as in January and February at $10 \text{ mg}\cdot\text{L}^{-1}$, alone or in combination with calcium (Ca), potassium (K) or gibberellic acid (GA_3). Application of 2,4-D directly after physiological fruit drop, either alone or in a tank-mix with K, consistently reduced the number of split mandarin fruit, with later applications in January and February generally being ineffective. Post physiological fruit drop application of $10 \text{ mg}\cdot\text{L}^{-1}$ 2,4-D significantly increased growth rate ($\text{mm}\cdot\text{day}^{-1}$) of all the mandarin cultivars, resulting in increased fruit size. Differences in sensitivity of cultivars to 2,4-D were evident, with the January application reducing the splitting in 'Midnight' Valencia. However, all the 2,4-D treatments reduced the fruit growth rate of the orange cultivars. The 2,4-D treatments, in terms of splitting, increased rind thickness, strength and coarseness of 'Marisol' Clementine throughout fruit development. In addition, fruit diameter and length increased to such an extent that the fruit shape was altered (reduced d/l-ratio), reducing the potential of the rind to crack and the fruit to split, however, rind coarseness of treated fruit was also increased. There were no major negative side effects on internal and external fruit quality, except for a possible reduction in juice content (%). Therefore, $10 \text{ mg}\cdot\text{L}^{-1}$ 2,4-D can be applied directly after physiological fruit drop on 'Marisol' Clementine and 'Mor' mandarin to reduce fruit splitting.

The full thesis is available on the University of Stellenbosch web site:
<http://hdl.handle.net/10019.1/79933>

Opsomming

Vruggrootte asook die integriteit van die skil is belangrike aspekte in die bepaling van 'n sitrusvrug se waarde. Die toediening van 2,4-dichlorofenoksie asynsuur (2,4-D) om vrugsplit, 'n fisiologiese defek wat tot die kraak van die sitruskil lei, te verminder is getoets op drie mandaryn- en twee lemoenkultivars. Hiermee saam is die potensiaal van 2,4-D om vruggrootte te verbeter ook geëvalueer. Die 2,4-D behandeling is direk na die fisiologiese vrugval periode toegedien, asook in Januarie en Februarie, teen $10 \text{ mg}\cdot\text{L}^{-1}$, alleen of in kombinasie met kalsium (Ca), kalium (K) of gibberelliensuur (GS_3). Al die mandarynkultivars het 'n vermindering in die totale aantal gesplete vrugte getoon indien die 2,4-D (enkel of in kombinasie met K) toegedien was direk na fisiologiese vrugval. Suksesvolle behandelings het ook 'n toename in vruggrootte tot gevolg gehad. Toediening van behandelings in Januarie en Februarie was oor die algemeen oneffektief.

Verskille in kultivar sensitiviteit teenoor 2,4-D is gevind, met vrugsplit in 'Midnight' Valencia wat verminder was deur die Januarie toediening van 2,4-D. Al die 2,4-D behandelings het vruggrootte van die lemoenkultivars verlaag. Daar is bevind dat die 10 mg.L⁻¹ 2,4-D, enkel of in kombinasie met K, 'n toename in beide skildikte en –sterkte van 'Marisol' Clementine teweeg bring asook 'n growwer skil. Behandelings met 2,4-D het vrugdeursnee en –lengte laat toeneem, wat 'n verandering in vrugvorm tot gevolg gehad het, tot so 'n mate dat vrugte minder geneig was om gesplete te wees. Behalwe vir 'n moontlike verlaging in die sapinhoud (%) van vrugte, was daar geen noemenswaardige negatiewe effekte op interne en eksterne vrugkwaliteit nie. Die toediening van 10 mg.L⁻¹ 2,4-D direk na fisiologiese vrugval kan dus aanbeveel word op mandaryn kultivars wat geneig is tot vrugsplit.

Die volledige tesis is beskikbaar op die Univ.Stellenbosch se biblioteek webwerf: <http://hdl.handle.net/10019.1/79933>

Introduction

Citrus fruit splitting is a physiological disorder in Clementine mandarin, mandarin hybrids as well as 'navel' and 'Valencia' orange and is a consequence of micro-cracks developing at the styler-end of the fruit rind. As the fruit matures, the split lesion extends towards the equatorial region of the fruit and eventually leads to premature drop. In severely affected orchards, fruit losses of as much as 30% have been reported (Barry and Bower, 1997). Citrus fruit growth follows a sigmoidal curve and consists of three development stages, as described by Bain (1958). Stage I is characterized by cell division, with the total volume of a single fruitlet predominantly consisting of the rind. Stress factors in this period, such as nutritional imbalances, water deficit, high flower number and fruit set hampers sufficient cell division and lead to the development of a weak and thin rind. During stage II, increase in fruit volume occurs due to cell enlargement of the pulp and physical splitting of the citrus fruit becomes visible. During stage III, the fruit starts to ripen, very little or no increase in fruit volume occurs and split fruit drop from the tree. Not only does splitting lead to unwanted reduction in crop load, but also requires additional labour to sanitize orchards as split fruit provide perfect conditions for manifestation of insect pests and decay. A commercial solution is therefore required to control fruit splitting as it occurs throughout South African production regions. Most of the studies on the control of fruit splitting focussed on increasing the rind thickness and strength of split-prone species by applying pre-harvest mineral nutrient sprays to the canopy or plant growth regulator (PGR) foliar sprays. Although results were erratic, PGR application of the synthetic auxin, 2,4-dichlorophenoxy acetic acid (2,4-D) were generally more successful. In this study the treatment effect on splitting of different combinations of 10 mg.L⁻¹ 2,4-D and calcium (Ca) and potassium (K) were evaluated in different split-prone cultivars in their different localities, over two production seasons. In addition to splitting, the effect of different timings of 10 mg.L⁻¹ 2,4-D applications, starting after physiological fruit drop, on fruit size and general fruit quality parameters, was also evaluated.

Synthetic auxins have a direct stimulatory effect on fruit growth and size. The isopropylester formulation of 2,4-D is registered as a fruit size enhancer in California. It is rapidly absorbed by roots, stems and leaves and translocated in the phloem to young meristematic tissue (Ashton et al., 1991). It accumulates in organs such as young leaves, flowers or fruitlets where it increases sink strength of these organs by stimulating cell expansion (Mitchell, 1961). However, except for difference in cultivar sensitivity to synthetic auxins, success of application is dependent on timing, as well as concentration and application rate.

Objective

In this study the effect of different timings of 2,4-D treatments on a variety of cultivars was evaluated, with the aim of providing producers with a commercial, viable solution to fruit splitting which at the same time increases fruit size and maintains acceptable fruit quality.

(Of each of the research chapters an abstract is supplied below).

Chapter 1: Foliar 2,4-dichlorophenoxy acetic acid (2,4-d) application after physiological fruit drop reduces fruit splitting and increases fruit size in mandarin

Citrus fruit splitting entails the initiation of cracks at the styler- or navel-end of the fruit, developing into splitting and eventual abscission of affected fruit. Although cultural and environmental factors contribute to the susceptibility of fruit to the disorder, split-prone cultivars develop a genetically weak and split-susceptible rind. Foliar applications of different combinations of 2,4-dichlorophenoxy acetic acid (2,4-D), gibberellic acid (GA₃) and mineral nutrients calcium (Ca) and potassium (K) to reduce the disorder in split-prone mandarin cultivars, were evaluated over two consecutive growing seasons, 2010/2011 and 2011/2012. Studies were carried out on three different mandarin cultivars, viz., Marisol Clementine, Mor and Orri mandarin, at three different localities, viz., Citrusdal, De Doorns and Paarl, South Africa. Treatments were applied directly after physiological fruit drop (November/December) and at two later dates in summer (January and February). The

application of 10 mg·L⁻¹ 2,4-D directly after physiological fruit drop, either single or in combination with K increased rind thickness and reduced splitting of 'Marisol Clementine' mandarin and 'Mor' mandarin by up to 50% in both seasons (Tables 1 and 2). Post physiological fruit drop application of 10 mg·L⁻¹ 2,4-D significantly increased growth rate (mm/day) of all the cultivars, resulting in bigger fruit at time of harvest and had no effect on rind coarseness and no styles remained attached. Except for a slight reduction in juice content (%) and total acidity (TA), there were no effects on the °Brix, and °Brix:TA at commercial harvest. A medium cover foliar spray of 10 mg·L⁻¹ 2,4-D directly after physiological fruit drop is recommended for 'Marisol Clementine' mandarin and 'Mor' mandarin orchards with a history of severe fruit splitting and after excessive fruit set.

Table 5.3.4.1. Effect of different combinations of chemical applications on total fruit splitting of various cultivars in the production season of 2010/11.

Treatment	Ave. nr. split fruit per tree			
	'Marisol Clementine'	'Marisol Clementine'	'Mor' mandarin	'Orri' mandarin
	Paarl mandarin	Citrusdal mandarin	Paarl	De Doorns
Control	209 ^{abcz}	15 ^{ns}	8 ^{abz}	22 ^{ns}
2,4-D*	121 ^{cd}	12	3 ^b	18
Bonus NPK*	154 ^{bcd}	13	5 ^{ab}	20
Ca(NO ₃) ₂ *	237 ^{ab}	-	9 ^a	24
GA ₃	-	15	-	-
2,4-D + Bonus NPK*	79 ^d	14	7 ^{ab}	20
2,4-D + Ca(NO ₃) ₂ *	151 ^{bcd}	14	5 ^b	21
2,4-D January	273 ^a	16	8 ^a	16
2,4-D February	184 ^{bcd}	21	7 ^{ab}	19
<i>P-value</i>	0.0038	0.1966	0.0140	0.4753

^z Means with a different letter within a column differ significantly at the 5% level (LSD)

^{ns} No significant differences

*after physiological fruit drop

Table 5.3.4.2. Effect of different application dates of 2,4-D on fruit growth rate of various mandarin cultivars in the production season of 2010/11.

Treatment	Fruit growth rate (mm.day ⁻¹)			
	'Marisol Clementine'	'Marisol Clementine'	'Mor' mandarin,	'Orri' mandarin,
	Paarl mandarin,	Citrusdal mandarin,	Paarl	De Doorns
Control	0.28 ^{bz}	0.30 ^{ns}	0.24 ^b	0.30 ^b
2,4-D*	0.30 ^a	0.30	0.26 ^a	0.32 ^a
2,4-D January	0.27 ^b	0.29	0.23 ^c	0.31 ^b
2,4-D February	0.27 ^b	0.30	0.23 ^c	0.30 ^b
<i>P-value</i>	0.0001	0.2101	0.0312	0.0441

^z Means with a different letter within a column differ significantly at the 5% level (LSD)

^{ns} No significant differences

*after physiological fruit drop

Chapter 2: Foliar 2,4-dichlorophenoxy acetic acid (2,4-d) application in January reduces fruit splitting and fruit growth rate of Valencia orange

Fruit splitting is a physiological disorder that involves the cracking of the rind at the styler- and/or navel-end of a fruit and eventual splitting and premature drop of affected fruit. 'navel' and 'Valencia' orange are particularly susceptible due to the presence of the navel structure in 'navel' orange and thin rind of 'Valencia' orange. Foliar applications of different combinations of 2,4-dichlorophenoxy acetic acid (2,4-D), gibberellic acid (GA₃) and mineral nutrients calcium (Ca) and potassium (K) to reduce the disorder in split-prone orange cultivars, were evaluated over two consecutive seasons, 2010/2011 and 2011/2012. Studies were carried

out on two different orange cultivars, viz., Bahianina Navel and Midnight Valencia, at two different localities, viz., Citrusdal and Clanwilliam. Treatments were applied directly after physiological fruit drop (November/December) and at two later dates in summer (January and February). January application of 10 mg·L⁻¹ 2,4-D reduced splitting in 'Midnight Valencia' orange. Post physiological fruit drop application of 10 mg·L⁻¹ 2,4-D had no effect on rind coarseness and no styles remained attached (Tables 5.3.4.3 and 5.3.4.4). Except for a reduction in juice content (%) and total acidity (TA), there were no effects on the °Brix, and °Brix:TA at commercial harvest. All the 2,4-D treatments reduced the growth rate of fruit, resulting in smaller fruit at time of harvest. Further research should be conducted in order to validate the foliar application of 2,4-D, later than blossom period on orange cultivars.

Table 5.3.4.3. Effect of different combinations of chemical applications on total fruit splitting of various orange cultivars and sites in the production season of 2010/11.

Treatment	Ave. nr. split fruit per tree	
	'Midnight Valencia', Citrusdal	'Bahianina Navel', Clanwilliam
Control	25 ^{ns}	0
5% Bonus-NPK*	17	0
2,4-D + 2% Ca(NO ₃) ₂ *	19	0
20 mg·L ⁻¹ GA ₃ January	25	0
10 mg·L ⁻¹ 2,4-D*	25	0
2,4-D January	13	0
2,4-D February	18	0
2,4-D and 5% Bonus-NPK*	28	0
<i>P-value</i>	0.0839	

^z Means with a different letter within a column differ significantly at the 5% level (LSD)

^{ns} No significant differences

*after physiological fruit drop

Table 5.3.4.4. Effect of treatments on fruit growth rate (mm·day⁻¹) of various orange cultivars in the production season of 2010/11.

Treatment	Ave. fruit growth rate (mm/day)	
	'Midnight Valencia', Citrusdal	'Bahianina Navel', Clanwilliam
Control	0.17a ^z	0.30a
10 mg·L ⁻¹ 2,4-D*	0.12c	0.29b
2,4-D January	0.14b	0.28b
2,4-D February	0.14b	0.29b
<i>P-value</i>	0.0021	0.0173

^z Means with a different letter within a column differ significantly at the 5% level (LSD)

*after physiological fruit drop

Chapter 3: Foliar 2,4-dichlorophenoxy acetic acid (2,4-d) and bonus-NPK application reduces fruit splitting in 'Marisol Clementine' mandarin (*Citrus reticulata* Blanco) by increasing rind thickness, strength and fruit diameter

'Marisol Clementine' mandarin fruit is prone to splitting, a physiological disorder which entails cracking of the rind at the styler-end and eventual splitting and abscission of the fruit. Foliar application of the synthetic auxin, 2,4-dichlorophenoxy acetic acid (2,4-D), has the potential to reduce the disorder in mandarin cultivars, although the method of action is unknown. The effect of foliar applications of 2,4-D on its own, or in combination with potassium (K) on fruit splitting and fruit anatomy of 'Marisol Clementine', was evaluated in an orchard prone to the disorder in Paarl, South Africa. Treatments consisted of 10 mg·L⁻¹ 2,4-D and a tank-mix of 10 mg·L⁻¹ 2,4-D + 5% Bonus-NPK, applied after the physiological fruit drop period. Fruit were sampled prior to visual split initiation, at the start of visual split initiation, one month after visual split initiation and at commercial harvest and the treatment effect on fruit anatomy determined (Fig. 5.3.4.1). Both treatments reduced fruit splitting significantly, but internal quality was unaffected. Treatments increased rind thickness and rind strength throughout fruit development. Both fruit diameter and length increased to such an extent that the fruit shape was altered (reduced d/l-ratio). Rind coarseness of treated fruit was also significantly increased, reducing cosmetic appearance.

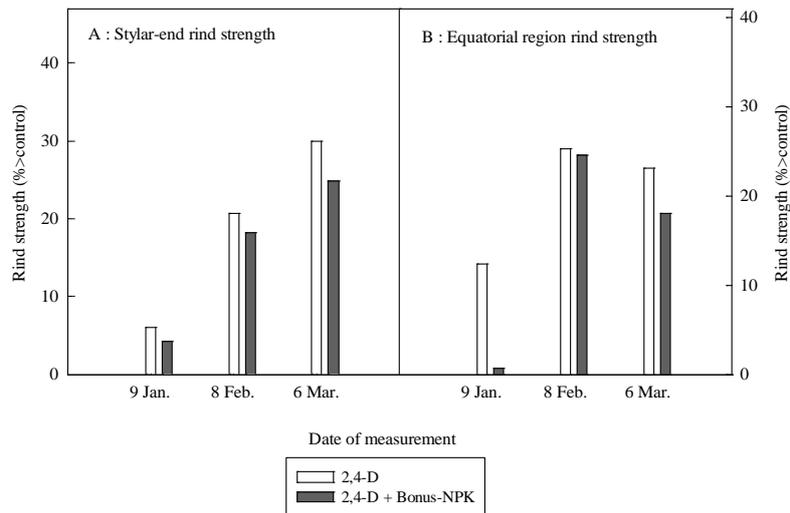


Figure 5.3.4.1. Effect of 2,4-D and 2,4-D in combination with 5 % Bonus-NPK on (A) rind strength at the stylar-end and (B) at the equatorial region of treated fruit expressed as the increase in % compared to the control

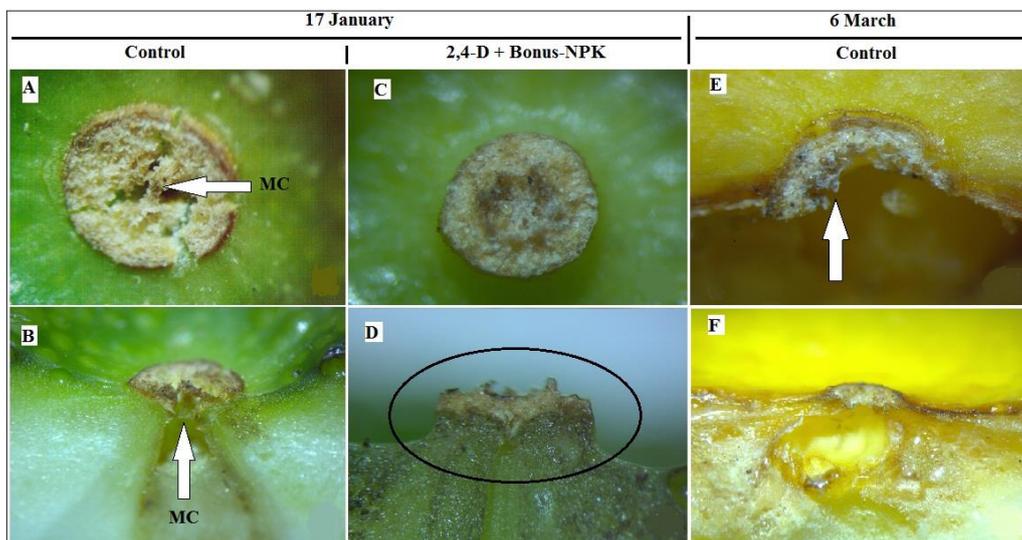


Figure 5.3.4.2. Control fruit showing micro-cracks at stylar-end of fruit (MC), two weeks prior to visual fruit split initiation, on 17 January (A-B). No micro-cracks were visible at the stylar-ends of fruit treated with 2,4-D + Bonus-NPK on the same evaluation date in January (C-D). The apical floral meristem extended into the style of the fruit (D) (circle), which after style abscission, resulted in a compact, solid tissue. In March the split lesion (arrow) extended into the flavedo and albedo of the control fruit as the split became more severe (E-F).

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Technology transfer

Grower presentation

Stander O.P.J., K.I. Theron J.S. Verreyne and P.J.R. Cronje. 2012. Novel usage of 2,4-D to increase citrus fruit quality. CRI research symposium, Champagne Castle, Kwa-Zulu Natal 19-22 Aug 2012.

International symposium

Stander O.P.J., K.I. Theron J.S. Verreyne and P.J.R. Cronje. 2012. Novel usage of 2,4-D to increase citrus fruit quality. 2nd All Africa Horticultural Congress. 15-20 Jan, Skukuza, Mpumalanga, South Africa.

Publication in peer reviewed journal

Cronje, P.J.R., Stander, O.P.J., Theron, K.I., 2013. Fruit Splitting in Citrus. Horticultural Review Vol 41. (*Accepted Manuscript*).

Grower advice

Except for a slight reduction in juice content (%) and total acidity (TA), there were no effects on the °Brix, and °Brix:TA of all the cultivars at commercial harvest. This slight reduction in juice content (%) was probably only due to the way juice % was calculated as the treated fruit generally had thicker rinds and actual juice content of sap vesicles was probably not affected. A medium cover foliar spray of 10 mg·L⁻¹ 2,4-D directly after physiological fruit drop could be used for 'Marisol Clementine' mandarin and 'Mor' mandarin orchards with a history of severe fruit splitting. Similar application of 2,4-D in January on 'Midnight Valencia' orange is not recommended, due to the negative effect on fruit size and juice content. This study offers proof that the post physiological fruit drop application of 2,4-D, between stage I and II of fruit development, increases rind thickness and -strength of mandarin and as a result reduces fruit susceptibility to the physiological disorder of the rind that is fruit splitting.

5.3.5 **PROGRESS REPORT: Study on the effect of humic and fulvic acids on fertiliser application in citrus**

Project 1028 (April 2011 – March 2016) by J.T. Vahrmeijer (CRI) and A. Gatabazi (UP)

Summary

Soil organic material consists mostly of humic acids, which play a role in soil fertility by enhancing the nutrient permeability of the cell membranes of plants and micro-organisms. This project was conducted at the microbial laboratory of the Department of Plant Production and Soil Science at the University of Pretoria. The research consists of nine different treatments in a completely randomized block design (CRBD) with each treatment replicated three times. The sandy clay loam soil used was supplemented with two different fertiliser application rates (220-50-80 and 165-37.5-60 N, P and K) with and without humates or fulvate (200 kg·ha⁻¹).

One gram of soil from each trial was added to 9 ml of Ringers solution, shaken at 25°C, 230 rpm for 20 minutes. Serial dilutions were performed in Ringers solution and 0.1 ml of each suspension was distributed in triplicate on 0.1 Trypticase soy agar and potato dextrose ager. Bacterial and fungal density were recorded after two and three days of incubation at 25°C and expressed as colony forming units (CFU) per gram of soil. The results demonstrated that humic and fulvic acids result in an increase in the culturable fraction of the soil microbial community (bacteria and fungi).

A project was started to determine the long term effect of potassium-humates and fulvate, together with reduced fertiliser applications, on the yield and nutrient content of the soil and leaves of commercial orchards. This project is currently in progress and will continue for three years.

Opsomming

Ontbinde organiese material in die grond bevat humiensure wat 'n rol speel in grondvrugbaarheid deur die permeabiliteit van sel- en mikro-organismemembrane te verhoog. 'n Projek om die rol van humate en fulvate op die mikro-organismeeiwigtheid in grond, onder laboratoriumtoestande te bepaal is in die mikrobiologiese laboratorium by die Departement van Plant Produksie and Grondwetenskappe by die Universiteit van Pretoria uitgevoer. Die navorsing het bestaan uit nege behandelings, wat in 'n ewekansige blokontwerp gerangskik, is. Elke behandeling is drie keer herhaal. 'n Sand-klei-leem grond is gebruik en die behandelings het bestaan uit gronde wat onderskeidelik met twee konsentrasies kunsmis (220-50-80 and 165-37.5-60 N, P and K) gemeng is. Humate en fulvate is by van die grond toegedien (200 kg·ha⁻¹).

Een gram grond van elke behandeling is onderskeidelik by 9 ml Ringers oplossing bygevoeg en vir 20 min (230 rpm) by 25°C geskud. 'n Reeks verdunnings met Ringers oplossing is gemaak en 0.1 ml van elke suspensie is in triplikaat op 0.1 Trypticase-soja-agar en aartappel-dekstrose-agar geplaas en vir twee tot drie dae by 25°C geïnkubeer. Daarna is die bakteriese -en swamdigheid, uitgedruk as kolonie vormende eenhede per gram grond, getel. Die resultate dui daarop dat humate en fulvate die mikro-organismekultuur in grond verhoog.

’n Projek is geïnisieer om die langtermyn effek van humien- en fulviensure, tesame met ’n verlaging in kunsmisstowwe, op die opbrengs en voedingstofkonsentrasies van die grond en blare van sitrusboorde te bepaal. Data word nog ingesamel en hierdie projek sal nog vir die volgende drie jaar voortduur.

5.3.6 **PROGRESS REPORT: The evaluation of different formulations of micronutrients on foliar uptake**

Project 1037 (April 2012 – March 2014) by J.T. Vahrmeijer (CRI) and S.M. Scholly (UP)

Summary

The experiment commenced in April 2012. Small citrus trees were planted in 20 litre bags and placed in a greenhouse. A method for sample preparation was validated, where plant sap press extraction and microwave assisted digestion were utilised. Leaves were treated with different formulations of Manganese (amino acid-chelate, EDTA-chelate, and $MnSO_4$), Boron (H_3BO_3) and a control treatment (containing only deionised water, buffer- and wetting agents). Treated leaves, as well as the leaf directly above and below the treated leaf which received no treatments, were sampled, washed with 10% acetone, and rinsed with deionised water. Microwave assisted digestion proved the most efficient of the two methods validated, and will be used in the remainder of the study. From the validation experiment, it was also found that manganese-amino acid treated leaves showed the highest increase in Mn-content, but the Mn-EDTA treatment showed the highest increase in the leaves above and below the treated leaves. For the main experiment, the different micronutrients were then applied to the different groups of citrus seedlings as well as a control spray containing only the additives and no micronutrient products. This procedure was repeated for each of the different formulations for the particular element at different sampling times. Leaves were sampled by the procedure as described by Coetzee *et al.* (2010), and the waxy layer removed by rinsing with 10% acetone. Samples were dried and digested by microwave assisted digestion and ICP-AES analysis is in progress. Leaf samples have been collected for the citrus leaf surface comparison study from commercial orchards in the Groblersdal area and will be prepared for SEM and TEM studies.

Opsomming

Die studie het in April 2012 in aanvang geneem. Klein sitrusbome is oorgeplant in 20 liter sakke en in ’n kweekhuis geplaas om te vestig. Die eksperiment sal voortgaan indien die temperature styg en die bome nuwe blare vorm. Twee metodes vir monster voorbereiding is geëvalueer, waar die een metode van plantsap gebruik maak, en die ander metode van ’n mikrogolf geassisteerde verteringsprosedure gebruik maak. Sitrusblare is behandel met verskillende formularies van mangaan (aminosuur-chelaat, EDTA-chelaat, en $MnSO_4$), boor (H_3BO_3) en ’n kontrole, wat slegs gedeïoniseerde water, benatter en buffer bevat. Behandelde blare, sowel as die blare onmiddelik bo en onder die behandelde blaar is geneem vir ontledings. Daarna is die blare gespoel met 10% asetoon, en afgespoel met gedeïoniseerde water. Mikrogolf geassisteerde verteerde monsters het die mees effektiewe resultaat getoon van die twee metodes, en sal dus verder in die studie gebruik word. Uit die metode-evaluasie eksperiment het dit ook geblyk dat die Mn-aminosuur behandelde blare die grootste toename in Mn-inhoud gehad het, maar die Mn-EDTA behandeling het die grootste toename in die blare onmiddelik bo en onder die behandelde blaar getoon. Verskillende mikro-elemente is op verskillende sitrusboompies toegedien en ’n kontrole behandeling wat slegs die byvoegmiddels en gedeïoniseerde water bevat, is by die eksperiment ingesluit. Hierdie prosedure is herhaal vir elke formulering van elke mikro-element en blaremonster is op verskillende tye geneem soos beskryf deur Coetzee *et al.* (2010). Die waslagie op die blare is verwyder deur die blare met 10% asetoon te was, blare is gedroog in drogingssonde en verteer deur mikrogolf geassisteerde vertering en konsentrasies van die verskillende elemente is met ’n geïnduseerde gekoppelde plasma atomiese emissie spektrum (ICP-AES) bepaal. Verskillende blaarmonsters is in kommersiële boorde in die Groblersdaldistrik geneem om die blaaroppervlak van die verskillende sitrus variëteite te vergelyk. Die blaarmonster wat geneem is sal voorberei word vir SEM en TEM studies.

5.3.7 **PROGRESS REPORT: The influence of silicon on brown spot (*Alternaria alternata*) control**

Project 1057 (Feb 2013 – Mar 2014) by J.T. Vahrmeijer (CRI) and N.M. Asanzi (UP)

Summary

The objectives of this study are to: 1) determine under laboratory conditions if silicon (Si) reduces *Alternaria alternata* infection of young leaves in mandarin ‘Nules’ (*Citrus reticulata*) trees; and 2) assess under field conditions the impact of root and foliar applied Si to lemon (*Citrus limon*) trees on *Guignardia citricarpa* infection. The first part of the study will be done in a glasshouse at the experimental farm of the University of

Pretoria from August 2013 - November 2013. The second part of the study is done in an orchard of 12 year-old 'Eureka' (*Citrus limon*) lemon trees grafted on rough lemon rootstocks in the Brits region. Potassium silicate (K_2SiO_3) has been applied as a drench ($2000 \text{ mg.L}^{-1} \text{ Si}$ at 5 L tree^{-1}) once a month since February 2013 to a section of the orchard to ensure a sufficient increase in the Si-content of the trees. Another section of the orchard will be sprayed with Si once *Guignardia citricarpa* is observed in the trial block. Silicon concentration in the leaves will be determined by using induced couple plasma optical emission spectrometry (ICP-OES) with a microwave-assisted acid-based digestion method adapted from Haysom & Ostatek-Boczynski (2006) and the effect of Si on the suppression of *Guignardia citricarpa* will be evaluated.

Opsomming

Die doel van hierdie studie is om: 1) onder laboratoriumtoestande die invloed van silikon (Si) op die onderdrukking van *Alternaria alternata* in die jong blare van mandaryn 'Nules' (*Citrus reticulata*) te bepaal en 2) in 'n boord die impak van blaar- en worteltoegediende Si op *Guignardia citricarpa* infeksie te bepaal. Die eerste gedeelte van die studie sal, van Augustus 2013 - November 2013, in 'n glashuis op die proefplaas by die Universiteit van Pretoria uitgevoer word. Die tweede gedeelte van die studie word in 'n boord met 12 jaar oue 'Eureka' (*Citrus limon*) suurlemoenbome, wat op growwe skil onderstokke ingeënt is, uitgevoer. Die eksperiment word in die Brits omgewing uitgevoer deur kaliumsilikaat (K_2SiO_3) wortelbehandeling ($2000 \text{ mg.L}^{-1} \text{ Si}$ teen 5 L tree^{-1}) toe te dien. Die behandeling geskied maandeliks vanaf Februarie 2013 om die Si-inhoud van die suurlemoenbome te verhoog. In 'n ander gedeelte van die boord sal Si as 'n blaarbespuiting toegedien word wanneer *Guignardia citricarpa* in die boord opgemerk word. Blare van die bome sal met behulp van 'n mikrogolf geassisteerde suur verteringstegniek voorberei word (Haysom & Ostatek-Boczynski, 2006) en die Si-konsentrasie sal met 'n geïnduseerde gekoppelde plasma atomiese emissie spektrum (ICP-AES) bepaal word. Die effek van Si op die onderdrukking van *Guignardia citricarpa* sal dan ge-evalueer word.

5.4 PROGRAMME: COLD CHAIN MANAGEMENT AND PACKAGING

Programme coordinator: Malcolm Dodd (SU)

5.4.1 Programme summary

The programme aims to improve the efficiency of the citrus cold chain. The research focuses on understanding the airflow dynamics within reefer containers in order to enable techniques such as ambient loading and easier management of 'Steri protocols' (5.4.2). Reducing the cost of landside preparation of grapefruit for Japan 'Steri' has also been investigated. There are ways of reducing these costs; however, there are significant barriers to overcome in order to achieve this such as getting the buy-in from DAFF, MAF (Department of Agriculture in Japan) and the PPECB (5.4.3). Measuring temperatures from the beginning to the end of a typical citrus supply chain has provided transparency where it has never existed before (5.4.4).

Programopsomming

Die program is daarop ingestel om die doeltreffendheid van die sitrus koue ketting te verbeter. Navorsing is daarop gefokus om die dinamika van lugvloei in verkoelde skeepshouers te verstaan, sodat tegnieke geskep kan word wat die laai van vrugte by kamertemperatuur en beter beheer van 'steri protokol' in staat stel (5.4.2). Daar is ook ondersoek ingestel na die vermindering van koste verbonde aan die voorbereiding van pomelo's vir Japan 'steri'. Daar is maniere om hierdie kostes te verlaag, maar groot struikelblokke moet eers oorkom word, soos om die inkoop van die Departement van Landbou, Bosbou en Visserye (DvLBV), die Departement van Landbou in Japan (MAF) en die PPECB te verkry (5.4.3). Deurlopende temperatuurmeting, van die begin tot die einde van 'n tipiese sitrus voorsieningsketting, het deursigtigheid verskaf waar dit nie voorheen bestaan het nie (5.4.4).

5.4.2 PROGRESS REPORT: Energy optimisation of refrigerated shipping containers and the measurement of temperature and humidity throughout the supply chain

Project C1/09 (April 2009 - March 2013) by M.C. Dodd (SU)

Summary

A series of trials were conducted testing the feasibility of using refrigerated shipping containers to pre-cool citrus and manage the pulp temperature according to the 'Systems approach partial steri programme'. This forms part of a strategic plan for the management of False Codling Moth for the EU markets. Three trials were conducted on grapefruit packed in open top display cartons as well as navels and Valencia's packed in A15C 'Super vent' cartons. With each fruit kind 20 pallets were placed in a standard container and another 20 were placed in a container with either built in reversed air flow technology or with a disposable kit that

creates horizontal airflow. Fifteen pulp temperature recorders were placed throughout the 20 pallets in pattern which allowed for analysis of the rate of pre-cooling and the evenness of temperature management. The only trial where the fruit was pre-cooled fast enough to allow for the 12 day 'Steri' temperature within the shipping time frame from RSA to Europe was with the horizontal air flow kit.

Opsomming

'n Reeks opeenvolgende proefnemings is uitgevoer om die doeltreffendheid van verkoelde skeepshouers te bepaal, vir die voorverkoeling en handhawing van vrugtemperatuur, volgens 'n 'stelselbenadering vir 'n gedeeltelike steri-program'. Sitrus wat teen kamertemperatuur gelaai is, is vir die toetse gebruik. Dit vorm deel van 'n strategiese plan vir die beheer van Vals Kodling Mot in die Europese Unie (EU). Drie proefnemings is uitgevoer op pomelo's wat in oop vertoonkartonne verpak is, sowel as op nawels en Valencia's wat in A15C 'Super vent'-kartonne verpak is. Twintig palette van elke vrugtesoort is in 'n standaard skeepshouer geplaas en 'n verdere twintig is in skeepshouers geplaas wat of met ingeboude omgekeerde lugvloei toegerus is, of toegerus is met 'n wegdoenbare apparaat wat horisontale lugvloei skep. Om vrugtemperatuur te meet, is vyftien opnemers deurlopend in die twintig palette geplaas. Die opnemers is in 'n spesifieke patroon geplaas om die analise van die voorverkoelingstempo en die eweredigheid van temperatuurbeheer moontlik te maak. Die enigste proefneming waarin die vrugte vining genoeg voorverkoel is om voorsiening te maak vir die twaalf-dag verskepingstyd tussen Suid-Afrika en Europa, was die ene waarin horisontale lugvloei gebruik is.

5.4.3 **PROGRESS REPORT: Using higher 'Steri' temperatures for specialised reefer vessels bound for Japan to reduce landside costs** Project 6/12 (Apr 2012 – Mar 2014) by M.C. Dodd (SU)

Summary

This project requires the cooperation and agreement between DAFF, MAF, PPECB and NYKCool shipping line. The initial ground work has been set back because NYKCool, contrary to the advice of the CRI, sent a letter in November 2012 outlining the project to DAFF. The latter organisation was confused by this letter as it was not sent to a specific official and consequently they did not know how to respond. The outcome was that nothing happened. The matter has been raised by CRI again with DAFF and, with the assistance of Mike Holtzhausen; the necessary discussions will take place. To this end, a meeting took place between CRI and PPECB on Thursday the 10th October 2013. The current status of this project is that the research on cooling the grapefruit in a vessel should be conducted on fruit destined for a market such as Russia. Discussions are underway with exporters and a shipping line to try and achieve this.

Opsomming

Hierdie projek vereis 'n ooreenkoms en samewerking tussen die Departement van Landbou, Bosbou en Visserye (DvLBV), die Departement van Landbou in Japan (MAF), die PPECB en die NYKCool skeepsredery. Die aanvanklike grondwerk is vertraag, omdat NYKCool, teenstrydig met die raad van die CRI, in November 2012 'n brief aan die DvLBV gerig het wat 'n oorsig van die projek verskaf. Hierdie brief het verwarring by die DvLBV veroorsaak, aangesien dit nie aan 'n spesifieke persoon gerig was nie en die DvLBV daarom nie geweet het hoe om daarop te reageer nie. Gevolglik het niks gebeur nie. Die CRI het die saak weer met die DvLBV aangeroer, en met die hulp van Mike Holtzhausen sal die nodige samesprekings plaasvind. In dié verband is 'n vergadering tussen die CRI en die PPECB op Donderdag 10 Oktober 2013 gehou. Die huidige stand van die projek is sodanig dat navorsing rakende die skeepsverkoeling van pomelos verkieslik uitgevoer moet word op vrugte wat bestem is vir 'n mark soos Rusland. Huidige samesprekings tussen uitvoerders en 'n skeepsredery poog om dit te vermag.

5.4.4 **PROGRESS REPORT: Using Radio Frequency Identification Technology (RFID) to get an understanding of the storage air and fruit pulp temperatures and relative humidity in a typical South African fruit export supply chain from the very beginning to very end over two seasons** Project (April 2012 – Mar 2014) by M.C. Dodd (SU)

Summary

A co-operative project between Stellenbosch University, Xsense (now BT9-Tech) of Israel and Sainsbury's in the UK set about using RFT enabled temperature and relative humidity data recorders (Tags) to map these parameters from the very beginning of the cold supply chain ("Birth" of a pallet) to the very end (Point of sale).

BT9-Tech provided:

1. Free of charge the 'Communication units' which were placed at five strategic locations across the supply chain, or hand held readers. These capture the data from the temperature recorders and upload it via the GSM network to a server where it is stored and read via the Internet.

Sainsbury's provided:

1. Access to receiving depots and distribution centres within the UK in which some readers will be placed.
2. Quality evaluator staff that retrieved the recorders at a Distribution Centre in the UK and then placed them into the reconstituted pallets of the same product that was sent to store. They followed the load to store where they ensured that they placed the recorders below the point of sale stand (This is because the recorders could be seen as a 'bomb threat'). They were provided with hand held Radio receivers that captured the data from in-store and uploaded said data to the BT9-Tech server.

Conclusion:

From the data analysed thus far it can be stated that this particular citrus cold chain is effective as the temperatures recorded were mostly within protocol. In addition there were no quality issues recorded in any of the soft citrus shipments.

Opsomming

'n Projek tussen die Universiteit Stellenbosch, in samewerking met Xsense (nou BT9-Tech) van Israel en Sainsbury's in die VK, het RFT-temperatuur-opnemers en relatiewe humiditeits data-opnemers gebruik om hierdie parameters aan te dui, van die aanvang ('geboorte' van 'n pallet) van die koue ketting tot aan die einde daarvan (verkoopspunt).

BT9-Tech het die volgende verskaf:

1. Gratis 'kommunikasie-eenhede' wat op vyf strategiese punte dwarsoor die handelsketting geplaas is, of hand-'lesers'. Hiermee word data vanaf die temperatuur-opnemers vasgelê en gelaai deur die GSM-netwerk na 'n bediener waar dit bewaar en gelees word via die Internet.

Sainsbury's het die volgende verskaf:

1. Toegang tot opslagplekke/depots en verspreidingsentra in die VK, waar party van die 'lesers' geplaas sou word.
2. Gehaltebeheer-personeel wat die opnemers gaan haal het by 'n verspreidingsentrum in die VK en dit daarna in heropgeboude pallette, wat voorsien was van dieselfde produk as wat na die winkel versend is, geplaas het. Hulle het die vraag na die winkel gevolg waar hulle seker gemaak het dat die opnemers op 'n plek gehou is wat laer as die verkoopspunt was. (Dit is omdat die opnemers dalk kon voorkom as 'n 'bomdreigement'). Hulle was voorsien van hand-radio-ontvangers wat data vasgelê het vanuit die winkel en dit dan gelaai het na 'n BT9-Tech bediener.

Slotsom:

Vanuit die data wat tot dusver ontleed is, blyk dit dat die bepaalde sitrus koue ketting doeltreffend is, aangesien die temperature wat opgeneem is meestal binne protokol was. Daar was ook geen probleme in terme van gehalte by enige van die sagte sitrus wat verskeep is nie.

5.5 PROGRAMME: CULTIVAR EVALUATION

Programme coordinator (Acting): Tim G. Grout (CRI)

5.5.1 Programme 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.

Programopsomming

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 bewes 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 hot humid inland areas (Onderberg)

Project 75 A by J. Joubert (CRI)

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 steeds 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 vruggroote produseer. Alpha of Midnight, aangevul deur Moosrivier Late 1 en Bend 8A2 (eksperimentele seleksies) verteenwoordig die middel van die Valencia seisoen vir hierdie area, gevolg deur McClean SL wat uitstekend vaar met goeie produksie, vruggroote en saadlose vrugte. Die later seleksies wat kan bydra tot die keuse om die seisoen te verleng, kan bestaan uit Henrietta (min saad) en Louisa (saadloos), opgevolg deur Lavalley 2, wat groot vrugte produseer, goeie oes verseker met belowende interne kwaliteit.

Henrietta, Louisa, Skilderkrans, Moosrivier Late 1 en Bend 8A2 is steeds eksperimentele/semi-kommersiele seleksies wat goed presteer. Hierdie seleksies kan in die toekoms ingesluit word soos meer en beter inligting beskikbaar word.

Summary

Recommendations for selections that performed well in this season, according to optimal maturity from early to late in this hot, humid production area, are as follows. Start the season 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, with the addition of Moosrivier Late 1 and Bend 8A2 (experimental selections) represent the middle of the Valencia season for this area, followed by McClean SL with good yield production, large size and seedless fruit. The later selections Henrietta (low seeded) and Louisa (seedless) can broaden the list of choices, followed by Lavalley 2, producing large fruit, excellent yield and promising internal quality.

Henrietta, Louisa, Skilderkrans and Bend 8A2 remain experimental/semi-commercial selections that performed well. These selections should be included in future plantings when more and better information becomes available.

Objective

To find suitable Valencia selections with superior characteristics for the hot inland citrus production areas.

Materials and methods

Field evaluations and laboratory analyses were conducted on Alpha, Bend 8A 1&2, Benny 2, Henrietta, Lavalle 2, Louisa, McClean SL, Midnight 1, Moosrivier Late 1&2, Nouvelle La Cotte, Ruby, Skilderkrans and Turkey (control) at Esselen Nursery, Malelane, Mpumalanga.

Table 5.5.2.1. Internal fruit quality minimum export requirements for Valencia types.

Variety	Juice %	Brix	Min Acid	Max Acids	Ratio	Colour
Valencia EU	48	8.5	0.6	1.8%	7.5:1	Colour plate 3 of set no. 34
Midnight	52	9.5	0.85	1.8%	7.5:1	Colour plate 3 of set no. 34
*Turkey	50	10.0	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34

*Interim internal fruit quality standards.

Table 5.5.2.2. List of Valencia selections evaluated at Esselen Nursery (Malelane) during 2012.

Selection	Rootstock	Year Planted	No. of trees
Alpha	CC	1996	1
Bend 8A1	SC	2003	1
Bend 8A2	SC	2003	1
Benny 2	C35	2003	1
Henrietta	MxT	2006	1
Lavalle	SC	2001 (Topwork)	1
Louisa	MxT	2006	1
McClean SL	C35	2001	1
Midnight	C35	2001	1
Moosrivier Late 1	SC	2003	1
Nouvelle La Cotte	MxT	2006	1
Ruby	SC	2003	1
Skilderkrans	MxT	2006	1
Turkey	C35	1998	1

Results and discussion

This project is ongoing – all evaluations and tasks have been completed to date. Trees were visually evaluated at Esselen Nursery (Malelane) during the 2012 season.

Alpha

Alpha produced a very good crop on the trees and the yield was estimated at 100 kg per tree. The trees' condition was very good on Carrizo rootstock, a well known rootstock with good replant qualities. With the second evaluation on 11 July 2012, the external colour was between T1 and T3. The fruit shape was fairly round with smooth skin texture and thin rind thickness, no navel end was visible and no thorns were on the bearing branches. Internally a deep yellow colour developed, the fibre strength was soft and the fruit peeled easily. There were split fruit on the trees evaluated. Medium to large fruit size and good internal quality were prominent for this selection, with Brix above 12 and juice above 60 (Table 5.5.2.3), complying with minimum export standards. Based on the internal quality results in Table 5.5.2.3 estimated maturity was end June to end July.

*Bend 8A1&2

Bend 8A1 produced 60 kg per tree, compared to 8A2 with 50 kg per tree. The fruit size on Bend 8A1 was bigger (between count 56 and 72), and Bend 8A2 peaked between count 88 and 58. Bend 8A2 produced fruit with a smoother rind and rounder shape, as well as better external colour (T1). The seed count per fruit varied from low (2.9 seeds per fruit) in 8A2 to medium in 8A1 (7.5 seeds per fruit). Bend 8A2 seems to be earlier maturing compared to 8A1, although the Brix:acid ratio on 8A1 was 8.3 and the Brix level 11.1 when the second evaluation was conducted. Both selections complied with the export minima. Based on the internal quality results in Table 5.5.2.3 estimated maturity was end June to mid-July.

Benny 2

The Benny 2 produced the optimal fruit size for Valencias peaking between count 72 and 56. The tree size on C35 rootstock is more compact and produces a good crop when taking tree volume into consideration (tree one third smaller in size). The fibre strength (rag) remains soft compared to the other Valencia selections. Benny 2 matures after Turkey and before Midnight or Delta so fits in well in the harvesting and packing programme. The external colour was delayed this season, remaining at T4 with acid levels at 1.09% after completing the second evaluation. Based on the internal quality results in Table 5.5.2.3 maturity was estimated as middle to end of June.

*Henrietta

Henrietta had an improved crop on the tree this season, bearing excellent yields of up to 80 kg per tree and tree condition was very good. Fruit size varied from medium to large (count 88 – 64), fruit shape was round, rind texture smooth and small thorns were visible on the bearing branches. Rind thickness was fairly thin; fruit peeled easily and contained medium rind oil content. Seed count averaged 5 seeds per fruit. Internal quality indicated that Henrietta matures late in the Valencia season, with an acid content of 1.5% and external colour from T2 to T4. Based on the internal quality results in Table 5.5.2.3 maturity was estimated as mid July to end August.

Lavalle 2

The higher acid level (1.5%) tested with the second evaluation, indicated that this selection was late maturing internally, although the external colour developed to T3/4. Yield production was good, measuring up to 60 kg per tree. Tree condition was good in combination with SC, an excellent replant rootstock developing a large tree size with good internal quality. There were no seeds present in the fruit evaluated this season. Internal colour of the fruit was yellow; the fruit developed a fairly thin rind with medium rind texture and peeled easily. The internal quality complied with the export standards, producing Brix levels of 12 and juice of 58%. Fruit size varied from count 72 to 56, excellent for Valencia production. Based on the internal quality results in Table 5.5.2.3 maturity was estimated as mid to end August.

*Louisa

This year the seed count per fruit was 1.2, in comparison with the trial site at Group 91 where the fruit was completely seedless. Future evaluations will determine the seediness of the selection. The yield production on the trees was 30 kg, not performing well at this stage compared to the other selection of the same age. Fruit shape was round, rind texture medium to fairly smooth and the rind was medium to fairly thick. The fruit peeled easily and the internal colour was yellow. Acid content was higher (1.54%) if compared to Lavalle 2, a late Valencia selection. Based on the internal quality results in Table 5.5.2.3 maturity was estimated as mid to end August.

McClellan SL

The yield on the tree this season was 60 kg, producing medium to large fruit (count 88-56), with orange internal colour and very good flavour. This year McClellan SL produced 0.6 seeds in comparison to 0.1 seeds per fruit the previous seasons. The standard McClellan will be included in future trials as a control to compare the SL selection's performance. The internal quality was good and complied with the export standards. McClellan SL produced fairly round fruit with soft fibre strength that peels easily, containing low rind oil levels. Many totally seedless selections have fruit set problems and bear less fruit, but this does not appear to be the case with this cultivar. Based on the internal quality results in Table 5.5.2.3 maturity was end July.

Midnight

C35 in combination with Midnight developed a medium sized tree, one third smaller compared to the tree size of Swingle. The tree produced 60 kg, with medium to large (count 88-56) fruit size. Fruit shape is fairly round, rind texture medium coarse, fibre strength fairly soft and fruit peels easily. Internally the flavour varied from good to very good, with juice levels around 60% and Brix 11. The acid level was higher this season, but complied with the export requirements (Max 1.8 to min 0.85). Based on the internal quality results in Table 5.5.2.3 estimated maturity was end of June to middle of July.

*Moosrivier Late 1&2

Moos Late 1 produced 100 kg yield per tree and Late 2 only 10 kg yield per tree. Moos Late 1 had promising performance, developed smooth round fruit with deep yellow internal colour, good flavour, peeled easily and fairly soft rag. Fruit quality on Moos Late 1 (Brix 11, juice 61%, acid 1.4%) is very good compared to Late 2 (Brix 10, juice 50%, acid 1.2%). Moos Late 2 develops a pale yellow/lime green internal colour, average flavour, medium coarse rind and large to extra large (count 72-40) fruit. Moos Late 2 is completely seedless compared to Late 1 with 3.8 seeds per fruit, probably explaining the excellent crop on the Late 1 trees. Based on the internal quality results in Table 5.5.2.3 estimated maturity was from the end of July to the middle of August.

*Nouvelle La Cotte

Yield production on the tree was 40 kg, resulting in small to medium (count 105-64) fruit size, compared to the previous season. Fruit shape was round, rind smooth and fairly thin, peeled easily and fair flavour. The acid (1.5%) level remained fairly high when the external colour developed to T1. There were small thorns visible on the bearing branches of the tree. Based on the internal quality results in Table 5.5.2.3, estimated maturity was the end of June to the middle of August.

Ruby

Ruby peaked between count 125 and 72, producing small to medium sized fruit on the tree. With the second evaluation the internal quality complied with the export standards, except for an acid content of 1.65% (recommendation 1.4%) and external colour was between T2 and T3. Yield production on the tree was very good, 100 kg per tree, explaining the smaller fruit size. Fruit characteristics consist of good flavour, round fruit shape, smooth skin texture, fairly thin rind, easy peeling and a deep red internal colour. For this season the Esselen trial site compared excellently to the Group 91 trial site with similar results and conclusions. There were some split fruit, but no creasing or sunburn visible. Based on the internal quality results in Table 5.5.2.3, estimated maturity was mid July to mid August.

*Skilderkrans

Skilderkrans produced small to large (count 88-40) fruit size, due to the very light crop on the trees. The internal quality was good, except for a high acid content of 1.54%, above the export maximum of 1.4%. External colour varied between T1 and T2 with the second evaluation. The fruit peeled fairly easy, rind thickness was thin, rag was medium tough (raggy/strong), fruit shape was round and the rind texture medium rough. 1.3 seeds per fruit were counted, considerably higher compared to the previous season. Based on the internal quality results in Table 5.5.2.3 estimated maturity was the middle of July to the beginning of August.

Turkey

Turkey produced 80 kg per tree this season: very good for a medium sized tree. Fruit size peaked between count 105 and 56, small to large size for this season. The smaller fruit size on the trees was caused by the heavier crop produced. Fruit characteristics for Turkey were round fruit shape, smooth rind texture, very good flavour, soft rag, fairly thin rind, fruit peeling easily and seed count per fruit of 8.5 on average. The internal colour was light yellow, and externally the fruit remained yellow up to completely overmatured fruit. It should be borne in mind that this selection is not a true Valencia and actually has the qualities of a mid-season orange, for instance the exceptionally soft rag of the fruit, and the soft rind that can result in rind problems if managed incorrectly. The Turkey should not be harvested over more than four weeks as extending the harvesting season can lead to rind disorders developing. Based on the internal quality results in Table 5.5.2.3, estimated maturity was the end of May to mid-June.

Conclusions

All the selections evaluated complied with the export standards, with the exception of the late maturing Henrietta, Lavalley 2, Louisa, Nouvelle La Cotte, Ruby and Skilderkrans where the acid levels were above 1.4%. These acid levels will decrease towards the end of the season, indicating extended shelf-life of the selections. Where the Brix: acid ratio was below 7:1 it was often associated with later maturing selections having higher acid levels. When the acid levels decrease, the ratio will increase. The average seed count for this season increased due to possible cross pollination in the mixed trial block caused by strong cross pollinating selections (soft citrus) and a possibility of more active bees. Better yield production resulted in smaller fruit size on the trees, between count 125 and increasing up to count 40 on selections with lighter yields.

*This was the second evaluation of Bend 8A1&2, Henrietta, Louisa, Moosrivier 1&2, Nouvelle La Cotte and Skilderkrans at this trial site, so information is limited and future evaluations will improve recommendations on these varieties.

Table 5.5.2.3. Internal fruit quality data for Valencia and late orange selections at Esselen Nursery (Malelane) during the 2012 season.

Selection	Root-stock	Date harvested	Size mm	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Alpha	CC	07/06/2012	75-85	72-56	58.0	11.0	1.41	7.8	1.1	T3-4
Alpha	CC	11/07/2012	68-78	88-64	61.8	12.1	1.29	9.4	0.4	T1/2/3
Bend 8A1	SC	11/07/2012	74-85	72-56	55.3	11.1	1.34	8.3	7.5	T1-2
Bend 8A2	SC	11/07/2012	71-85	88-56	60.2	9.9	1.27	7.8	2.9	T1

Benny 2	C35	16/05/2012	77-85	72-56	55.9	10.1	1.29	7.8	0.4	T5-6
Benny 2	C35	07/06/2012	74-79	72-64	55.6	11.1	1.09	10.2	6.5	T4
Henrietta	MxT	11/07/2012	68-79	88-64	57.7	10.4	1.51	6.9	7.2	T2/3/4
Lavalle	SC	11/07/2012	73-85	72-56	58.0	11.9	1.52	7.8	0.0	T3-4
Louisa	MxT	11/07/2012	64-79	125-64	57.3	11.8	1.54	7.7	1.3	T1
McClean SL	C35	07/06/2012	74-85	72-56	56.1	11.0	1.29	8.5	0.6	T4-5
McClean SL	SC	11/07/2012	72-84	88-56	55.8	11.8	1.48	8.0	0.0	T1-4
Midnight	C35	07/06/2012	70-85	88-56	57.6	11.0	1.39	7.9	1.3	T4-5
Midnight	C35	11/07/2012	71-83	88-56	59.1	10.9	1.46	7.5	1.1	T1-2
Moosrivier Late 1	SC	11/07/2012	67-79	105-64	60.8	10.6	1.41	7.5	3.8	T3-4
Moosrivier Late 2	SC	11/07/2012	74-93	72-40	49.6	9.9	1.20	8.3	0.0	T4/5/6
Nouvelle La Cotte	MxT	11/07/2012	65-80	105-64	59.5	9.8	1.51	6.5	1.4	T1
Ruby	SC	11/07/2012	64-75	125-72	58.5	11.4	1.65	6.9	8.2	T2-3
Skilderkrans	MxT	11/07/2012	72-95	88-40	60.5	10.6	1.54	6.9	1.3	T1-2
Turkey	C35	16/05/2012	75-83	72-56	50.7	11.5	1.09	10.6	8.8	T5-6
Turkey	C35	07/06/2012	71-80	88-64	56.8	12.1	1.01	12.0	8.5	T3-4
Turkey	CC	07/06/2012	73-79	72-64	52.2	11.7	1.16	10.1	8.4	T3
Turkey	C35	11/07/2012	65-70	105-88	56.2	13.2	1.15	11.5	5.6	T1-2

5.5.3 PROGRESS REPORT: Evaluation of Valencia selections in the hot dry inland areas (Letsitele) Project 75 B by J. Joubert (CRI)

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. Optimum plukvenster is binne die eerste vier weke van piek rytheid. Benny 2 volg na Turkey met goeie produksie en medium tot groot vuggroote. Midnight 1 vul die middel van die Valencia seisoen met goeie interne kwaliteit vrugte, groot vruggroote, gladde skille en lae saadtellings per vrug. Lavalle 2 is tot op datum die laatste Valencia seleksie wat semi-kommersieel aangeplant word, met uitstekende vruggroote 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 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. Ruby sal meer vir die lokale mark of sap aanleg aangewend kan word, a.g.v. die uitstekende produksie op die bome, kleiner vruggroote en saad inhoud. Laat in die seisoen kan aangevul word met Moosrivier Late 1, soos meer inligting beskikbaar word uit verdere evaluasies.

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. 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. Lavalle 2 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 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. Ruby will be more suitable for the local or juice market, due to excellent yield production, small fruit size and seed content. Late in the season you could possibly add Moosrivier Late 1 to the options, when more information becomes available from future evaluations.

Objective

To find suitable Valencia selections with superior characteristics for the hot inland citrus production areas.

Materials and methods

Field evaluations and laboratory analyses were conducted on Benny 1&2, Bend 8A 1&2, Henrietta, Jassie, Lavalle 1, Louisa, Midnight 1, Moosrivier Late 1 and 2, Ruby, Skilderkrans and Turkey at Group 91, Letsitele.

Table 5.5.3.1. List of Valencia selections evaluated at Group 91 (Letsitele) during 2012.

Selection	Rootstock	Tree Age	No. of trees
Bend 8A 1	CC/SC	2005	10/10
Bend 8A 2	CC/SC	2005	10/10
Benny 1	CC/SC	2005	10/10
Benny 2	CC/SC	2005	10/10
Henrietta	CC	2006	Semi-Com
Jassie	CC/SC	2005	10/7
Lavalle 1	CC	2005	100
Louisa (Letaba Oranje)	CC	2007	Semi-Com
Midnight 1	CC/SC	2005	10/10
Moosrivier Late 1	CC/SC	2005	10/10
Moosrivier Late 2	CC/SC	2005	10/10
Ruby	CC/SC	2005	10/10
Skilderkrans	CC/SC	2005	10/6
Turkey	C35	2005	2
Turkey	CC	2005	4
Turkey	SC	2005	10

Results and discussion

Bend 8A1

Bend 8A1 on Carrizo produced 60 kg per tree, compared to Swingle with 90 kg per tree and the tree condition on both combinations was very good this season. The fruit size on Carrizo and Swingle varied from small to large (count 88-48), with a tendency of smaller fruit size on Carrizo. Internally the juice levels were on the lower side, but still above 48% for export. Acids remained fairly high after the second evaluation was completed, and on Swingle tested as high as 1.7%. Fruit characteristics of Bend 8A1 concluded to fairly round fruit shape, fairly smooth rind, fruit peels easily, the fibre strength is fairly soft and the internal colour light yellow. The seed count per fruit was higher on Bend 8A1 (4 seeds per fruit) when compared to 8A2 (1.3 seeds per fruit). Maturity seems to be mid July and beginning of August (Table 5.5.3.2).

Bend 8A2

Bend 8A2 performed well and internally produced a higher juice content (57%) than 8A1, lower acids (1.5%) and better Brix:acid ratios (7.6:1). Trees on Carrizo averaged 100 kg per tree with medium fruit size (count 72-56), and on Swingle 70 kg per tree with medium to large fruit size (count 88-48). Fruit shape was round, fairly smooth rind texture, and deep yellow internal colour; it peeled easily with fairly thin rind. The seed count per fruit was low, between 1.0 and 1.7, 3 seeds per fruit on average lower compared to 8A1. Fruit maturity was estimated at mid to end of July (Table 5.5.3.2).

Benny 1 and 2

Benny 1 and 2 produced an average yield of 100 kg per tree on Carrizo and Swingle, except for Benny 1 on Swingle with 120 kg. Fruit size on both selections peaked between count 88 and 56/48. Benny 1 internally produced higher juice levels (avg. 55) and seed counts (avg. 4 seeds per fruit) compared to Benny 2, where Benny 2 produced higher Brix (avg. 11.7), acid (1.4%) and Brix:acid ratio (8.5:1). External colour on both selections by the time of harvest varied between T1 and T2. The internal colour of the fruit was deep yellow, fruit shape round, rind texture fairly smooth, high rag content and medium rind thickness. Based on ratios, Benny 2 matures end-June to early July while Benny 1 only matures mid-June to mid-July.

Henrietta

Henrietta bore 100 kg fruit per tree this season where the selection was planted on Carrizo and the trees on average were 2.8 m high, and 2.85 m wide. Fruit size peaked at count 88 to 64, good size for Valencia

production and export. The fruit shape was slightly oblong with a smooth rind texture, deep yellow internal colour and very good flavour. Fibre strength was medium with a medium thick rind, and the fruit peeled easily with fairly low rind oil. The internal quality was good with high Brix (12.3) and acid (1.68%) levels by the second evaluation, indicating the shelf life potential of the selection. There were 4.2 seeds per fruit counted on average. The external colour of the fruit developed into a deep orange, very favourable for the export markets. Maturity was end of July to beginning of August.

Jassie

Fruit size peaked between count 105 and 56 with Jassie on Swingle producing the smaller fruit size due to bigger tree size and growth. Production on Carrizo was 100 kg per tree and on Swingle 80-90 kg. Swingle produced a higher Brix (12.2) and acid (1.68%) level in combination with Jassie, where Carrizo produced a higher juice (54%) level and seed count (5.1 seeds per fruit). Fruit shape was round with a smooth rind texture, internal colour was light yellow, juice flavour varied between very good on Carrizo to excellent on Swingle. Fibre strength was fairly soft, rind thickness was medium to smooth and the fruit peeled easily. Maturity was middle to end of July in this area.

Lavalle 1

This season Lavalle produced 3 seeds per fruit compared to last year's 0.5. The yield on the trees averaged 60 kg, very good when you consider the tree size of 3 metres in height. The internal quality complied with export requirements, except for the acid level being on the higher side (1.54%), but keep in mind that Lavalle is a late Valencia selection with good shelf life and the optimal harvest time will be in August. The navel end on some fruit seems to develop a button. There was split fruit on some of the trees evaluated. From the ratio on this date it is apparent that Lavalle 1 maturity is middle to end of August.

Louisa

The fruit set was lighter compared to Henrietta, trees were bearing 50 kg per tree. Fruit size was optimal for Valencia production between count 72 and 56, better than the previous season's large fruit size (count 56-40). Internal quality complied with the requirements, although the juice content of 51% was low and the acid level of 1.33% indicated that Louisa remained the latest Valencia selection available currently. Louisa remained seedless; the internal colour of the fruit was light yellow, fruit shape round, medium smooth rind texture and fairly thick rind. There were small thorns on the bearing branches and the tree height on Carrizo measured 2.75 m. Acid levels and ratios indicate that this cultivar matures mid to end of July.

Midnight 1

Midnight 1 on Carrizo bore a yield of 50 kg per tree and Swingle 80 kg per tree, considering that the Swingle tree was bigger than the Carrizo. The fruit size on both rootstocks varied between count 88 and 56, juice content around 55%, Brix levels around 11.5 and acids at 1.2%. Swingle outperformed Carrizo with the better Brix:acid ratio of 11.3:1. Seed counts on Carrizo were higher (3 seeds per fruit), compared to Swingle with 0.1 seeds per fruit. Fruit shape tends to be slightly oblong, rind texture was fairly smooth; fruit was raggy with a medium rind thickness and peeled moderately. Midnight 1 developed very good internal quality early in the season with ratios indicating maturity to be mid-July on Carrizo and 1 to 2 weeks earlier on Swingle.

Moosrivier Late 1 and 2

Crop production for Moos Late 1 on Carrizo was 70 kg and on Swingle was 60 kg per tree, compared to Moos Late 2 on Carrizo being 30 kg and on Swingle 70 kg per tree. Moos Late 1 on both rootstocks produced small to medium fruitsize (count 125-64), and Moos Late 2 on both rootstocks medium to large fruit size (count 88-56). Moos Late 1 performed well, developing internal qualities that meet export standards, except for high acids (1.8 -1.9%) indicating a late maturing Valencia selection. The seed count per fruit varied from 2 up to 3.6. Moos Late 2 developed lower Brix and acid levels and was completely seedless. When internal quality was taken into consideration, Moosrivier Late 1 was the later maturing selection, estimated maturity end-July to middle August. Moosrivier Late 2 matured mid to end-July, with delayed external colour.

Ruby

Ruby performed similarly on both rootstocks with Brix content of 11.5, juice levels of 55% and acids around 1.5%. External colour on the fruit was between T2 and T3. Fruit size was on the smaller size (count 105-64), due to the excellent yield on the trees (70 kg). Seeds per fruit varied from 3.0 up to 4.8. Fruit shape was round with fairly smooth rind texture, medium strong fibre internally, medium rind thickness and fruit peeled easily. Internal colour was dark red and well developed. Ruby's estimated maturity time will be mid-July to mid-August.

Skilderkrans

This selection at Group 91, as well as at Esselen nursery performed poorly this season. The yield on both rootstocks averaged between 20 and 30 kg per tree, with an average tree size of 3 metres high. Fruit size varied from medium to large (count 88-56); with this light crop a bigger fruit size was expected. Internally the Brix content was good (12.7) and the acid level of 1.9% indicated a late maturing Valencia selection. Juice level was slightly above the minimum required export figures. External colour was delayed on Swingle between T1 and T5 beginning July. The fruit developed a smooth rind, fibre strength was fairly soft and the fruit shape was round. Ratios indicate maturity to be early August for Carrizo and mid-August for Swingle.

Turkey

Turkey was planted on three rootstocks, Carrizo, Swingle and C35. The Carrizo combination outperformed the other two with ideal fruit size distribution (count 72-56), highest Brix content (11.4), best Brix:acid ratio of 11.2:1 and lowest seed count per tree of 2.5 seeds. The external colour was similar on all three rootstocks between T2 and T3 in the middle of June. Yield production and tree size showed Carrizo to be the best rootstock combination for Turkey. Both Swingle and C35 produced 20 kg per tree, but Carrizo produced 80 kg per tree. Tree height measured 2.16 metres on C35, 2.76 metres on Swingle and 3.27 metres on Carrizo. Based on the ratios, maturity was end-May for C35 and mid-June for Carrizo and Swingle.

Conclusions

Bend 8A2 produced fruit with higher Brix levels, lower acid levels, lower seed counts per fruit and better Brix:acid ratios than Bend 8A1. Bend 8A1 (90 kg per tree) produced a higher yield on Swingle compared to 8A2 (70 kg per tree) and the opposite scenario on Carrizo, with Bend 8A1 60 kg per tree and 8A2 100 kg per tree. Benny 1 and 2 produced similar quality fruit this season, with Benny 1 outperforming 2 with regards to better yield production on Swingle rootstock, 120 kg per tree.

Henrietta produced a better fruit size and peaked between count 88 and 64. The internal quality of the fruit was excellent, developing Brix as high as 12.3. Louisa was completely seedless, developed the ideal fruit size (count 72-56) for Valencias, setting a lighter crop on the trees (50 kg per tree), due to the more compact tree development (2.7 metres).

Jassie produced an excellent internal quality on Swingle, good fruit size (count 105-56) for Valencias and very good yield (80-100 kg per tree). Skilderkrans performed poorly this season compared to the previous season, bearing a very light crop with average internal quality, and less seeds (0.2 per fruit).

Midnight 1 developed good yields of good fruit size with very good internal quality. Normally, Carrizo produces lower acids and develops better external colour compared to Swingle, but with the Midnight 1 selection the opposite seems to be true. Future evaluations will confirm the conclusion, but this was the second year with these results indicating that the Swingle combination matured before Carrizo.

Moosrivier Late 1 is later maturing when compared to Moosrivier Late 2, with high juice and acid percentages, but the external fruit colour on Moos Late 2 was delayed. Moosrivier Late 2 was completely seedless.

Lavalle 1 is also late maturing with very good Brix levels and optimal fruit size. Ruby produced very good quality fruit and excellent yields on the trees.

Turkey performed best in combination with Carrizo when Brix:acid ratio and yield production were considered.

Table 5.5.3.2. Internal fruit quality data for Valencia orange selections at Groep 91 (Letsitele) during the 2012 season.

Selection	Root-stock	Date harvested	Size mm	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Bend 8A 1	CC	12/06/2012	73-84	72-56	53.5	11.5	1.61	7.1	4.4	T2/3/4/5
Bend 8A 1	CC	02/07/2012	71-86	88-48	51.5	11.8	1.47	8.0	4.9	T1-2
Bend 8A 1	SC	12/06/2012	72-88	88-48	50.9	11.4	1.64	7.0	2.1	T3-4
Bend 8A 1	SC	02/07/2012	72-80	88-64	53.2	12.3	1.70	7.2	4.3	T1-2
Bend 8A 2	CC	12/06/2012	74-86	72-48	55.1	11.2	1.46	7.7	1.4	T3/4/5
Bend 8A 2	CC	02/07/2012	74-83	72-56	56.0	11.8	1.43	8.3	1.7	T1-2
Bend 8A 2	SC	12/06/2012	73-87	72-48	56.0	10.6	1.58	6.7	1.2	T3/4/5

Bend 8A 2	SC	02/07/2012	72-79	88-64	59.0	11.4	1.51	7.5	1.0	T1-2
Benny 1	CC	12/06/2012	70-80	88-64	55.0	11.2	1.28	8.8	3.4	T2-3
Benny 1	CC	02/07/2012	74-85	72-56	56.9	11.6	1.22	9.5	3.8	T1-2
Benny 1	SC	12/06/2012	73-88	72-48	54.1	11.2	1.48	7.6	4.3	T3-4
Benny 1	SC	02/07/2012	71-85	88-56	54.7	11.6	1.38	8.4	4.2	T1-2
Benny 2	CC	12/06/2012	72-82	88-56	55.8	11.4	1.43	8.0	3.8	T2-3
Benny 2	CC	02/07/2012	72-78	88-64	54.1	12.0	1.20	10.0	3.2	T1-2
Benny 2	SC	12/06/2012	74-85	72-56	54.1	11.6	1.51	7.7	2.3	T2-3
Benny 2	SC	02/07/2012	73-83	72-56	54.3	11.9	1.44	8.3	1.9	T1-2
Henrietta	CC	02/07/2012	72-78	88-64	52.3	12.3	1.68	7.3	4.2	T1-3
Jassie	CC	12/06/2012	67-80	105-64	53.8	11.2	1.45	7.7	5.4	T2-3
Jassie	CC	02/07/2012	70-85	88-56	54.0	11.6	1.24	9.4	4.8	T1-2
Jassie	SC	12/06/2012	67-75	105-72	50.7	12.0	1.82	6.6	4.0	T3-4
Jassie	SC	02/07/2012	74-80	72-64	50.8	12.4	1.54	8.1	3.7	T1-2
Lavalle	CC	02/07/2012	72-85	88-56	58.3	11.2	1.51	7.4	3.0	T1-4
Louisa	CC	02/07/2012	76-83	72-56	50.8	11.7	1.33	8.8	0.0	T1-4
Midnight 1	CC	12/06/2012	73-80	72-64	53.9	11.4	1.24	9.2	3.0	T2/3/4
Midnight 1	CC	02/07/2012	70-79	88-64	55.2	11.5	1.21	9.5	0.9	T3-4
Midnight 1	SC	12/06/2012	70-79	88-64	54.2	11.0	1.21	9.1	0.1	T3-4
Midnight 1	SC	02/07/2012	72-84	88-56	54.6	11.6	1.03	11.3	0.0	T1-2
Moos Late 1	CC	12/06/2012	66-77	105-72	52.2	11.6	2.12	5.5	2.0	T3/4/5
Moos Late 1	CC	02/07/2012	69-78	88-64	55.3	12.3	1.79	6.9	3.4	T1/2/3
Moos Late 1	SC	12/06/2012	64-73	125-72	52.0	11.3	1.77	6.4	3.6	T3/4/5
Moos Late 1	SC	02/07/2012	62-81	125-64	53.7	12.6	1.93	6.5	3.5	T1/2/3
Moos Late 2	CC	02/07/2012	70-82	88-56	49.7	12.3	1.60	7.7	0.0	T4-5
Moos Late 2	SC	02/07/2012	73-83	72-56	51.9	11.7	1.40	8.4	0.0	T5-6
Ruby	CC	12/06/2012	67-75	105-72	53.0	10.9	1.42	7.7	4.8	T2-3
Ruby	CC	02/07/2012	69-82	88-56	55.9	11.6	1.16	10.0	3.0	T2
Ruby	SC	12/06/2012	68-76	88-72	53.2	11.2	1.61	7.0	4.8	T2-3
Ruby	SC	02/07/2012	71-80	88-64	55.7	11.5	1.36	8.5	4.5	T2
Skilderkrans	CC	02/07/2012	70-84	88-56	51.8	11.7	1.46	8.0	0.0	T1-3
Skilderkrans	SC	02/07/2012	73-85	72-56	49.8	12.6	1.92	6.6	0.2	T1-5
Turkey	CC	12/06/2012	75-83	72-56	50.9	11.4	1.02	11.2	2.5	T2-3
Turkey	C35	12/06/2012	80-92	64-40	51.7	10.0	0.93	10.8	5.3	T2-3
Turkey	SC	12/06/2012	77-88	72-48	52.3	10.2	1.11	9.2	4.4	T2-3

5.5.4 PROGRESS REPORT: Evaluation of Valencia selections in the hot inland areas (Swaziland) Project 740A by J. Joubert (CRI)

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 Portsgate 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. Turkey op C35 in die koeler produksie areas (Ngonini) ontwikkel te stadig wat 'n te klein boomgrootte tot gevolg het, in vergelyking met die warm areas, maar vir die Tambuti area was die bome gesond en in 'n goeie kondisie, met goeie produksie. Hoër digtheid aanplantings in kombinasie met C35 sal soortgelyke opbrengste per hektaar verseker, in vergelyke met die grootter bome op Carrizo. Die kleiner boomgrootte het verskeie voordele, bv. makliker en vinniger oes praktyke en laer bespruitings volumes met beter boom bedekking. 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, in kombinasie met Carrizo, 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.

Summary

This production area is classified as hot and humid and the establishment of Valencia and grapefruit selections is very favourable. Turkey, followed by Portsgate 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 Black spot problems, so look out for this on young trees and adapt spray programmes accordingly. Turkey on C35 in the cooler production areas (Ngonini) developed too slowly and the tree size was smaller compared to the hot areas, but for the Tambuti area the trees were healthy and in good condition, bearing a good crop. Planting higher density orchards in combination with C35 will result in similar yield production per hectare, compared to the bigger tree size of Carrizo. The smaller trees have numerous advantages, for example easier harvesting practices and lower spray volumes with better tree coverage. 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, in combination with Carrizo rootstock, remaining 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.

Objective

To find suitable Valencia selections with superior characteristics for the hot inland citrus production areas.

Materials and methods

Field evaluations and laboratory analyses were conducted on Alpha, Delta, Jassie, McClean SL, Portsgate and Turkey at Tambuti Estate, Swaziland.

Table 5.5.4.1. Internal fruit quality data was compared with the minimum export requirements for Valencia types.

Variety	% Juice	Brix ^o	Min % Acid	Max % Acids	Ratio	Colour
Valencia	48	9.0	0.6	1.6%	8.0:1	Colour plate 3 of set no. 34
Midknight	52	9.5	0.85	1.8%	7.5:1	Colour plate 3 of set no. 34
*Turkey	50	10.0	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34
Delta SL	52	10.5	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34

*Turkey – interim internal fruit quality standards

Table 5.5.4.2. List of Valencia selections evaluated at Tambuti Estate (Swaziland) during 2012.

Selection	Rootstock	Tree Age	No. of trees
Alpha	CC	2003	5
Delta	CC	2003	5
Jassie	C35/CC	2005	10/10
McClean SL	CC	2003	5
Portsgate	CC	2003	5
Turkey	C35/CC	2005	10/10

Results and discussion

These comments are based on the second evaluation date (29/6/2012). Maturity/estimated maturity are based on a Brix acid ratio of 10.0.

Alpha

Alpha produced small to medium sized fruit (count 88-64), decreased in fruit size by one count, due to an average yield production of between 150 and 200 kg/tree. There were no seeds present in the fruit tested this season, as with the previous season. Internally the fruit quality complied with the export requirements, developing the highest juice content for this Valencia trial (59%). Tree condition was good, considering that the trees were 10 years old this season. The internal colour was deep yellow, fibre strength fairly soft, thin rinds, fruit peeled easily with medium rind oil. Estimated maturity date was beginning to end of July.

Delta (control)

Delta produced between 100 and 150 kg per tree, and the tree condition was good to very good. There was no sunburn, splitting or creasing noticeable on the fruit. The fruit size on the trees was small to medium, between counts 125 and 64. Delta tends to produce small fruit but the advantage of this selection is the fact that it is completely seedless. The external colour range was from T3 to T5. Internal quality was good with a Brix: acid ratio of 9.7, the ratio will increase later in the season when the Brix improves and the acids decrease. Peak maturity is estimated to be end July.

Jassie

Jassie was planted on Carrizo and C35 at this trial site to determine the impact of the semi-dwarfing C35 rootstock on production, internal quality, scion/rootstock compatibility and fruit size. The yield production on C35 peaked between 100 and 120 kg per tree, and on Carrizo at 80 kg per tree. The external colour on C35 (T3-5) was delayed compared to Carrizo (T2-4). The average seed count of the fruit varies from 4.8 to 5.6 seeds per fruit. The internal quality related to juice, acid levels and seed count per fruit evaluated, was similar on both rootstocks. The Brix content for Jassie on Carrizo was higher (average 10.9) and the tree size on C35 (3.5 m) was becoming smaller than Carrizo (4 to 4.5 m). Once mature (10 years plus), tree size will be approximately one third smaller in height. Maturity is estimated to be mid July.

McClellan SL

McClellan SL remained completely seedless at this trial site and produced 250 kg per tree. This scenario is very favourable for this Valencia selection, due to the fact that the seedless varieties generally do not bear good crops. The fruit size this season was smaller, and peaked from count 88 to count 72. Tree condition was excellent, and the internal fruit colour developed into a deep orange. The Brix: acid ratio peaked above 12, resulting in an excellent internal quality (Juice 58%, Brix 11.1 and acid 0.9%). There was no sunburn, splitting or creasing problems with the fruit, the fruit peels moderately with high rind oil content. Maturity is estimated to be the middle of July.

Portsgate

Portsgate produced 200 kg per tree this season and peaked from count 88 to 64 (medium to large). The tree condition in combination with Carrizo was very good, developing a large tree size. Fruit shape was round with smooth skin texture and no thorns visible on the bearing branches of the trees. Fruit size peaked between count 88 and 64, producing medium sized fruit on the trees. The internal colour developed to a deep yellow, with good internal quality and Brix: acid ratios above 10.3. The fruit were completely seedless. Maturity is estimated to be mid to end of June.

Turkey

Turkey was also planted on Carrizo and C35 to compare the impact of the two different rootstocks on the selection, as well as scion/rootstock compatibility. The dwarfing effect on the C35 trees was visible, with a tree size difference between the two combinations, C35 measured between 2.5 and 3 m high and Carrizo 4.5 m high. C35 produced 120 kg per tree, compared to Carrizo with 150 kg per tree. Both rootstock combinations produced good internal quality fruit, and peaked at a Brix reading of 12, juice content above 58% and an acid level of above 1.0%. The external colour was between T1 and T2. Future evaluations will determine the extent and effects of C35 dwarfing on Turkey. Rind texture remained smooth and internal fibre strength (rag) was very soft. Based on a ratio of 10.0 maturity is end of May to mid-June.

Conclusions

Turkey remains a very good and currently the only choice as an early maturing commercial Valencia type, but keep in mind that Weipe and Valearly are two new experimental varieties that are being evaluated with possible future value as early maturing Valencia selections. Jassie proved to be very promising in the other trial sites where the selection was included. Jassie on Carrizo for the Tambuti trial site outperformed the C35 trees. Future trials and evaluations will confirm this statement. Portsgate matures after Turkey and before Benny, when more information becomes available in future this will be a selection to consider for new plantings. Alpha performed well this season, but there was a slight decrease in fruit size: medium to large fruit in 2011 but more medium sized fruit in the 2012 season. McClellan developed excellent crops on the trees for a completely seedless variety. The fruit size was medium to large and will be in demand for exporting larger fruit.

Table 5.5.4.3. Internal fruit quality data for Valencia orange selections at Tambuti Estate (Swaziland) during the 2012 season.

Selection	Root-stock	Date harvested	Size mm	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Alpha	CC	29/06/2012	69-80	88-64	59.3	9.5	1.25	7.60	0.0	T3/4/5
Delta	CC	29/06/2012	63-79	125-64	56.9	10.2	1.05	9.71	0.0	T1/2/3/4
Jassie	CC	29/06/2012	73-83	72-56	57.4	10.9	1.12	9.73	4.8	T2/3/4
Jassie	C35	29/06/2012	72-85	88-56	57.5	9.8	1.15	8.52	5.6	T3/4/5
McClellan SL	CC	29/06/2012	69-77	88-72	57.9	11.1	0.92	12.07	0.0	T1-2
Portsgate	CC	29/06/2012	72-80	88-64	57.2	9.2	0.89	10.34	0.0	T4/5/6
Turkey	CC	24/05/2012	75-81	72-64	54.9	12.5	1.11	11.26	2.4	T4-5
Turkey	CC	29/06/2012	72-81	88-64	58.3	12.1	1.00	12.10	2.5	T1-2
Turkey	C35	24/05/2012	70-81	88-64	57.6	11.8	1.19	9.92	2.6	T4-5
Turkey	C35	29/06/2012	74-83	72-56	58.4	12.0	1.05	11.43	5.4	T1-2

5.5.5 PROGRESS REPORT: Evaluation of Valencias on new imported rootstocks in the Malelane area

Project 416 A by J. Joubert (CRI)

Opsomming

Midnight, met 'n gesonde entlas verbinding, het bewys dis verenigbaar met Sunki x Beneke 812, 'n hibried onderstam cruising tussen Sunki manderyn en Beneke trifoliaat. Die boomgrootte van hierdie kombinasie word as medium beskou, alhoewel Sunki onderstam as boom op sy eie groeikragtig is en 'n groot boom oplewer. Die produksie hierdie seisoen het toegeneem, met 'n ooreenkomstige afname in vruggrootte met pieke by telling 72.

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. Sunki 812 het hierdie seisoen die beste oes op die bome gelewer, gevolg deur 802 en FF-6. Vruggrootte op al drie onderstam kombinasies het gepiek by telling 105/125.

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.

Summary

Visual evaluations of the Midnight: Sunki 812 bud-union, indicated that the union was in good condition. Sunki 812 is a hybrid rootstock cross between a Sunki mandarin and Beneke trifoliolate. The tree size of this combination was described as medium, although Sunki rootstock as a tree on its own is aggressive and develops into a fairly large tree. Yield production this season increased with a corresponding decrease in fruit size, peaking at count 105/125.

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. Sunki 812 produced the best yield on the trees this season, followed by 802 and FF-6. Fruit size on all three rootstock combinations peaked at count 105/125.

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.

Objectives

- To investigate the performance of Midnight and Delta Valencias on new, imported rootstocks on replant soils.
- To improve production, internal quality, rind colour and fruit size count distributions.

Materials and methods

Seed of HRS 802, HRS 812, HRS 809 and C61 were imported and propagated in 1996 by Esselen Nursery, a CIS accredited nursery in the Malelane region of Mpumalanga.

Delta Valencia was budded onto the following three newly imported rootstock hybrids at Esselen nursery in 1997: HRS 802 (Siamese pummelo x Gotha Road trifoliolate), HRS 812 (Sunki mandarin x Beneke trifoliolate) and FF-6 (Sunki x MTO trifoliolate orange). Midnight Valencia was budded onto HRS 812 (Sunki x Beneke). The trees were planted at Esselen Nursery in March 1999.

Table 5.5.5.1. Number of trees per rootstock in the Delta and Midnight Valencia trial at Malelane.

Selection	Rootstock	No. of trees
Midnight	Sunki 812	4
Delta	Sunki 812	4
Delta	HRS 802	4
Delta	FF-6	5

Results and discussion

Midnight Valencia

This is the first trial of Midnights planted on Sunki x Beneke 812 in South Africa. Internally fruit quality was excellent with high Brix (12.1) and juice (56.8%) levels (Table 5.5.5.2). The acid content remained even higher this season (1.5%) compared to the previous season (1.3%), keeping the Brix: acid ratio way below 10 (7.86). Yields increased for the 2012 season from 34.1 to 47.6 kg/tree in comparison with the previous season (Table 5.5.5.4), and the 7 year mean was 52.8 kg/tree (Table 5.5.5.4). Delta produced a 30% better crop on Sunki Beneke 812 over the 7 year period compared to Midnight. Although Midnight is usually a smaller tree than Delta this is not the case in this trial and the difference in yields cannot be attributed to vigour and tree size. Fruit size peaked at count 72 (28%), followed by count 88 (23%) and count 105/125 (20%), producing a smaller fruit size on the trees for this season.

Delta Valencia

Delta on 802 produced the best juice content (58%) and Brix:acid ratio of 10.9:1, followed by FF-6 with the highest Brix level (12.3) and Sunki 812 the highest acids (1.7%), resulting in the lowest Brix:acid ratio of 7.3:1, slightly delaying maturity, but also extending the picking period and increasing the shelf life of the fruit (Table 5.5.5.2), as well as a delayed external colour between T1-2. Fruit size on all three rootstocks peaked at count 105/125, followed by count 72 and then count 88. Delta on Sunku 812 was the only scion:rootstock combination to increase the yield on the trees from 43 kg to 57 kg this season. FF-6 decreased from 61 kg to 33 kg, a 45% decrease in yield, and 802 from 75 kg to 51 kg, a 30% drop in yield.

Conclusions

Midnight on Sunki 812 performed well, producing a better crop compared to the previous season (from 34 to 48 kg per tree), with smaller fruit sizer due to better yield and good internal qualities. The acid levels (1.5%) increased even more this season compared to 2011 (1.3%), 2010 (1.0%) and 2009 (1.05%). The Brix: acid ratio was even lower due to the higher acid level, delaying the maturity of the selection with the external colour at T1.

Delta was evaluated on three hybrid rootstocks, HRS Sunki 812, HRS 802 and FF-6. The more important combination of the above mentioned was Sunki 812. Sunki 812 was selected for replant conditions, very specific high pH and calcareous soils. Delta performed well on Sunki 812 and HRS 802, producing fruit with good internal quality. Delta on Sunki 812 was the only combination with a better crop on the trees, improving by up to 30%. FF-6 internally performed well except for a very low juice content of 39% which did not meet the export requirements.

The FF-6 rootstock was also selected for high pH soils, future trials in the Musina and Kakamas areas must be conducted to determine these characteristics.

Table 5.5.5.2. Internal fruit quality of Midnight and Delta Valencias on different rootstocks at Esselen Nursery (Malelane) on 20 July 2012.

Selection	Root-stock	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Midnight	Sunki 812	56.8	12.10	1.54	7.86	1.6	T1
Delta	Sunki 812	55.6	12.10	1.66	7.29	0.0	T1-2
Delta	HRS 802	58.2	11.10	1.02	10.88	0.0	T1
Delta	FF-6	38.5	12.30	1.47	8.37	0.2	T1

Table 5.5.5.3. Fruit size distribution at Esselen nursery during the 2012 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Midnight	Sunki 812	48	5.85	Delta	HRS 802	48	1.52
Midnight	Sunki 812	56	19.76	Delta	HRS 802	56	14.92
Midnight	Sunki 812	72	27.92	Delta	HRS 802	72	26.05
Midnight	Sunki 812	88	23.40	Delta	HRS 802	88	22.15
Midnight	Sunki 812	105/125	20.42	Delta	HRS 802	105/125	32.13
Midnight	Sunki 812	144	2.65	Delta	HRS 802	144	3.23
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	Sunki 812	48	4.30	Delta	FF-6	48	3.53
Delta	Sunki 812	56	15.58	Delta	FF-6	56	18.98
Delta	Sunki 812	72	21.00	Delta	FF-6	72	25.30
Delta	Sunki 812	88	21.51	Delta	FF-6	88	22.38
Delta	Sunki 812	105/125	30.38	Delta	FF-6	105/125	26.40
Delta	Sunki 812	144	7.23	Delta	FF-6	144	3.41

Table 5.5.5.4. Production per tree of Midnight and Delta Valencia trees on different rootstocks at Esselen Nursery (Malelane) during the 2012 season.

Cultivar	Rootstock	Kg/tree (2006)	Kg/tree (2007)	Kg/tree (2008)	Kg/tree (2009)	Kg/tree* (2010)	Kg/tree* (2011)	Kg/tree (2012)	7 Year Total	7 Year Mean
Midnight	Sunki 812	46.8	98.7	50.4	51.7	40.2	34.1	47.6	369.5	52.8
Delta	Sunki 812	65.4	120.5	66.4	78.6	58.7	42.7	56.5	488.8	69.8
Delta	HRS 802	58.5	120.6	102.3	102.4	81.4	74.7	51.1	591.0	84.4
Delta	FF-6	62.9	134.1	97.2	80.7	79.8	61.4	33.3	549.4	78.5

*Note: Heat wave experienced during fruit set period (fruit also stolen from this specific section)

5.5.6 PROGRESS REPORT: Evaluation of Grapefruit varieties on different rootstocks in the Swaziland area

Project 416 B by J. Joubert (CRI)

Opsomming

Marsh, Star Ruby en Nelruby op al vier onderstam kombinasies se gemiddelde opbrengs was laer as die vorige seisoen, behalwe vir Marsh en Nelruby op C35. Star Ruby op C35 en MxT is 'n uitstekende kombinasie, as die boomgrootte van C35 (derde kleiner as ander bome) in ag geneem word, en die feit dat vir hierdie seisoen in totaal dieselfde opbrengs per boom as Swingle geproduseer het.

Nelruby het die hoogste Brix: suur verhouding vir hierdie proef behaal met hoë Brix en laer sure, maar Star Ruby het die hoogste sap persentasie gelewer. Die algemene vrug kwaliteit vir hierdie seisoen was beter in vergelyking met die 2011 seisoen. Star Ruby, en meer spesifiek in kombinasie met MxT, het die laagste Brix: suur verhouding van 6.54% opgelewer.

Die algemene tendens wat vruggrootte aanbetref was kleiner vrugte, met 'n hoër obrengs op die bome. Hierdie seisoen was die scenario omgekeerd met minder vrugte op die bome, sowel as kleiner vruggrootte. Al drie kultivars het op telling 48 gepiek, behalwe Star Ruby in kombinasie met MxT en 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.

Summary

Marsh, Star Ruby and Nelruby on all four rootstock combinations produced a lower yield compared to the previous season, except for Marsh and Nelruby on C35. Star Ruby on C35 and MxT is a excellent combination, when tree size of C35 (third smaller than other trees) were taken into account, and the fact that for this season in total the same yield was produced compared to Swingle.

Nelruby produced the highest Brix: acid ratio for this trial with high Brix and acids, but Star Ruby developed the highest juice percentage levels. The average fruit quality for this season was better compared to the 2011 season. Star Ruby, and more specific in combination with MxT, developed the lowest Brix: acid ratio of 6.54%.

The general tendency for fruit size was heading towards smaller fruit, and a heavier crop on the trees. This season the typical scenario was different with less fruit on the trees, as well as smaller fruit size. All three cultivars peaked at count 48, except for Star Ruby in combination with MxT and X639.

Note: The trees in this trial are generally smaller than expected due to problems encountered with the irrigation scheme.

Objective

- Investigate the performance of grapefruit cultivars on different rootstocks on heavy, replant soils.
- Improve production, fruit size, internal quality and rind colour.

Materials and methods

Trees were planted in 2003, 10 trees Marsh, Nelruby and Star Ruby all on C35, MxT, SC, and X639.

Table 5.5.6.1. Number of trees per rootstock in the grapefruit trial at Tambuti, Swaziland.

Planted 2003		
Selection	Rootstock	No. of trees
Marsh	C35	10
Marsh	MxT	10
Marsh	SC	10
Marsh	X639	10
Nelruby	C35	10
Nelruby	MxT	10
Nelruby	SC	10
Nelruby	X639	10
Star Ruby	C35	10
Star Ruby	MxT	10
Star Ruby	SC	10
Star Ruby	X639	10

Results and discussion

Internal quality and colour

Star Ruby gave the highest juice percentages (mean 58.9%) followed by Nelruby (mean 53.2%) and Star Ruby (mean 52.9%); Juice percentages were acceptable (export standards) for all cultivars and rootstocks. Marsh gave the lowest internal quality with Brix levels from 8.6 on X639 to 9.6 on C35 (mean 9.1) with acid levels and ratios averaging 1.32% and 6.89:1 respectively. Marsh ratios were below 7.0 on X639 and Swingle rootstocks.

Nelruby gave the highest Brix levels from 10.4 on MxT and SC to 9.7 on C35 (mean 10.1), the lowest acid levels (mean 1.25%) and highest ratios (mean 8.2:1) followed by Star Ruby with Brix levels of 8.5 on MxT to 10.3 on SC (mean 9.4) with acid levels and ratios averaging 1.35% and 6.97 respectively. Star Ruby seed counts were the lowest at 0.2 seeds/fruit followed by Nelruby at 2.5 seeds/fruit and Marsh at 5.2 seeds/fruit. External colour was T1-2 for Star Ruby, T2-3 for Nelruby and T3-5 for Marsh.

Rootstock effect on internal quality and colour was variable and did not show any specific trends.

Fruit size

The fruit size increased this season on Marsh, Nelruby and Star Ruby; where all three selections on all four rootstock combinations peaked at count 48, followed by count 40 except for Star Ruby on MxT with 42% and on X639 with 32% at count 64. The average fruit size on all the combinations decreased compared to 2011.

Yield

Marsh produced the highest yield on Swingle with 126.5 kg/tree, followed by Star Ruby on C35 with 113.9 kg/tree and Nelruby on Swingle with 115.8 kg/tree. The only yield increase this season was Marsh and Nelruby on C35 with 92.6 and 51.0 kg per tree respectively. All the other rootstock: scion combinations produced less fruit on the trees.

Taking the total yield production of the trial into consideration per rootstock, Swingle bore the most fruit (258.1 kg/tree) followed by C35 (257.5 kg/tree), X639 (200.5 kg/tree) and MxT (162.4 kg/tree).

Conclusions

Nelruby on MxT developed the highest Brix (9.5) content for this trial, with Star Ruby on Swingle the highest juice (59.1%) and Star Ruby on MxT the highest acid (1.34%). Fruit size on all three cultivars peaked at count 48, except for Star Ruby on MxT and X639 (peaked at count 64), developing smaller fruit size this season, as well as a lighter crop on most combinations.

The fruit production on the trees decreased this season; except for Marsh and Nelruby on C35. All the problems were addressed with regard to red scale and irrigation. When compared to the poor crops of the 2010 season, the 2012 season was substantially better, even with the yield decreases. The best yield production was Star Ruby on all four rootstock combinations (mean 94.3 kg per tree), and the lowest was Nelruby (mean 40.7 kg per tree). The lowest crop for this trial was Nelruby on MxT with 17.9 kg per tree. External colour of the Nelruby was paler than Star Ruby with fewer blushes on the rind. Internal pigmentation of Nelruby was also slightly less than Star Ruby.

Nelruby on C35 developed small trees when compared to Marsh and Star Ruby. This might be a specific characteristic of this combination. No incompatibility has been detected at the bud-union, but future evaluations will determine if there might be a problem.

Note: The trees in this trial are generally smaller than expected due to problems encountered with the irrigation scheme.

Table 5.5.6.2. Internal fruit quality data of grapefruit on different rootstocks at Tambuti Estates on 24 May 2012.

Selection	Root-stock	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Marsh	C35	52.4	9.6	1.34	7.16	5.1	T4-5
Marsh	MxT	52.7	9.4	1.34	7.01	4.6	T4-5
Marsh	SC	52.7	8.8	1.33	6.62	5.4	T3-4
Marsh	X639	53.9	8.6	1.29	6.67	5.8	T4-5
NelRuby	C35	56.5	9.7	1.13	8.58	2.5	T2-3
NelRuby	MxT	50.5	10.4	1.42	7.32	2.5	T2-3
NelRuby	SC	51.4	10.4	1.30	8.00	2.7	T2-3
NelRuby	X639	54.2	10.0	1.15	8.70	2.3	T2-3
TSR	C35	59.8	9.0	1.33	6.77	0.1	T1-2
TSR	MxT	58.6	8.5	1.30	6.54	0.1	T1
TSR	SC	59.3	10.3	1.30	7.92	0.3	T1-2
TSR	X639	57.7	9.7	1.46	6.64	0.1	T1-2

Table 5.5.6.3. Fruit size distribution per rootstock at Tambuti Estate during the 2012 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Marsh	C 35	27	4.16	Nelruby	SC	27	1.59
Marsh	C 35	32	5.08	Nelruby	SC	32	2.90
Marsh	C 35	36	16.53	Nelruby	SC	36	12.43
Marsh	C 35	40	27.17	Nelruby	SC	40	25.85
Marsh	C 35	48	34.98	Nelruby	SC	48	42.77
Marsh	C 35	64	12.08	Nelruby	SC	64	14.46
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Marsh	MxT	27	3.88	Nelruby	X639	27	1.29
Marsh	MxT	32	4.87	Nelruby	X639	32	2.11
Marsh	MxT	36	18.94	Nelruby	X639	36	9.37
Marsh	MxT	40	26.15	Nelruby	X639	40	24.70
Marsh	MxT	48	34.81	Nelruby	X639	48	36.00
Marsh	MxT	64	11.36	Nelruby	X639	64	26.54
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Marsh	SC	27	1.48	Star Ruby	C 35	27	4.32
Marsh	SC	32	2.35	Star Ruby	C 35	32	3.47
Marsh	SC	36	11.18	Star Ruby	C 35	36	11.10
Marsh	SC	40	24.49	Star Ruby	C 35	40	20.58
Marsh	SC	48	45.55	Star Ruby	C 35	48	34.98
Marsh	SC	64	14.94	Star Ruby	C 35	64	25.55
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Marsh	X639	27	3.50	Star Ruby	MxT	27	0.46
Marsh	X639	32	4.66	Star Ruby	MxT	32	1.06
Marsh	X639	36	16.24	Star Ruby	MxT	36	4.81
Marsh	X639	40	29.03	Star Ruby	MxT	40	12.41
Marsh	X639	48	35.82	Star Ruby	MxT	48	39.72
Marsh	X639	64	10.75	Star Ruby	MxT	64	41.54
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Nelruby	C 35	27	1.73	Star Ruby	SC	27	3.12
Nelruby	C 35	32	3.07	Star Ruby	SC	32	2.80
Nelruby	C 35	36	16.44	Star Ruby	SC	36	12.42
Nelruby	C 35	40	22.97	Star Ruby	SC	40	22.84
Nelruby	C 35	48	37.24	Star Ruby	SC	48	40.09
Nelruby	C 35	64	18.55	Star Ruby	SC	64	18.74
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Nelruby	MxT	27	0.35	Star Ruby	X639	27	0.79
Nelruby	MxT	32	1.58	Star Ruby	X639	32	0.70
Nelruby	MxT	36	6.15	Star Ruby	X639	36	4.16
Nelruby	MxT	40	24.08	Star Ruby	X639	40	13.85
Nelruby	MxT	48	50.62	Star Ruby	X639	48	48.58
Nelruby	MxT	64	17.22	Star Ruby	X639	64	31.92

Table 5.5.6.4. Production per tree of grapefruit on different rootstocks at Tambuti Estates during 2012.

Cultivar	Rootstock	Kg/tree					5 Years	Mean
		(2008)	(2009)	(2010)	(2011)	(2012)		
Marsh	C35	57.4	22.5	66.0	78.1	92.6	316.6	63.3
Marsh	MxT	64.4	44.3	34.5	62.4	37.9	243.5	48.7
Marsh	SC	79.3	95.4	140.3	157.8	126.5	599.3	119.9
Marsh	X639	66.7	27.3	44.7	95.9	81.7	316.3	63.3
Nelruby	C35	50.3	9.0	22.0	50.1	51.0	182.4	36.5
Nelruby	MxT	69.0	51.4	34.7	61.2	17.9	234.2	46.8
Nelruby	SC	95.2	35.3	18.4	115.8	59.6	324.3	64.8
Nelruby	X639	71.4	46.2	39.5	81.2	34.1	272.4	54.5
Star Ruby	C35	63.7	17.7	36.9	119.6	113.9	351.8	70.4
Star Ruby	MxT	74.2	60.9	100.3	150.6	106.6	492.6	98.5
Star Ruby	SC	60.2	63.7	44.8	119.8	72.0	360.5	72.1
Star Ruby	X639	31.4	31.3	41.2	102.0	84.7	290.6	58.1

5.5.7 PROGRESS REPORT: Evaluation of various Valencia selections on different rootstocks in the Komatipoort area
Project 590 B by J. Joubert (CRI)

Opsomming

Delta se vruggrootte op al die onderstam kombinasies het by telling 105/125 gepiek, waarna telling 88 gevolg het. Die oes produksie op al die kombinasies het toegeneem, met Swingle die hoogste (70.1 kg/boom), gevolg deur X639 (69.1 kg/boom). Gemiddeld vir elke boom het die opbrengs van 39 kg/boom na 56 kg/boom verhoog. C35 was die enigste onderstam wat se sap inhoud te laag was, en het nie aan die uitvoer standaard voldoen nie. Delta op Terrabella het 'n uitstekende 13.8 Brix : suur verhouding opgelewer, die hoogste vir die totale proef. Geen onverenigbaarheids tekens was by enige van die kombinasies sigbaar nie.

McClellan SL het op twee onderstamme nie aan die uitvoer standaard voldoen nie, met C35 en Carrizo wat te lae sap hoeveelhede gelever het. Die gemiddelde Brix: suur verhouding van al die kombinasies het toegeneem na 10.1: 1. Vruggrootte was uitstekend vir Valencias gewees, en die tellings het gepiek tussen 56, 72 en 88. McClellan SL op X639 was die engste kombinasie wat 'n laer oes opbrengs gelever het, die res het almal toegeneem, met weereens Swingle die hoogste opbrengs (74 kg/boom).

Midnight op C35 en X639 het van die hoogste sap vlakke vir hierdie proef geproduseer. Die hoogste suurvlak was op Swingle gewees, wat dan ook 'n laer Brix: suur vlak van 8.96 tot gevolg gehad het. Hierdie kombinasie kan later in die seisoen geoes word, hou wel die eksterne kleur ontwikkeling goed dop. Midnight was die enigste seleksie met saad tellings in die vrugte. Die vruggroottes het tussen telling 56 en 72 gepiek, behalwe vir X639 (telling 105/125). Oes produksie op al die kombinasies was beter, met C35 die hoogste, maar steeds laag as die gemiddeld van 41.7 kg/boom in ag geneem word. Warm temperature tydens blomset in hierdie produksie area het 'n groot invloed op die prestasie.

Portsgate het hierdie seisoen uitstekend gevaar. Die interne kwaliteit van die vrugte op al die onderstam kombinasies het aan die uitvoer standaard voldoen, behalwe vir Swingle met 'n laer Brix: suur verhouding a.g.v hoër suurvlakke. Vruggroottes het gepiek by telling 72 (C35 en TB), telling 105/125 (CC, KC, SC en X639) en telling 88 (MxT). Die hoogste oes obrengs in kombinasie met Portsgate, asook in totaal vir hierdie proef, was op Swingle geproduseer (106 kg/boom). Die kleiner vruggroottes kan direk aan die grootter oeste toegeskryf word, en al die vrugte was saadloos gewees.

Summary

Delta peaked on all the rootstock combinations with fruit size at count 105/125, followed by count 88. Yield production increased on all rootstock selections, with Swingle the best (70.1 kg/tree), followed by X639 (69.1 kg/tree). The average yield per tree increased from 39 kg to 56 kg per tree compared to the previous season. C35 produced low juice content and did not comply with the export requirements. Delta on Terrabella developed an excellent Brix: acid ratio of 13.8, the highest ratio for this trial. There were no incompatibility problems on the rootstock combinations visible.

McClellan SL on C35 and Carrizo produced low juice levels, not complying with the minimum export standards. The average Brix: acid ratio on all the rootstock combinations increased to 10.1:1. Fruit size for Valencias was excellent, fruit counts peaked between count 56, 72 and 88. McClellan SL on X639 was the only scion: rootstock combination with a lower crop on the trees, the rest increased, with Swingle the highest yield (74 kg/tree).

Midnight on C35 and X639 developed the best juice levels for this trial, above 61%. Midnight on Swingle produced the highest acid level, causing a low Brix: acid ratio of 8.96. Harvest this scion: rootstock combination later to improve ratio, but keep external colour development in mind. Midnight was the only selection to develop seeds in the fruit for this trial. The fruit sizes peaked between count 56 and 72, except for X639 (count 105/125). Yield production on all seven combinations was better, with C35 the best, although low in general when you consider an average yield of 41.7 kg/tree. High temperature during the flower set period in this production area played a role.

Portsgate performed excellently this season. The internal quality of the fruit on all the rootstocks complied with the export requirements, except for Swingle with a lower Brix: acid ratio due to high acid levels. Fruit size peaked at count 72 (C35 and TB), count 105/125 (CC, KC, SC and X639) and count 88 (MxT). The highest yield production in combination with Portsgate, as well as in total for this trial, was produced on Swingle (106 kg/tree). The smaller fruit size can be related to the higher crops on the trees, and all the fruit was seedless.

Objectives

- Evaluate and assess the horticultural performance and capability of various new Valencia selections on different rootstocks.
- Determine the superior rootstock combinations for these new selections.
- Be able to make credible commercial recommendations.

Materials and methods

Five trees of each cultivar rootstock combination were planted in 2002.

Evaluate visually to determine production per tree, trueness to type and compatibility with scion and harvest each tree with the sizer to determine production per tree as well as fruit size distribution per tree. Samples will be taken and internal quality tested and analysed. Fruit colour will be evaluated and analysed.

Table 5.5.7.1. List of cultivar and rootstock combinations in the Valencia trial at Golden Frontiers Citrus, Hectorspruit in the Komatipoort area.

Selection	Rootstock	Qty Trees
Delta (Control)	C35	5
Delta (Control)	CC	5
Delta (Control)	KC	5
Delta (Control)	MxT	5
Delta (Control)	SC	5
Delta (Control)	Terrabella	5
Delta (Control)	X639	5
McClellan SL	C35	5
McClellan SL	CC	5
McClellan SL	KC	5
McClellan SL	MxT	5
McClellan SL	SC	5
McClellan SL	Terrabella	5
McClellan SL	X639	5
Midnight	C35	5
Midnight	CC	5
Midnight	KC	4
Midnight	MxT	5
Midnight	SC	5
Midnight	Terrabella	5
Midnight	X639	5
Portsgate	C35	5
Portsgate	CC	5

Portsgate	KC	5
Portsgate	MxT	5
Portsgate	SC	5
Portsgate	Terrabella	5
Portsgate	X639	5

Results and discussion

Delta

Internal fruit quality analysis (Table 5.5.7.2)

- Juice %: The juice levels in the fruit produced varied from 43.2% on C35 up to 59.2% on MxT (mean 56.2%). The low juice content on C35 was due to poor quality, dry fruit on the trees.
- Brix°: Similar to the 2011 season, the highest Brix was on Terrabella (12.3) and the lowest on X639 (10.4). All the rootstocks ranged from 10.4 to 12.3 (mean 11.3). This Brix value was the highest for all scion cultivars in the trial.
- Acid: All the rootstock combinations produced acceptable acid levels above 0.85 and below 1.8, varying from 0.89 (Terrabella) to 1.15 (Koethen) and complied with export standards.
- Ratio: Ratios averaged 10.9 with Terrabella highest (13.8) and Koethen lowest (9.57).

Fruit size distribution (Table 5.5.7.3)

- Fruit size evaluations based on counts 56 and 72 (the optimal counts for Valencia production), showed all the rootstocks in combination with Delta peaked at count 105/125, followed by count 88 and 72, except for Koethen at count 144.

Note: Yields must be taken into consideration with fruit size due to the effect yield can have on fruit size.

Production per tree (Table 5.5.7.4)

- Yield production with all the rootstocks was better than in 2011. The lowest yield was on Terrabella with 44.4 kg/tree up to 70.1 kg/tree on Swingle. Keep the tree size of Swingle in mind. The mean yield improved from 39 kg/tree up to 55.7 kg/tree.

McClellan SL

Internal fruit quality analysis (Table 5.5.7.2)

- Juice %: McClellan SL on Swingle produced the highest juice content of 59.9% and on C35 the lowest at 43.46% (mean 55.1%).
- Brix°: Swingle produced the lowest level at 10.7 and C35 the highest at 11.8. Variation was not substantial with the other rootstocks falling close to the mean of 11.2.
- Acid: The acid levels this season were similar to 2011. All the selections complied with the minimum standards varying from 1.0 (Carrizo & Koethen) to 1.22 (MxT) averaging 1.1.
- Ratio: Ratios averaged 10.1 with Koethen highest (11.0) and MxT lowest (9.1).

Fruit size distribution (Table 5.5.7.3)

- Fruit size evaluations based on counts 56 and 72 (the optimal counts for Valencia production), showed that more than half of the rootstocks peaked at count 72 (Carrizo, Koethen, MxT and Terrabella) and C35, as well as X639 at count 56, with only Swingle at count 88. The second highest count spread was between count 56, 72 and 88, followed by count Count 105/125.

Note: Yields must be taken into consideration with fruit size due to the effect yield can have on fruit size.

Production per tree (Table 5.5.7.4)

- Production improved this season on six of the seven rootstocks from the lowest on MxT (16.1 kg/tree) up to Swingle (74.0 kg/tree) with the highest yield. X639 was the only rootstock that dropped in yield from 80.6 to 49.2 kg/tree. The mean yield was 45.7 kg/tree.

Midnight

Internal fruit quality analysis (Table 5.5.7.2)

- Juice %: All the rootstocks complied with the minimum export standard of 52%. Koethen produced the lowest level of 58.5%. MxT improved this season and increased from 56.6 to 59.3%. All the rootstocks were well above the minimum (58.5% to 61.4%), averaging 59.8%. C35 remained the highest producer for this season with 61.4%, compared to last year's 59.7%.
- Brix°: Brix levels tested between 11.1° (Koethen) and 12.2° (Carrizo) with a mean of 11.7. The export minimum is 9.5 indicating the good quality of the fruit produced.
- Acid: All the rootstock combinations produced an acid content above 0.85 and complied with the export standards averaging 1.2 overall. MxT for the following year produced the highest acid level of 1.35%, increasing by 0.11%, resulting in a ratio of 8.96 which is above the minimum of 7.5.
- Ratio: Ratios averaged 9.8 with X639 highest (10.4) and MxT lowest (8.96).

Fruit size distribution (Table 5.5.7.3)

- Fruit size evaluations based on counts 56 and 72 (the optimal counts for Valencia production), showed C35, MxT and SC peaked at count 56, Carrizo and Terrabella peaked at count 72, and Koethen and X639 peaked at count 105/125. With C35 the highest (47.86%) average at count 56.
Note: Yields must be taken into consideration with fruit size due to the effect yield can have on fruit size.

Production per tree (Table 5.5.7.4)

- In comparison with the previous year all the rootstocks increased in production from MxT the lowest (30.3 kg/tree) up to Swingle (53.0 kg/tree) the highest. Carrizo improved from 12.3 up to 41.8 kg/tree this season, an excellent 70% growth in yield.

Portsgate

Internal fruit quality analysis (Table 5.5.7.2)

- Juice %: All the selections complied with the export standards above 48% juice content. MxT produced the lowest at 58.3% and C35 the highest at 60.4%. The mean for all rootstocks was 59.6%. Portsgate outperformed Delta and McClean SL, with only Midnight producing on average higher juice content (mean 59.8%)
- Brix°: Koethen and C35 produced the highest Brix levels of 11.7 with SC the lowest at 10.5. The mean for this season was 11.2, compared to last season with 11.1, a slight improvement in quality.
- Acid: All the combinations complied with the minimum standards for this season with an overall average of 1.2; very similar to the previous season's 1.2%. Harvesting was planned to obtain optimal internal quality. Highest acid was on Swingle with 1.28%
- Ratio: Ratios averaged 9.8 compared to last years 10.3, resulting in a 0.5 decrease, with Terrabella highest (10.96) and Swingle lowest (8.2). The low ratio on Swingle was because of the high acids by the time of harvest, and by delaying the harvest time when Portsgate was planted on Swingle will improve the Brix: acid ratio considerably.

Fruit size distribution (Table 5.5.7.3)

- Fruit size evaluations based on counts 56 and 72 (the optimal counts for Valencia production), showed that more than half of the rootstocks peaked at count 105/125, except for C35 and

Terrabella that peaked at count 72, and MxT that peaked at count 88. The second highest count was 88 with five of the rootstock, except for Carrizo (count 144) and MxT (count 72).

Note: Yields must be taken into consideration with fruit size due to the effect yield can have on fruit size.

Production per tree (Table 5.5.7.4)

- In comparison with the previous season all the rootstocks increased in production and averaged 65.2 kg/tree, compared to 30 kg/tree, doubling yield production. The lowest yield was on MxT with 25.1 kg/tree, and Portsgate on Swingle produced the best crop on the trees, as well as the best yield production for the trial in total, measuring 106.0 kg/tree.

Note: External colour was the same (T1-2) for all scion rootstock combinations on 18/7/2012.

Conclusions

Delta on all the rootstocks produced very good internal quality and, compared to the previous season, all the selections developed a Brix: acid ratio above 10.0, except for Koethen citrange with 9.57. The highest ratio was in combination with Terrabella, peaking at 13.8 for this trial. Delta peaked on all the rootstock selections at 105/125 fruit count, followed by 88. Production was even better this season, all the combinations increased their yield on the trees, with Swingle outperforming the rest at 70 kg per tree. The production mean improved from 39 kg per tree to 56 kg per tree. Terrabella was the lowest at 44 kg per tree, although the highest for this rootstock over the last five years.

McClelland seedless on Swingle produced the highest juice levels (59.9%) for this trial. C35 outperformed the other rootstocks with the highest Brix level (11.8) for the second time this season. The average Brix: acid ratio average was 10.1, excellent for export quality and meets the requirements. Four of the rootstocks peaked at count 72, followed by the next two with count 56, and SC at count 88. McClelland seedless produces on average a good fruit size for a Valencia selection. Swingle and X639 bore a crop of 305 kg per tree averaged over a 5 year period, excellent production for this trial. McClelland seedless in combination with MxT performed poorly this season, producing a yield of only 16.1 kg per tree, and a 5 year mean of 76.7 kg per tree, not a combination to consider based on fruit production.

Midnight performance was better this season and the internal quality improved. The juice and Brix levels increased; C35 with 61.4% juice and Carrizo with 12.2 Brix. Acids on MxT were high (1.35%), but still acceptable and below the export maximum, lowest was on X639 (1.1%). Midnight always tends to have larger fruit, but this season was an exception, with only three rootstocks peaking at count 56 (C35, MxT, SC). Carrizo and Terrabella peaked at count 72, followed by Koethen citrange and X639 peaking at count 105/125. Crop set on the trees increased and all seven combinations were higher, with Swingle the best, producing 53 kg per tree. Taking tree size into consideration, C35 developed into a smaller tree and after ten years the tree volume will be between 20 to 30% smaller.

Portsgate improved substantially for the 2012 season with juice levels on three of the combinations above 60% (C35, KC, X639). The highest juice was on C35 (61.4%), Brix on Terrabella and Koethen citrange (11.7) and Brix: acid ratio on Terrabella (10.96%), also developing the lowest acid (1.04%). Portsgate peaked at various counts from 105/125 up to 72, with C35, MxT and Terrabella the best size for the trial. The yield on the trees improved and all seven combinations bore better crops. Swingle outperformed the rest, as well as producing the highest crop in total for this trial with 106 kg per tree. The improvement in yield production for Portsgate was excellent with a crop increase of 24 to 65 kg per tree average when taking all seven rootstocks into consideration.

Table 5.5.7.2. Internal fruit quality data for Valencias on different rootstocks at Golden Frontiers Citrus, Hectorspruit on 19 July 2012.

Selection	Root-stock	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Delta	C35	43.2	11.4	1.06	10.75	0.0	T1
Delta	CC	57.8	10.7	0.96	11.15	0.0	T1
Delta	KC	58.7	11.0	1.15	9.57	0.0	T1
Delta	MxT	59.2	11.0	1.05	10.48	0.0	T1

Delta	SC	58.5	10.9	1.06	10.28	0.0	T1
Delta	TB	57.3	12.3	0.89	13.82	0.0	T1
Delta	X639	58.5	10.4	1.01	10.30	0.0	T1
McClellan SL	C35	43.4	11.8	1.14	10.35	0.0	T1
McClellan SL	CC	46.3	11.2	1.03	10.87	0.0	T1
McClellan SL	KC	59.5	11.3	1.03	10.97	0.0	T1
McClellan SL	MxT	58.7	11.1	1.22	9.10	0.0	T1
McClellan SL	SC	59.9	10.7	1.16	9.22	0.0	T1
McClellan SL	TB	58.7	11.3	1.14	9.91	0.0	T1
McClellan SL	X639	59.2	11.2	1.07	10.47	0.0	T1
Midnight	C35	61.4	11.7	1.15	10.17	0.0	T1
Midnight	CC	59.1	12.2	1.19	10.25	0.2	T1
Midnight	KC	58.5	11.1	1.17	9.49	1.0	T1-2
Midnight	MxT	59.3	12.1	1.35	8.96	0.3	T1
Midnight	SC	59.5	11.8	1.19	9.92	0.1	T1-2
Midnight	TB	59.6	11.4	1.21	9.42	0.2	T1
Midnight	X639	61.1	11.4	1.10	10.36	0.0	T1-2
Portsgate	C35	61.4	11.7	1.15	10.17	0.0	T1
Portsgate	CC	58.4	11.5	1.19	9.66	0.0	T1-2
Portsgate	KC	60.1	11.7	1.11	10.54	0.0	T1-2
Portsgate	MxT	58.3	10.7	1.12	9.55	0.0	T1
Portsgate	SC	59.6	10.5	1.28	8.20	0.0	T1
Portsgate	TB	58.7	11.4	1.04	10.96	0.0	T1-2
Portsgate	X639	60.7	10.9	1.16	9.40	0.0	T1

Table 5.5.7.3. Fruit size distribution per rootstock at Golden Frontiers Citrus, Hectorspruit during the 2012 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	C35	48	0.44	Midnight	C35	48.0	24.19
Delta	C35	56	9.53	Midnight	C35	56.0	47.84
Delta	C35	72	25.06	Midnight	C35	72.0	17.14
Delta	C35	88	28.31	Midnight	C35	88.0	4.84
Delta	C35	105/125	31.04	Midnight	C35	105/125	2.52
Delta	C35	144	5.62	Midnight	C35	144.0	3.47
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	CC	48	0.17	Midnight	CC	48.0	4.48
Delta	CC	56	3.31	Midnight	CC	56.0	24.79
Delta	CC	72	12.96	Midnight	CC	72.0	34.27
Delta	CC	88	21.21	Midnight	CC	88.0	19.90
Delta	CC	105/125	47.64	Midnight	CC	105/125	14.58
Delta	CC	144	14.70	Midnight	CC	144.0	1.98
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	KC	48	0.20	Midnight	KC	48.0	4.44
Delta	KC	56	2.83	Midnight	KC	56.0	17.96
Delta	KC	72	10.80	Midnight	KC	72.0	18.84
Delta	KC	88	21.28	Midnight	KC	88.0	20.89
Delta	KC	105/125	50.86	Midnight	KC	105/125	29.78
Delta	KC	144	14.03	Midnight	KC	144.0	8.09
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit

Delta	MxT	48	0.85	Midnight	MxT	48.0	6.61
Delta	MxT	56	8.47	Midnight	MxT	56.0	25.21
Delta	MxT	72	27.43	Midnight	MxT	72.0	20.80
Delta	MxT	88	28.59	Midnight	MxT	88.0	17.63
Delta	MxT	105/125	30.23	Midnight	MxT	105/125	21.49
Delta	MxT	144	4.43	Midnight	MxT	144.0	8.26
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	SC	48	0.58	Midnight	SC	48.0	22.56
Delta	SC	56	7.34	Midnight	SC	56.0	35.24
Delta	SC	72	24.74	Midnight	SC	72.0	21.43
Delta	SC	88	27.52	Midnight	SC	88.0	10.90
Delta	SC	105/125	35.12	Midnight	SC	105/125	9.02
Delta	SC	144	4.72	Midnight	SC	144.0	0.85
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	TB	48	0.75	Midnight	TB	48.0	8.72
Delta	TB	56	9.63	Midnight	TB	56.0	27.40
Delta	TB	72	24.20	Midnight	TB	72.0	29.98
Delta	TB	88	27.64	Midnight	TB	88.0	18.92
Delta	TB	105/125	31.24	Midnight	TB	105/125	13.64
Delta	TB	144	6.53	Midnight	TB	144.0	1.35
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	X639	48	0.10	Midnight	X639	48.0	1.53
Delta	X639	56	3.09	Midnight	X639	56.0	15.48
Delta	X639	72	14.86	Midnight	X639	72.0	18.13
Delta	X639	88	24.89	Midnight	X639	88.0	19.14
Delta	X639	105/125	44.96	Midnight	X639	105/125	32.38
Delta	X639	144	12.11	Midnight	X639	144.0	13.34
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
McClean SL	C35	48	6.04	Portsgate	C35	48.0	0.52
McClean SL	C35	56	37.12	Portsgate	C35	56.0	14.20
McClean SL	C35	72	30.48	Portsgate	C35	72.0	29.44
McClean SL	C35	88	16.10	Portsgate	C35	88.0	26.63
McClean SL	C35	105/125	9.36	Portsgate	C35	105/125	25.60
McClean SL	C35	144	0.91	Portsgate	C35	144.0	3.61
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
McClean SL	CC	48	2.32	Portsgate	CC	48.0	0.18
McClean SL	CC	56	17.20	Portsgate	CC	56.0	2.52
McClean SL	CC	72	27.07	Portsgate	CC	72.0	8.96
McClean SL	CC	88	22.07	Portsgate	CC	88.0	18.71
McClean SL	CC	105/125	22.07	Portsgate	CC	105/125	47.18
McClean SL	CC	144	9.27	Portsgate	CC	144.0	22.45
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
McClean SL	KC	48	1.01	Portsgate	KC	48.0	0.06
McClean SL	KC	56	14.31	Portsgate	KC	56.0	3.01
McClean SL	KC	72	28.79	Portsgate	KC	72.0	10.97
McClean SL	KC	88	27.95	Portsgate	KC	88.0	21.84

McClellan SL	KC	105/125	23.57	Portsgate	KC	105/125	51.25
McClellan SL	KC	144	4.38	Portsgate	KC	144.0	12.87
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
McClellan SL	MxT	48	5.51	Portsgate	MxT	48.0	2.31
McClellan SL	MxT	56	27.82	Portsgate	MxT	56.0	18.68
McClellan SL	MxT	72	29.75	Portsgate	MxT	72.0	33.55
McClellan SL	MxT	88	25.07	Portsgate	MxT	88.0	24.63
McClellan SL	MxT	105/125	11.02	Portsgate	MxT	105/125	19.17
McClellan SL	MxT	144	0.83	Portsgate	MxT	144.0	1.65
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
McClellan SL	SC	48	1.57	Portsgate	SC	48.0	0.03
McClellan SL	SC	56	9.89	Portsgate	SC	56.0	2.29
McClellan SL	SC	72	22.57	Portsgate	SC	72.0	7.78
McClellan SL	SC	88	29.11	Portsgate	SC	88.0	20.32
McClellan SL	SC	105/125	32.15	Portsgate	SC	105/125	54.96
McClellan SL	SC	144	4.72	Portsgate	SC	144.0	14.62
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
McClellan SL	TB	48	2.52	Portsgate	TB	48.0	2.95
McClellan SL	TB	56	25.35	Portsgate	TB	56.0	20.54
McClellan SL	TB	72	36.77	Portsgate	TB	72.0	33.91
McClellan SL	TB	88	22.83	Portsgate	TB	88.0	22.66
McClellan SL	TB	105/125	10.94	Portsgate	TB	105/125	18.50
McClellan SL	TB	144	1.57	Portsgate	TB	144.0	1.44
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
McClellan SL	X639	48	11.70	Portsgate	X639	48.0	0.23
McClellan SL	X639	56	30.47	Portsgate	X639	56.0	5.74
McClellan SL	X639	72	28.30	Portsgate	X639	72.0	19.13
McClellan SL	X639	88	18.02	Portsgate	X639	88.0	30.50
McClellan SL	X639	105/125	9.34	Portsgate	X639	105/125	40.24
McClellan SL	X639	144	2.17	Portsgate	X639	144.0	4.15

Table 5.5.7.4. Production per tree of Valencia selections on different rootstocks at Golden Frontiers Citrus, Hectorspruit during the 2012 season.

Cultivar	Rootstock	Kg/tree					5 Years	Mean
		(2008)	(2009)	(2010)	(2011)	(2012)		
Delta Valencia	C35	60.1	45.6	51.7	37.6	50.5	245.5	49.1
Delta Valencia	CC	33.4	25.6	21.5	38.4	58.3	177.2	35.4
Delta Valencia	KC	38.5	26.2	10.4	32.6	49.0	156.7	31.3
Delta Valencia	MxT	39.4	40.0	6.4	13.8	48.4	148.0	29.6
Delta Valencia	SC	73.9	76.8	65.7	41.7	70.1	328.2	65.6
Delta Valencia	TB	34.9	30.5	32.3	31.0	44.4	173.1	34.6
Delta Valencia	X639	35.1	51.8	44.7	51.1	69.1	251.8	50.4
McClellan Seedless	C35	81	64.3	32.5	46.3	46.1	270.2	54.0
McClellan Seedless	CC	29.2	54.6	22.6	27.5	32.4	166.3	33.3
McClellan Seedless	KC	24.7	58.3	21.6	38.8	46.7	190.1	38.0
McClellan Seedless	MxT	19.4	26.6	10.1	4.5	16.1	76.7	15.3
McClellan Seedless	SC	35.7	98.1	49.7	47.5	74.0	305.0	61.0

McClellan Seedless	TB	46.9	39.4	49.6	37.2	55.5	228.6	45.7
McClellan Seedless	X639	29.3	96.2	49.9	80.6	49.2	305.2	61.0
Midnight Valencia	C35	33.9	83.4	54.8	39.0	49.7	260.8	52.2
Midnight Valencia	CC	20.9	32.1	3.6	12.3	41.8	110.7	22.1
Midnight Valencia	KC	11.3	29.7	15.2	16.9	44.0	117.1	23.4
Midnight Valencia	MxT	8.2	27.0	1.2	8.6	30.3	75.3	15.1
Midnight Valencia	SC	13.8	57.7	8.1	30.7	53.0	163.3	32.7
Midnight Valencia	TB	19.6	53.7	14.3	16.1	36.6	139.7	27.9
Midnight Valencia	X639	5.2	17.9	3.3	13.0	36.3	75.7	15.1
Portsgate	C35	33.6	53.5	22.3	26.2	68.4	204.0	40.8
Portsgate	CC	26.6	31.3	9.6	4.2	50.9	122.6	24.5
Portsgate	KC	19	31.3	4.9	11.8	58.2	125.2	25.0
Portsgate	MxT	30.5	19.4	3.3	7.5	25.1	85.8	17.2
Portsgate	SC	55.2	44.0	19.9	15.3	106.0	240.4	48.1
Portsgate	TB	48	40.8	30.4	31.8	55.8	206.8	41.4
Portsgate	X639	35.6	73.0	37.1	73.1	91.7	310.5	62.1

5.5.8 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Satsuma mandarins in a cold production region (East Cape Midlands)
Project 57A by J. Joubert, R. Fenwick and S. Meeding (CRI)

Opsomming

Na aanleiding van die 2012 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 twee tellings 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, het Okitsu Wase ook kleiner vruggroottes gehad. Sonet kan nou hier ingevoeg word, met goeie interne kwaliteit, vruggrootte en uitstekende opbrengs, selfs nadat heelwat vrugval voorgekom het a.g.v. oopbars. Sonet was die enigste seleksie met saad gewees vir hierdie proef. Die Satsuma seisoen kan afgesluit word met die laat seleksie Ueno. Ueno se hoë suurvlakke dui aan dat hierdie seleksie vir 'n langer tydperk aan die bome 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.

Summary

The following recommendation can be made according to the results obtained from the 2012 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 two counts larger than Miho Wase, both have 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. Sonet would follow these with good internal quality, size and excellent yields, even after dropping fruit due to splitting. However, for this trial Sonet was the only selection with seeds in the fruit. The Satsuma season ends with the late selection Ueno, 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 good internal quality and prevent fruit from becoming puffy. All Satsuma selections would require degreening after harvest as all were internally mature but had inadequate external colour.

Objectives

- To select Satsuma cultivars with improved and consistent productivity, fruit size, rind colour, and internal fruit quality (Brix, acidity and ratio).
- To extend the harvest period (both earlier and later maturity).
- To describe the characteristics of new Satsuma cultivars and determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Satsuma selections from the Adelaide region of the East Cape Midlands and included the following varieties: Okitsu Wase, Miyagawa Wase, Kuno, Ueno, Miho Wase and Sonet.

Table 5.5.8.1. List of Satsuma selections evaluated at Saxfold (Adelaide) during 2012.

Selection	Rootstock	Planted
Kuno	Carrizo	1995
Miho Wase	Swingle	1991
Miyagawa Wase	Swingle	2000
Okitsu Wase	Carrizo	2000
Sonet	Carrizo	
Ueno	Carrizo	1999

Results and discussion

These results are from commercial orchards of various ages in the area. Please note that the same rootstock was not used for these selections and may affect maturity and internal quality.

When the ratio between sugar and acid is 10:1, the fruit is considered to be at peak maturity. A ratio of 9:1 is considered to be the build-up towards peak maturity of 10:1. After reaching the peak, the ratio increases to 11:1, after which it is considered overmature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in greater instances of quality and rind issues.

According to the results shown in Table 5.5.8.2, Miyagawa Wase was the earliest to mature. This was followed by Miho Wase, Kuno, Okitsu Wase, Sonet and lastly Ueno. Fruit size was found to be largest in Miyagawa Wase (Count 1x); with smallest fruit size present in Miho Wase, Okitsu Wase and Ueno (Count 2). The fruit size increased relative to the previous season. Sonet had the highest juice level (60.6%), as well as the lowest acid content (0.85%) with a Brix: acid ratio of 10.5, but it was the only selection producing three seeds per fruit. Colour was poor on all varieties with Miyagawa Wase and Miho Wase having the poorest colour when harvested (T5-6). Degreening of fruit would be required following harvest.

Conclusion

Miho Wase and Miyagawa Wase were the earliest selections and had the greatest yields. Okitsu Wase and Kuno were towards the middle of the Satsuma season and had slightly lower yields than the earlier varieties. Sonet followed with good yields and good quality. Ueno was the latest selection and had slightly lower yields than the mid-maturing Satsuma selections. There was an average external colour delay on all the selections.

Table 5.5.8.2. Internal fruit quality data for Satsuma selections in the Adelaide region (Saxfold) of the East Cape Midlands (ECM) during the 2012 season.

Selection	Root-stock	Date harvested	Count	Juice %	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Kuno	CC	22-Mar-12	1	56.6	9.0	1.21	7.4	0	5
Kuno	CC	04-Apr-12	1	57.8	9.6	1.04	9.2	0	5-6
Miho Wase	SC	22-Mar-12	2	50.9	9.8	0.98	10.0	0	5
Miyagawa Wase	SC	22-Mar-12	1	57.2	9.7	1.23	7.9	0	5-6
Miyagawa Wase	SC	04-Apr-12	1x	56.4	10.1	1.01	10.0	0	4-5
Okitsu Wase	CC	22-Mar-12	2	57.5	10.1	1.43	7.1	0	4-5
Okitsu Wase	CC	04-Apr-12	1	59.4	10.7	1.31	8.2	0	5
Sonet		13-Apr-12	1	60.6	8.9	0.85	10.5	0.3	5-6
Ueno	CC	22-Mar-12	2	51.5	9.9	1.98	5.0	0	7-8
Ueno	CC	04-Apr-12	2	58.6	11.1	1.57	7.1	0	6-7

Ueno	SC	12-Apr-12	1	58.7	11.3	1.52	7.4	0	5-6
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5.5.9 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Satsuma mandarins in a cold production region (Western Cape)

Project 57D by J. Joubert, R. Fenwick and S. Meeding (CRI)

Opsomming

Ueno Satsuma was die seleksie wat eerste rypgeword het, maar in die ander produksie areas word dit aangedui as laat rypwordend (Adelaid). Die resultate van die vorige seisoen verskil van die huidige inligting. Die seisoen moer eerder met Aoshima begin word, met goeie opbrengs, alhoewel die seleksie nie te lank op die bome gehou moet word nie as gevolg van die vinnige daling in suurvlakke. Ohtsu volg met goeie opbrengs en interne kwaliteit. 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 laatste Satsuma seleksie, maar word nie aanbeveel nie as gevolg van groeikragtige bome en swak opbrengs. Kleur het goed ontwikkel en interne kwaliteit was goed, maar hierdie seleksie sal verder ge-evalueer moet word om vas te stel of dit enige waarde inhou vir die sitrusbedryf. Pluk periodes moet nie langer as 2 tot 3 weke wees nie, om hoë interne kwaliteit te behou en powwerige vrugte te vermy. Alle Satsuma seleksies moes ontgroen word na oes, alhoewel sekere seleksies effens beter opgekleur was as van die ander, by interne optimum.

Summary

Ueno Satsuma was shown to be the earliest maturing variety in this trial, but in the other production areas it was a late maturing selection (Adelaide). The results obtained from the previous year in this area also differ from the current results. The season should rather start with Aoshima, which has good yields but should not be kept too long on the trees because acid drops rapidly towards the end of the season. This can then be followed up by Ohtsu, 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 for the industry. Picking periods should be limited to 2 to 3 weeks to maintain good internal quality and to avoid puffiness. All Satsuma selections would require degreening after harvest, although there were some selections that had slightly better colour than others when internally mature.

Objectives

- To select Satsuma cultivars with improved and consistent productivity, fruit size, rind colour, and internal fruit quality (Brix, acidity and ratio).
- To extend the harvest period (both earlier and later maturity).
- To describe the characteristics of new Satsuma cultivars and determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Late Satsuma selections from the Paarl region of the Western Cape. The following selections were evaluated: Aoshima, Dobashi Beni, Imamura, Ohtsu, Owari and Ueno.

Table 5.5.9.1. List of Satsuma selections evaluated at Lustigaan (Paarl) during 2012.

Selection	Rootstock	Planted
Aoshima	Carrizo	2006
Aoshima	Swingle	2006
Dobashi Beni	Carrizo	2006
Imamura	Carrizo	2006
Ohtsu	Carrizo	2006
Owari	Carrizo	2006
Ueno	Carrizo	2006

Results and discussion

These results are from a six-year-old trial block in the area.

When the ratio between sugar and acid is 10:1, the fruit is considered to be at peak maturity. A ratio of 9:1 is considered to be the build-up towards peak maturity of 10:1. After reaching the peak, the ratio increases to 11:1, after which it is considered overmature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in greater instances of quality and rind issues.

The results from the 2012 season (Table 5.5.9.1) show that Ueno was the earliest to mature. This was followed by Aoshima on Carrizo, Ohtsu, Dobashi Beni, Owari, Aoshima on Swingle and Imamura which was the latest selection. Aoshima on Swingle was approximately two weeks later than Aoshima on Carrizo, with similar Brix levels, but Brix: acid ratio difference of 2.4. In the other production areas Ueno Satsuma was the latest selection to mature, for example in the East Cape Midlands (Adelaide) during 2012 season. Internal quality was better this season and Brix at the second evaluation tested above 10, juice levels on all the selections were above 50% and Brix: acid ratios were as high as 15.8 (Ueno). Fruit size peaked at count 1X on all the selections, and there were seeds in the Aoshima, Ohtsu and Ueno fruit. The best colour development was present on Owari (T1), followed by Dobashi between T1 and T2.

Conclusion

Ueno was the earliest selection with good yields and external colour. This was followed by Aoshima on Carrizo, Ohtsu and Dobashi Beni, producing good internal quality and acceptable external colour. Owari were next to mature, followed by Aoshima on Swingle. Imamura remained the latest maturing Satsuma for this trial site and had very poor yields, similar to the previous season, although colour development and internal quality were good (lowest Brix: acid ratio of 10.7).

Table 5.5.9.1. Internal fruit quality data for Satsuma selections in the Paarl region (Lustigaan) of the Western Cape during the 2012 season.

Selection	Root-stock	Date harvested	Count	Juice %	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Aoshima	CC	27-Mar-12	3	50.8	9.6	1.50	6.4	0.1	8
Aoshima	CC	21-May-12	1x	48.6	10.5	0.71	14.8	0	2-3
Aoshima	SC	21-May-12	1x	51.4	10.3	0.83	12.4	0.3	2-3
Dobashi Beni	CC	27-Mar-12	3	53.5	9.9	1.59	6.2	0	7-8
Dobashi Beni	CC	21-May-12	1x	51.0	10.8	0.78	13.8	0	1-2
Imamura	CC	27-Mar-12	3	56.0	9.6	1.96	4.9	0	8
Imamura	CC	21-May-12	1x	56.0	10.3	0.96	10.7	0	3-4
Ohtsu	CC	27-Mar-12	2	54.2	9.5	1.30	7.3	0	8
Ohtsu	CC	21-May-12	1x	51.4	10.9	0.72	15.1	0.1	2-3
Owari	CC	27-Mar-12	3	57.4	9.5	1.48	6.4	0	8
Owari	CC	21-May-12	1x	50.6	10.4	0.80	13.0	0	1
Ueno	CC	27-Mar-12	1	55.9	9.7	1.38	7.0	0.1	8
Ueno	CC	21-May-12	1x	54.4	10.9	0.69	15.8	0	2

5.5.10 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Clementine mandarins in a cold production region (Western Cape)

Project 1000D by J. Joubert, R. Fenwick and S. Meeding (CRI)

Opsomming

Die Bonanules, wat 'n eksperimentele seleksie is, kan 'n moontlike opsie wees 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. (moontlike oopbars van vrugte word gemonitor). Marisol volg met die beste

vruggrootte vir die seisoen, asook baie goeie interne kwaliteit. Clemenpons se rypwordings tyd is volgende, met baie goeie opbrengs, goeie interne kwaliteit en kleiner vruggrootte as Marisol. Nules sal die Clementine seisoen eindig en vrugte kan goed hang aan die bome. Al vier seleksies het 'n vertraging in eksterne kleur ontwikkeling gehad, hierdie waarneming was nie in 2011 'n probleem nie.

Summary

The Bonanules, an experimental selection, would be a possible option to start off the Clementine season, with flatter fruit than the other selections as well as having good size, internal quality and yields, but occasional fruit splitting.. This could be followed by Marisol, with the biggest fruit size for the season, and with very good internal quality. Clemenpons follows closely with very good yields, good internal quality but small fruit size compared to Marisol. Nules will end the Clementine season as the fruit hangs well. All four selections experienced a delay in external colour, whereas colour development was not noted as a problem in the 2011 season.

Objectives

- To select Clementine cultivars with improved and consistent productivity, fruit size, rind colour, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the cultivar characteristics of new Clementine cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Clementine selections from the Wellington region of the Western Cape; the planting age is unknown. The following varieties were evaluated: Bonanules Clemenpons, Marisol and Nules.

Table 5.5.10.1. List of Clementine selections evaluated at Bonathaba (Wellington) during 2012.

Selection	Rootstock	Planted
Bonanules	Troyer	Unknown
Clemenpons	Carrizo	Unknown
Marisol	Troyer	Unknown
Nules	Troyer	Unknown

Results and discussion

These results are from commercial orchards in the area. This project is ongoing and additional varieties are planned for inclusion in the future.

According to the results shown in Table 5.5.10.2, Bonanules (experimental selection) is the earliest maturing selection followed by Marisol for this season, which matures approximately two weeks later. Clemenpons follows next in line and the latest maturing selection for this site was Nules. The fruit size increased for this season and peaked at count 1X for Marisol, followed by Bonanules (count 1) and Clemenpons as well as Nules with count 3. The highest Brix level was on Nules (13.1) taking the time of maturity into consideration, and the lowest was Marisol with 10.4. Nules had the highest acid content for this season as well as the highest Brix, resulting in a fairly low Brix: acid ratio of 8.7. Yields were very good for all cultivars with Marisol and Nules having the greatest yield, followed by Bonanules and Clemenpons with slightly lower, but similar yields. The external colour development was delayed this season compared to 2011 and peaked between T4 and T8. Fruit shape for Bonanules is flat whereas Marisol, Clemenpons and Nules have rounder fruit. There were no seeds in any of the evaluated fruit this season.

Conclusion

Bonanules was the earliest to mature followed by Marisol, Clemenpons and then Nules. Fruit size increased and the largest count size was on Marisol (1X) There was a delay in external colour compared to the 2011 season. All four selections were seedless when evaluated.

Table 5.5.10.2. Internal fruit quality data for Clementine selections in the Wellington region (Bonathaba) of the Western Cape during the 2012 season.

Selection	Root-stock	Date harvested	Count	Juice %	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Bonanules	TC	27-Mar-12	1	59.6	11.1	0.99	11.2	0	5
Clemenpons	CC	27-Mar-12	3	58.0	11.8	1.42	8.3	0	7
Marisol	TC	27-Mar-12	1x	64.1	10.4	1.10	9.5	0	4-5
Nules	TC	27-Mar-12	3	56.7	13.1	1.51	8.7	0	7-8

5.5.11 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Valencia Oranges in a cold production region (Sundays River Valley)

Project 75C by J. Joubert, R. Fenwick and S. Meeding (CRI)

Opsomming

Die resultate van die 2012 seisoen het bewys dat Delta Valencia 'n goeie opbrengs gehad het en dat dit voor Turkey rypgeword het, met Volckameriana as onderstam. Op 'n goeie interne kwaliteit induserende onderstam, sal Delta na Turkey en Delicia ryppword, alhoewel die twee verskillende areas waar die bome ge-evalueer was, asook tipe onderstam invloed het. Turkey het die beste interne gehalte gehad met goeie opbrengs. Na Turkey was dit Delicia gewees wat goeie opbrengs behaal het, met aanvaarbare interne kwaliteit, suurvlaakte was aan die hoër kant gewees. Vruggrootte was goed gewees vir Turkey en Delicia, maar kleiner vir Delta. Kleur het goed gevorder in alle seleksies en vrugte was opgekleur voor vrugte intern gereed 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, veral in die geval van Turkey.

Summary

The results from the 2012 season show that Delta Valencia had good yields and matured earlier than Turkey, although it is on Volckameriana. On a good internal quality inducing rootstock, Delta Valencia will mature after Turkey and Delicia, although the two different areas where the trees were evaluated can have an impact and the rootstock combination. Turkey had the best internal quality and also had good yields. After Turkey the selection that matured next was Delicia, which had good yields and acceptable internal quality; acid being on the high side. Fruit size on Turkey and Delicia was good but fruit size on Delta was smaller. 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.

Objectives

- To select Valencia cultivars with improved and consistent productivity, fruit size, rind colour, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new Valencia cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Valencia selections from the Sundays River Valley region. A range of new Valencia selections will be included in a trial in the area shortly. The following varieties were evaluated: Delicia, Delta and Turkey.

Table 5.5.11.1. List of Valencia selections evaluated at Invercloy and H. Ehlers (SRV) during 2012.

Selection	Rootstock	Planted
Turkey	Carrizo	2001
Delicia	Carrizo	2001
Delta	Volckameriana	2001

Results and discussion

These results are from commercial orchards of various ages and rootstocks. This project will be replaced by a new trial with additional selections on the same rootstock, once trees are bearing fruit.

When the ratio between sugar and acid is 10:1, the fruit is considered to be at peak maturity. A ratio of 9:1 is considered to be the build-up towards peak maturity of 10:1. After reaching the peak, the ratio increases to 11:1, after which it is considered overmature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in greater instances of quality and rind issues.

These selections were evaluated to obtain information and establish differences between the commercial Valencia selections in the area. These evaluations will be replaced with a trial including several new and existing Valencia selections once trees are bearing, in order to determine suitability in the Sundays River Valley as the commercially used cultivars in the area are limited. According to the results shown in Table 5.5.11.2, Delta had the highest Brix: ratio for this season. Turkey matured first, followed by Delicia and Delta. The internal quality indicates a different scenario, showing that Delta matures first, followed by Turkey and Delicia, but keep in mind that these Delta trees were planted on Volk which induces lower acid levels in the fruit. On average Turkey had the highest Brix levels (11.9°). Delta had good Brix levels (10.1°) and fairly low acid levels (0.95) compared to the other selections. Delicia had on average good Brix levels (10.1°) compared with acid of above 1.2% fairly late in the season. Seed was present in Turkey Valencia (average 1.4 seeds per fruit) and Delicia had no seeds compared to the previous season. Colour development was good for all varieties (T1). Yields were also good for all varieties.

Conclusion

The internal quality for Delta indicated that this selection matures first, although planted on Volk that induces low acids. There were two different sites and the Delta trees might be in an earlier area. Due to experience from other trial blocks and production areas, Turkey Valencia will mature first, being the earliest commercial Valencia selection available, with very good internal quality, good fruit size and some seed. This is followed by Delicia also with good fruit size and acceptable internal quality. Delta then normally ends the Valencia season with good internal quality and yields but smaller fruit size.

Table 5.5.11.2. Internal fruit quality data for Valencia selections in the Sundays River Valley during the 2012 season.

Selection	Root-stock	Date harvested	Site	Count	Juice %	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Delicia	CC	31-Jul	Invercloy	56	53.3	9.6	1.28	7.5	0	1
Delicia	CC	21-Aug-12	Invercloy	56	57.8	10.1	1.22	8.3	0	1
Turkey	CC	31-Jul-12	Invercloy	56	54.2	11.9	1.29	9.2	1.4	1
Delta	Volk	31-Jul-12	H Ehlers	72	57.1	10.1	0.95	10.6	0	1

5.5.12 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Valencia Oranges in a cold production region (Western Cape)

Project 75D by J. Joubert, R. Fenwick and S. Meeding (CRI)

Opsomming

Vir die 2012 seisoen was Limpopo SL die vroegste Valencia seleksie gewees om ryp te word. Interne kwaliteit was goed, met klein tot medium vruggrootte en effens dikker skille by tye. Midnight 1 volg vir hierdie proef perseel (word na Benny 1&2 ryp in die Noordelike produksie areas) met goeie interne kwaliteit, klein tot medium vruggrootte en goeie opbrengs. Benny 1 word voor Benny 2 ryp en eindig die Valencia seisoen vir hierdie proef. Albei seleksies het baie goeie opbrengs, uitstekende interne kwaliteit en goeie vruggrootte gehad. Saad is in albei seleksies gevind, met groter hoeveelhede in Benny 1 teenoor Benny 2. 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.

Summary

The results from the 2012 season showed that Limpopo SL was the earliest Valencia to mature with good internal quality, small to medium fruit size, moderate yield but with occasional thicker rinds. Midnight 1 was the next to mature at this trial site (it matures after Benny 1&2 in Northern production areas), with good internal quality, small to medium fruit size and good yield. Benny 1 matured before Benny 2 which brought the Valencia season at this trial 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 good internal quality and to avoid rind disorders.

Objectives

- To select Valencia cultivars with improved and consistent productivity, fruit size, rind colour, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new Valencia cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Valencia selections from the Citrusdal region of the Western Cape. A range of new Valencia selections will be added to this trial and will be planted in September 2013. The following varieties were evaluated: Benny 1, Benny 2, Limpopo SL and Midnight 1.

Table 5.5.12.1. List of Valencia selections evaluated at Patrysburg (Citrusdal) during 2012.

Selection	Rootstock	Planted
Limpopo SL	Carrizo	2008
Midnight 1	Carrizo	2008
Benny 1	Carrizo	2008
Benny 2	Carrizo	2008

Results and discussion

These results are from the first crop of a four-year-old trial block in the Citrusdal region of the Western Cape. This project will be boosted by the addition of several new Valencia selections in September 2013.

This is the second crop from this trial block. Additional cultivars will be added to these existing selections. According to the results in Table 5.5.12.2, Limpopo SL is the first to mature, followed by Midnight 1 almost five weeks later and then Benny 1 and Benny 2. Midnight 1 and Benny 1 had the smallest fruit size (count 72 & 64), with Benny Limpopo SL and Benny 1 having fruit size one count larger (count 64). On average, Limpopo SL had the lowest juice percentage (46%) compared to the other selections (on average all over 55%). This may be attributed to the thicker than usual rinds found in Limpopo SL. On average, Benny 2 had the highest Brix levels (13.4°) followed by Benny 1 and Midnight 1 (12.4°) with Limpopo SL having the lowest Brix levels (11.9°). Yields were poorest on the Limpopo SL, with Midnight 1 having better yields. Both Benny 1 and Benny 2 had very good to excellent yields. Colour development was good for all selections. Midnight 1 and Limpopo SL were completely seedless, with Benny 2 having on average less seed (0.1 seed) per fruit than Benny 1 (1.75 seeds).

Conclusion

Limpopo SL was the earliest to mature, followed by Midnight 1 and then Benny 1 and 2, with Benny 1 slightly before 2. Benny 1 and Midnight 1 had smallest fruit size and Benny 2 and Limpopo SL the largest. Benny 2 had the highest Brix levels and Benny 1 the most seeds per fruit. Limpopo SL and Midnight 1 were completely seedless.

Table 5.5.12.2. Internal fruit quality data for Valencia selections in the Citrusdal region (Patrysburg) of the Western Cape during the 2012 season.

Selection	Root-stock	Date harvested	Count	Juice %	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Benny 1	CC	19-Jun-12	72	59.1	12.6	2.06	6.1	1.9	1
Benny 1	CC	31-Jul-12	64	56.4	12.4	1.45	8.6	1.6	1
Benny 2	CC	19-Jun-12	64	51.9	12.8	2.13	6.0	0	1
Benny 2	CC	31-Jul-12	64	57.4	13.4	1.71	7.8	0.1	1
Limpopo seedless	CC	19-Jun-12	64	46.2	11.2	1.01	11.1	0	1
Limpopo seedless	CC	31-Jul-12	64	45.4	11.9	1.05	11.3	0	1
Midnight 1	CC	19-Jun-12	72	54.2	10.6	1.28	8.3	0	1
Midnight 1	CC	31-Jul-12	64	55.0	12.4	1.21	10.2	0	1

5.5.13 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (East Cape Midlands)

Project 997A by J. Joubert, R. Fenwick and S. Meeding (CRI)

Opsomming

Die resultate van die 2012 seisoen het aangedui dat Tahoe Gold die vroegste ryp geword met die tweede grootste vruggrootte, 0.3 sade per vrug en goeie interne kwaliteit. Yosemite Gold het die grootste vrugte geproduseer met 0.7 sade per vrug gemiddeld, en die seleksie was deel van die reeks Manderyn Hibriede wat in die middel van die manderyn seisoen ryp geword het vir hierdie proef persele. Shasta Gold was een van die later seleksies, met 0.2 sade per vrug gemiddeld en dieselfde vruggrootte as Tahoe Gold (1XX). Gold Nugget was basies saadloos en was gereed vi roes teen einde Julie tot middel Augustus. Tango presteer goed, was totaal saadloos met die kleinste vruggrootte in vergelyking met die ander seleksies. Winnola was die laatste seleskie gereed vir oes teen einde Augustus tot middel September, wat die Manderyn Hibried seisoen afsluit vir hierdie proef. Winnola was totaal saadloos met goeie inetrne kwaliteit. Daar word aanbeveel om nie die oesperiode langer as 2 tot 3 weke te verleng nie om goeie interne kwaliteit te verseker met minimum skil probleme.

Summary

The results of the 2012 season indicate that Tahoe Gold matures first with the second largest fruit size, 0.3 seeds per fruit and good internal quality. Yosemite Gold had the largest fruit size, produced 0.7 seeds per fruit average and matured in the middle of the Mandarin Hybrid range evaluated at these trial sites. Shasta Gold was one of the later maturing selections, had 0.2 seeds per fruit on average and the same fruit size as Tahoe Gold (1XX). Gold Nugget was virtually seedless, maturing at the end of July to the beginning of August. Tango performed well, was completely seedless with the smallest fruit size compared to the other selections. Winnola was the last selection to mature, at the end of August to the middle of September, ending of the Mandarin Hybrid season for this trial. Winnola was completely seedless as well with very good internal quality. Picking periods should not be longer than 3 - 4 weeks to maintain good internal quality and to avoid rind disorders.

Objectives

- To select Mandarin Hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new Mandarin Hybrid cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Mandarin Hybrid selections from the Cookhouse and Fort Beaufort region of the East Cape Midlands. A range of new Mandarin Hybrids have

been added to this area and should be bearing fruit in the 2013 season. The following varieties were evaluated: Gold Nugget, Nadorcott, Shasta Gold, Tahoe Gold, Tango, Winnola and Yosemite Gold.

Table 5.5.13.1. List of Mandarin Hybrid selections in the Cookhouse (J&B) region of the East Cape Midlands during the 2012 season.

Selection	Rootstock	Topwork
Gold Nugget	CC	2010
Nadorcott	CC	2010
Shasta Gold	CC	2010
Tahoe Gold	CC	2010
Tango	CC	2010
Winnola	CC	2010
Yosemite Gold	CC	2010

Table 5.5.13.2. List of Mandarin Hybrid selections in the Fort Beaufort (Riverside) region of the East Cape Midlands during the 2012 season.

Selection	Rootstock	Topwork
Gold Nugget	CC	2010
Shasta Gold	CC	2010
Tahoe Gold	CC	2010
Tango	CC	2010
Yosemite Gold	CC	2010

Results and discussion

These results are from semi-commercial orchards in the area. This project has been boosted by the addition of several new Mandarin Hybrid selections.

When the ratio between sugar and acid is 12:1, the fruit is considered to be at peak maturity for Mandarin Hybrids. This ratio is raised as a result of the high sugar levels associated with the new selections. A ratio of 11:1 is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which it is considered overmature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in a greater instance of quality and rind issues.

According to the results shown in Tables 5.5.13.3 and 5.5.13.4, Tahoe Gold matured first, followed by Nadorcott, Gold Nugget and Yosemite Gold maturing on average at the same time. This was followed by Tango and Shasta Gold which matured at the same time. The latest selection to mature at the two trial sites was Winnola, ending off the Mandarin Hybrid season. The maturity times for these experimental selections will be determined for the different production regions according to climate. Internal Quality on all the selections was good to very good. Fruit size was largest for Yosemite Gold (1XXX), followed by Shasta- and Tahoe Gold (1XX) and a combination of Gold Nugget and Winnola with count 1-1. Nadorcott followed by Tango had the smallest fruit size. All mandarins were of exceptional quality with Winnola having the highest Brix on average of 11.6°. This was followed by Shasta Gold (10.8) and Tango (10.6). Yosemite Gold had the lowest average Brix levels (10.0°) although this is still acceptable for young trees. Colour was very good for all the selections with no degreening required as all selections were fully coloured when harvested (T1). Shasta (0.2 seeds), Tahoe (0.3 seeds) and Yosemite Gold (0.7 seeds) had the highest incidence of seed per fruit, followed by Gold Nugget with less than 0.1 seeds. Tango and Winnola were completely seedless.

Conclusion

Tahoe Gold was first to mature, followed by Nadorcott, Gold Nugget and Yosemite Gold in the same time frame. The next Mandarin selection to mature was Tango and Shasta Gold, with Winnola ending of the Mandarin season. Yosemite Gold had the largest fruit size, followed by Shasta- and Tahoe Gold, then Gold Nugget, Winnola and Nadorcott. The smallest fruit size was produced on Tango. The three TDE selections Tahoe-, Shasta- and Yosemite Gold had the highest incidence of seed, with Tango and Winnola being seedless. Colour development was good on all selections.

Table 5.5.13.3. Internal fruit quality data for Mandarin hybrid selections from the Cookhouse (J&B) region of the East Cape Midlands during the 2012 season.

Selection	Root-stock	Date harvested	Count	Juice %	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Gold Nugget	CC	06-Jun-12	1	51.0	9.8	1.27	7.7	0	6-7
Gold Nugget	CC	22-Jun-12	1x	53.8	10.7	1.23	8.7	0	5
Gold Nugget	CC	11-Jul-12	1x	56.3	10.5	1.03	10.2	0	2
Gold Nugget	CC	07-Aug-12	1xx	50.0	12.0	0.95	12.6	0	1
Gold Nugget	CC	22-Aug-12	1x	47.5	11.4	0.79	14.4	0.1	1
Gold Nugget	CC	03-Sep-12	1x	51.5	12.0	0.76	15.8	0.2	1
Gold Nugget	CC	25-Sep-12	1x	51.2	11.4	0.64	17.8	0	1
Nadorcott	CC	06-Jun-12	1	55.2	9.1	1.12	8.1	0	5-6
Nadorcott	CC	22-Jun-12	1	58.3	9.2	1.16	7.9	0.1	2
Nadorcott	CC	11-Jul-12	1x	53.8	10.0	1.11	9.0	0	1
Nadorcott	CC	07-Aug-12	1	53.8	10.8	0.90	12.0	0	1
Nadorcott	CC	22-Aug-12	1x	47.8	11.1	0.88	12.6	0	1
Shasta Gold	CC	06-Jun-12	1xx	56.0	9.0	1.53	5.9	0.6	6
Shasta Gold	CC	22-Jun-12	1xx	52.9	9.7	1.52	6.4	0	3-4
Shasta Gold	CC	11-Jul-12	1xx	55.6	10.2	1.45	7.0	0.1	1-2
Shasta Gold	CC	07-Aug-12	1xx	55.0	10.9	1.22	8.9	0.2	1
Shasta Gold	CC	22-Aug-12	1xx	53.6	11.0	1.03	10.7	0.4	1
Shasta Gold	CC	03-Sep-12	1xx	54.2	12.6	1.04	12.1	0.1	1
Shasta Gold	CC	25-Sep-12	1xxx	54.0	11.9	0.84	14.2	0.3	1
Tahoe Gold	CC	06-Jun-12	1xx	61.2	8.7	1.06	8.2	0.6	6-7
Tahoe Gold	CC	22-Jun-12	1xx	55.6	9.1	1.01	9.0	0	4-5
Tahoe Gold	CC	11-Jul-12	1xx	55.6	9.8	0.91	10.8	0.2	1-2
Tahoe Gold	CC	07-Aug-12	1xx	55.6	10.1	0.85	11.9	0.4	1
Tahoe Gold	CC	22-Aug-12	1xx	52.1	11.0	0.73	15.1	0.3	1
Tahoe Gold	CC	03-Sep-12	1xx	58.0	11.8	0.83	14.2	0.2	1
Tango	CC	06-Jun-12	2	55.4	9.6	1.10	8.7	0	5-6
Tango	CC	22-Jun-12	2	63.6	10.3	1.21	8.5	0	4
Tango	CC	11-Jul-12	1	58.3	10.2	1.13	9.0	0	1-2
Tango	CC	07-Aug-12	2	54.5	11.2	1.05	10.7	0	1
Tango	CC	22-Aug-12	1	51.1	11.2	0.94	11.9	0	1
Tango	CC	03-Sep-12	1	55.3	11.3	0.89	12.7	0	1
Winola	CC	06-Jun-12	1	56.5	11.1	1.89	5.9	0	4
Winola	CC	11-Jul-12	1	57.1	10.8	1.66	6.5	0	1
Winola	CC	22-Aug-12	1x	59.4	11.3	1.34	8.4	0	1
Winola	CC	03-Sep-12	1x	59.7	12.2	1.33	9.2	0	1
Winola	CC	25-Sep-12	1	57.7	12.0	1.34	9.0	0	1
Winola	CC	07-Aug-12	1x	53.3	12.4	1.56	7.9	0	1
Yosemite Gold	CC	06-Jun-12	1xxx	53.7	8.7	1.31	6.6	1.2	7-8
Yosemite Gold	CC	22-Jun-12	1xxx	55.6	9.0	1.17	7.7	0.7	5
Yosemite Gold	CC	11-Jul-12	1xxx	54.5	9.1	1.07	8.5	0.5	4-5
Yosemite Gold	CC	07-Aug-12	1xxx	50.0	10.6	0.99	10.7	0.3	1
Yosemite Gold	CC	22-Aug-12	1xxx	51.4	10.5	0.79	13.3	0.5	1
Yosemite Gold	CC	03-Sep-12	1xxx	51.3	10.9	0.77	14.2	0.9	1
Yosemite Gold	CC	25-Sep-12	1xxx	54.2	10.3	0.70	14.7	0.9	1

Table 5.5.13.4. Internal fruit quality data for Mandarin hybrid selections from the Fort Beaufort (Riverside) region of the East Cape Midlands during the 2012 season.

Selection	Root-stock	Date harvested	Count	Juice %	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Gold Nugget	CC	30-Jul-12	1	54.5	12.9	1.03	12.5	0	1
Gold Nugget	CC	22-Aug-12	1xx	44.9	13.6	1.01	13.5	0	1
Shasta Gold	CC	06-Jun-12	1xx	57.5	10.6	1.81	5.9	0	4-5
Shasta Gold	CC	11-Jul-12	1x	55.6	12.4	1.64	7.6	0	1-2
Shasta Gold	CC	16-Jul-12	1xx	54.5	11.4	1.57	7.3	0	1
Shasta Gold	CC	30-Jul-12	1	53.3	11.5	1.09	10.6	0.2	1
Shasta Gold	CC	22-Aug-12	1xx	50.2	12.7	1.32	9.6	0	1
Shasta Gold	CC	13-Sep-12	1x	54.0	11.8	1.20	9.8	0	1
Tahoe Gold	CC	06-Jun-12	1	62.6	10.4	1.18	8.8	0.1	6
Tahoe Gold	CC	11-Jul-12	1xx	55.6	10.8	0.93	11.6	0	1
Tahoe Gold	CC	16-Jul-12	1x	58.3	11.3	0.99	11.4	0	1
Tahoe Gold	CC	30-Jul-12	1x	57.1	9.3	0.68	13.7	0	1
Tahoe Gold	CC	22-Aug-12	1xx	56.5	11.4	0.82	13.9	0.2	1
Tahoe Gold	CC	13-Sep-12	1x	55.1	11.1	0.83	13.4	0	1
Tango	CC	06-Jun-12	4-5	55.2	10.1	1.06	9.5	0	4-5
Tango	CC	11-Jul-12	1	58.3	10.0	1.02	9.8	0	1-2
Tango	CC	16-Jul-12	2	60.0	11.8	1.18	10.0	0	1
Tango	CC	22-Aug-12	1	50.4	12.1	1.00	12.1	0	1
Yosemite Gold	CC	30-Jul-12	1xx	53.3	10.5	1.10	9.5	0.4	1

5.5.14 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (Sundays River Valley)

Project 997B by J. Joubert, R. Fenwick and S. Meeding (CRI)

Opsomming

Die uitslae van die 2012 seisoen het bewys dat HE Mandarin die vroegste van die seleksies ryppgeword het. Vruggrootte was groot met goeie opbrengs. Nova was volgende om ryp te word met uitstekende interne kwaliteit en saadlose vrugte. Clemcott volg met die hoogste saadtelling per vrug, groot tot baie groot vruggrootte, asook die hoogste Brix inhoud. African Sunset het gevolg met 'n gemiddelde opbrengs en groot tot baie groot vrugte. Valley Gold was volgende om ryp te word, met die kleinste vruggrootte en hoogste suur vlakke. Gold Nugget eindig die Manderyn seisoen af, vrugkwaliteit was gemiddeld en kan toegeskryf word aan die bome se eerste drag. Daar word aanbeveel dat die oes periode nie langer as 2 tot 3 weke neem nie, om goeie interne kwaliteit te behou en na-oes skil probleme te vermy.

Summary

The results of the 2012 season show that HE Mandarin was the earliest of the selections to mature. Fruit size was large and yields were good. Nova was next to mature with excellent internal colour and seedless fruit. Clemcott followed with the greatest number of seed per fruit, large to very large fruit size, as well as the highest Brix content. African Sunset was next to mature in the season, also with relatively average yields and large to very large fruit. Valley Gold followed with the smallest fruit size and highest acid levels. Gold Nugget ended the Mandarin Hybrid season, fruit quality was average and will improve because this was the first crop on the trees. Picking periods should not be longer than 3 - 4 weeks to maintain a good internal quality standard and to avoid rind disorders.

Objectives

- To select Mandarin Hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).

- To describe the characteristics of new Mandarin Hybrid cultivars and to determine the climatic suitability of these cultivars in cold production regions

Materials and methods

Field evaluations and laboratory analyses were conducted on Mandarin Hybrid selections from the Sundays River Valley. A range of new Mandarin Hybrids have been added to this area and should be bearing fruit in the 2013 season. The following varieties were evaluated: Valley Gold, African Sunset, Clemcott, Nova, HE Mandarin and Gold Nugget.

Table 5.5.14.1. List of Mandarin hybrid selections evaluated in the Sundays River Valley region during the 2012 season.

Selection	Rootstock	Planted
HE Mandarin	Carrizo	2004
African Sunset	Carrizo	1997
Clemcott	Carrizo	2004
Valley Gold	Carrizo	1997
Nova	Carrizo	Unknown
Gold Nugget	Carrizo	2011

Results and discussion

These results are from commercial orchards in the area. This project has been boosted by the addition of several new Mandarin Hybrid selections which should have a crop in the 2012/3 season.

According to the results in Table 5.5.14.2, the HE Mandarin is first to mature, followed by Nova, Clemcott, and African Sunset. Valley Gold was the second latest selection to mature in this trial, and Gold Nugget ended the mandarin season by maturing last. Fruit size was extra large for the Clemcott, Gold Nugget and African Sunset (1XXX), with HE Mandarin being slightly smaller (1X) and Valley Gold, as well as Nova having the smallest size (2 to 1). Internal qualities were good for all selections. Clemcott had on average the highest Brix levels (14.4%) followed by Nova (13.7%), HEM (12.8%), Valley Gold (12.6%), African Sunset (12.3%) and lastly Gold Nugget with the lowest Brix levels (10.9%). The Gold Nugget trees were bearing their first crop so internal quality will improve. Acid levels were highest for Valley Gold (1.76%) and remained steady. These were followed by African Sunset (1.30%). Clemcott followed with 1.09%, and HE Mandarin, as well as Nova had the second lowest acid (0.98%) but there were insufficient internal quality tests to determine how acid would hold over time. Gold Nugget had on average the lowest acid levels (0.73%). Colour development was excellent with all selections being fully coloured with a deep orange rind when internally mature (T1). No seeds were present in Nova, Gold Nugget or African Sunset. Valley Gold and HE Mandarin had a low incidence of seed (average 0.7/0.9 seed per fruit) with Clemcott having the highest number of seed per fruit (average 5.7).

Conclusion

HE Mandarin was the earliest to mature, followed by Nova, Clemcott and then African Sunset and Valley Gold. Gold Nugget was the latest selection for this trial. Fruit size was largest in African Sunset and Clemcott. Valley Gold produced the smallest fruit size. Nova, Gold Nugget and Valley Gold were completely seedless and Clemcott was the seediest of them all. External colour development was very good: a deep orange colour.

Table 5.5.14.2. Internal fruit quality data for Mandarin hybrid selections from various regions of the Sundays River Valley during the 2012 season.

Selection	Root-stock	Date harvested	Site	Count	Juice %	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Valley Gold	CC	24-May-12	Dunbrody	2	64.8	11.9	2.01	5.9	1.1	4-5
Valley Gold	CC	04-Jun-12	Dunbrody	1	65.7	13.2	1.76	7.5	0.3	1
African Sunset	CC	24-May-12	Dunbrody	1x	59.9	11.7	1.33	8.8	0	2-3
African Sunset	CC	04-Jun-12	Dunbrody	1xxx	61.1	13.0	1.30	10.0	0	1
Clemcott	CC	04-Jun-12	Bonnievale	1xx	59.6	14.7	1.19	12.4	5.9	1
Clemcott	CC	04-Jul-12	Bonnievale	1xxx	54.5	14.1	1.09	12.9	5.5	1

HEM	CC	04-Jun-12	Bonnievale	1x	55.2	12.8	0.99	12.9	0.9	1
Nova		04-Jul-12	Bonnievale	1	58.3	13.7	0.98	14.0	0	1
Gold Nugget	CC	27-Sep-12	Bonnievale	1xx	59.2	10.9	0.73	14.9	0	1

5.5.15 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (Gamtoos River Valley)

Project 997C by J. Joubert, R. Fenwick and S. Meeding (CRI)

Opsomming

Die uitslae van die 2012 seisoen toon aan dat Tasty dogterbome die vroegste seleksie is met goeie opbrengs en groot vruggrootte, asook die laagste saadtelling per vrug. Tasty 1 is volgende op die lys met goeie tot baie goeie produksie, alhoewel vruggrootte baie groot is en die hoeveelheid saad per vrug aansienlik afgeneem het in vergelyking met 2011. Tasty 2 word laaste 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. Die hoeveelheid saad per vrug het ook aansienlik afgeneem. 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, veral in die geval van Tasty 2.

Summary

The results of the 2012 season show that the Tasty daughter trees were the first to mature with good yields and large fruit size, as well as the lowest number of seeds per fruit. Tasty 1 was next to mature with good to very good yields, but fruit size was extra large and the seed count per fruit decreased considerably compared to 2011. Tasty 2 matures last with excellent yields, good fruit size and intense orange-red coloured rind; however, the fruit tends to be externally over-mature (puffy) when internals are still immature. The seed count per fruit also decreased considerably for Tasty 2. 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.

Objectives

- To select Mandarin Hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new Mandarin Hybrid cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Mandarin Hybrid selections from the Gamtoos River Valley (Hankey). A range of new Mandarin Hybrids have been added to this area and should be bearing fruit in the 2013/14 season. The following varieties were evaluated: Tasty 1, Tasty 2 and Tasty daughter trees. The next season will include Shasta, Tahoe and Yosemite Gold, as well as Gold Nugget and Tango.

Table 5.5.15.2. List of experimental Mandarin hybrid selections evaluated in the Hankey region of the Gamtoos River Valley during the 2012 season.

Selection	Rootstock	Planted
Tasty Daughter		
Tasty 1	Seedling	Unknown
Tasty 2	Seedling	Unknown

Results and discussion

These results are from commercial orchards in the area. These selections are mostly natural mutations that are being evaluated to determine their value to the citrus industry. Tasty 1 & 2 have been planted from seed, but the Tasty daughter trees were topworked. The age of the mother trees is unknown. The next season (2013/2014) will have some additional Mandarin hybrid selections coming into production.

According to the results in Table 5.5.15.2, the Tasty daughter trees were the earliest to mature. These were followed by Tasty 1 and then Tasty 2. Tasty 2 seems to be fairly late to mature internally; over four weeks later. Fruit remained firm throughout the evaluation period and the external appearance is maintained. However, this does not apply to Tasty 2. Although this selection is regarded as being internally mature in August, long before this time fruit becomes puffy and the rind begins breaking down. This selection is far beyond over mature externally by the time it is regarded as internally mature. Tasty 1 had the largest fruit size (1XXX) with Tasty daughter trees 1XX and Tasty 2 having the smallest fruit size for this trial site (1X). The Tasty daughter trees had the highest average Brix levels (13.0°) followed by Tasty 2 (12.7°) and lastly Tasty 1 with the lowest average Brix levels (12.3°). Colour development was excellent on all selections. The best colour was displayed by Tasty 2, which has an almost red-orange colour and stands out when compared to the surrounding selections. Due to these trees being planted in a mixed planting, there is a high incidence of seed present in fruit. The selection with the highest number of seed is Tasty 1 with an average of 9.0 seeds per fruit, a substantial decrease compared to last season with 24.1 seed per fruit, almost making the fruit inedible. This is followed by Tasty 2 with an average of 5.9 seed per fruit. These selections will have to be planted in solid plantings to determine the level of seediness in a controlled environment as the current seed levels in some of these selections are unacceptable for an otherwise promising cultivar.

Conclusion

The Tasty daughter trees mature first followed by Tasty 1 and then Tasty 2. Tasty 1 had the highest number of seed with the daughter trees having the lowest amount. All three selections had similar colour development, although Tasty 2 had delayed internal quality compared to the other selections. Tasty 2 had the deepest red-orange colour.

Table 5.5.15.2. Internal fruit quality data for experimental Mandarin hybrid selections from the Hankey region of the Gamtoos River Valley region during the 2012 season.

Selection	Root-stock	Date harvested	Count	Juice %	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Tasty Daughter		24-Jul-12	1xx	50.0	13.0	0.93	14.0	4.8	1
Tasty 1		05-Jul-12	1xxx	48.0	11.7	1.08	10.8	10.3	1
Tasty 1		24-Jul-12	1x	50.0	11.9	1.13	10.5	7	1
Tasty 1		20-Aug-12	1xxx	39.7	13.8	1.08	12.8	9.8	1
Tasty2		05-Jul-12	1x	53.3	12.7	1.66	7.7	5.9	1

5.5.16 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (South Western Cape)

Project 997E by J. Joubert, R. Fenwick and S. Meeding (CRI)

Opsomming

Die resultate van 2012 toon aan dat 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 bv. Carrizo. Die bevinding was dat die kleiner vrugte 'n swakker interne kwaliteit gelewer het as die groter vrugte, asook later intern ryp as die groot vrugte. Bome is nog jonk so interne gehalte behoort te verbeter soos die bome ouer word. Sweet Spring se opbrengs was uitstekend gewees. Die oes periode moet nie langer wees as 2 tot 3 weke nie om goeie interne gehalte te verseker en skilprobleme te voorkom. Daar was bemarkingsprobleme met met die vrugte gewees, en toekomstige nish markte gaan krities wees vir die Sweet Spring se winsgewendheid en voortbestaan.

Summary

The results from the 2012 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 for example Carrizo. Smaller fruit were of poorer quality this season than the larger fruit and were internally mature later than the bigger fruit. The quality of Sweet Spring will improve as the trees get older. 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. There were problems with the

marketing of the fruit, and future niche markets for this selection might be crucial to ensure profitability and survival of the Sweet Spring cultivar.

Objectives

- To select Mandarin Hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new Mandarin Hybrid cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on a Mandarin Hybrid selection from the Rheenendal region from the South Western Cape: the Sweet Spring, a relatively unknown but unique selection.

Table 5.5.16.1. List of Sweet Spring selections evaluated in the Rheenendal region of the Western Cape during the 2012 season.

Selection	Rootstock	Planted
Sweet Spring (large)	Swingle	2008
Sweet Spring (small)	Swingle	2008

Results and discussion

These results are from commercial orchards in the area. This selection was evaluated due to its unique environmental requirements and new-found interest for the overseas market. The rootstock trial has been postponed until further notice, due to marketing problems with the Sweet Spring selection. The rootstocks will be used for a Lemon trial in the SRV region of the Eastern Cape. Other Mandarin selections were topworked at the Western Cape trial site to expand the variety of cultivars being evaluated to determine the suitability in that area.

Unlike other Mandarin Hybrid selections, the Sweet Spring has lower acid and Brix levels. As a result when the ratio between sugar and acid is 10:1, the fruit is considered to be at peak maturity. A ratio of 9:1 is considered to be the build-up towards peak maturity of 10:1. After reaching the peak, the ratio increases to 11:1, after which it is considered overmature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in greater instance of quality and rind issues.

Although the large and small fruit were harvested from the same trees, there is a difference in maturity. Large fruit tends to mature almost two weeks earlier than smaller fruit. The older trees on Troyer mature almost two months later than the large fruit. Colour development was poor in all instances with fruit having to hang long on the trees to allow for colour. Fruit was still not completely coloured up when harvested. Swingle may not be recommended for this selection as it brings about delayed colour development. Brix levels were highest on the young trees with the smaller fruit having slightly better levels (10.5°) than the large fruit (10.4°). However, acid was low for fruit on the young trees dropping to below 1.0% early in the season and going as low as 0.84% on the large fruit. Acid was better on the older trees and maintained a steady concentration throughout the season. Overall seed counts were low (<0.2 seed per fruit). Juice percentages were average for this selection, almost on the lower side (avg 46.3%). This may be attributed to thicker than normal rinds in the young trees. Juice levels were on average higher on the older trees that had thinner rinds. Yields were excellent for the Sweet Spring.

Conclusion

Swingle is not a good rootstock for Sweet Spring as it delays colour development (between T3 and T4). The opposite scenario occurred this season with small fruit being of poorer quality (lower juice levels) than bigger fruit and the bigger fruit were internally mature earlier (lower acid levels) than the smaller fruit. The quality of the Sweet Spring will improve as the trees get older.

Table 5.5.16.2. Internal fruit quality data for Sweet Spring from the Rheenendal region of the Western Cape during the 2012 season.

Selection	Root-stock	Date harvested	Count	Juice %	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Sweet Spring (Small)	SC	21-Jun-12	1x	44.4	10.5	0.89	11.8	0	3
Sweet Spring (Large)	SC	21-Jun-12	1xxx	48.1	10.4	0.84	12.4	0.2	4

5.5.17 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Navel oranges in a cold production region (Sundays River Valley)

Project 998B by J. Joubert, R. Fenwick and S. Meeding (CRI)

Opsomming

Die vroeë seleksies het beter suur vlakke gehad in vergelyking met 2011 en verbeterde kleur ontwikkeling, met die uitsondering van Palmer en Washington. Obrengs was soortgelyk op al die vroeë seleksies gewees. Die vroegste seleksie was Lina (TC) 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. Newhall (TC) se Brix vlakke was bo 10.1 gewees en was volgende ryp. Tulegold was volgende om ryp te word, het klein vrugte geproduseer (count 64) met die laagste Brix vlakke vir die vroeë seleksies, gevolg deur Washington (SC) met Brix (10.2°). Palmer was die laatste gereed vir oes van die vroeë seleksies, maar het saam met Washington die swakste kleurontwikkeling opgelewer. Uit die laat seleksies was Cambria nawel die eerste ryp en het baie goeie opbrengste gelewer, maar met swak eksterne kleur ontwikkeling en die laagste sure. Witkrans was volgende, met effens beter opbrengste en beter kleur, asook effens hoër interne kwaliteit. Autumn Gold was volgende met baie goeie opbrengs, asook verbeterde eksterne kleurontwikkeling en goeie interne kwaliteit. Summer Gold se rypwordings tyd is volgende met effens ligter drag, aanvaarbare interne kwaliteit, maar swak eksterne kleur ontwikkeling. Dan volg Lane Late met baie goeie opbrengs, goeie interne kwaliteit en goeie kleur ontwikkeling. Powell Summer was die tweede laaste seleskie om ryp te word. Hierdie seleksie het goeie opbrengste geproduseer met goeie kleur ontwikkeling en die beste interne kwaliteit in vergelyking met die ander seleksies. Die laatste seleksie om ryp te word is Glen Ora Late met die tweede beste interne gehalte en goeie kleur ontwikkeling. Die pluk periode moet nie langer as 2 tot 3 weke wees nie om goeie interne gehalte standarde te behou en na-oes skil probleme te vermy.

Summary

The early selections had better acid levels compared to 2011 and improved colour development, with the exception of Palmer and Washington. Yields were similar for all the early selections. Lina (TC) 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. Newhall (TC) matured next and had average Brix levels of 10.1. Tulegold was next to mature and had the lowest Brix levels (9.7) with smaller fruit size (count 64) of the early selections, followed by Washington (SC) with good Brix (10.2°). Palmer was the latest to mature and had acceptable Brix levels but together with Washington the poorest colour development. For the late selections, Cambria was the earliest to mature with very good yields but poor colour development and lowest acids. Witkrans was next to mature, with slightly higher yields but better colour and slightly higher internal quality. Autumn Gold matured next with very good yields, improved colour development and good internal quality. Summer Gold was next to mature, with slightly less yields and acceptable internal quality but with delayed colour development. This was followed by Lane Late with very good yields, good internal quality and good colour development. Powell Summer was the second latest to mature with good yields as well as good colour development and the best internal quality compared to the other selections. Glen Ora Late was the latest selection with the second best internal quality and good colour development. Picking periods should not be longer than 3 - 4 weeks to maintain a good internal quality standard and to avoid rind disorders.

Objectives

- To select Navel cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).

- To describe the characteristics of new Navel cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Navel selections from the Sundays River Valley region of the Eastern Cape. The following early to mid maturing selections were evaluated: Fukumoto, Lina, Newhall, Palmer, Tulegold and Washington. The following late maturing selections were evaluated: Autumn Gold, Cambria, Glen Ora Late, Lane Late, Powell Summer, Summer Gold and Witkrans.

Table 5.5.17.1. List of Navel selections evaluated at Sundays River Valley (Penhill) during 2012.

Selection	Rootstock	Planted
Fukumoto	Troyer	2007
Lina	Troyer	2007
Newhall	Troyer	2007
Palmer	Swingle	2007
Tulegold	Troyer	2007
Washington	Swingle	2007

Table 5.5.17.2. List of Navel selections evaluated at Sundays River Valley (Dunbrody) during 2012.

Selection	Rootstock	Planted
Autumn Gold	Carrizo	2004
Cambria	Rough Lemon	2004
Glen Ora Late	Rough Lemon	2004
Lane Late	Carrizo	2004
Powell Summer	Carrizo	2004
Summer Gold	Carrizo	2004
Witkrans	Carrizo	2004

Results and discussion

These results are from commercial orchards in the area on various rootstocks.

When the ratio between sugar and acid is 10:1, the fruit is considered to be at peak maturity. A ratio of 9:1 is considered to be the build-up towards peak maturity of 10:1. After reaching the peak, the ratio increases to 11:1, after which it is considered overmature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in greater instance of quality and rind issues.

According to the results in Table 5.5.17.3 at the Penhill trial site for the 2012 season, Lina (TC) was the earliest selection to mature; this was followed by Fukumoto (TC). Newhall (TC) was slightly later than Fukumoto (TC), followed by Tule Gold (TC) and Washington (SC). Washington had fairly low acids when taking into consideration the selection was planted on Swingle rootstock. Palmer was the selection that matured the latest, where for the 2011 season Washington was last maturing. Fukumoto (TC), Lina (TC) and Nwehall (TC) had the largest fruit size (count 56), followed by Tule Gold and Washington with count 56/64 except for Palmer which had the smallest fruit size (count 56/64/72). Overall Brix levels were good. On average, Fukumoto (TC) had the highest Brix levels (10.8°) followed by Lina (TC) (10.4°), Washington (SC) (10.2°), Newhall (TC) (10.1°), Palmer (SC) (9.9°) and Tule Gold (TC) having the lowest Brix levels (9.7°). Overall acid levels were higher compared to 2011. This may be attributed to a combination of older tree age and lower amount of rain during the season. Average acid level for all selections was above 1.0%, except for Lina with 0.9%. Colour levels were good to very good on most selections by peak maturity and time of harvest. Washington and Palmer had the poorest colour development with fruit being externally immature (T5-6) even though internally mature, only colouring up when overmature (T1 to T2). This may be as a result of the rootstock. Trees are all fairly young and yields were similar for all selections.

According to the results in Table 5.5.17.4, Cambria was the first to mature, followed by Witkrans and Autumn Gold. Summer Gold and Lane Late matured next at the same time, followed by Powell Summer. Glen Ora Late being the latest selection. All of the selections were of similar size (count 56) with only Lane Late being slightly smaller (count 56/64). Colour development was poorest for the Cambria, with colour levels at T4-5

towards the end of peak maturity. Autumn Gold also had poor colour development at T2-3 during peak maturity. This was followed by Summer Gold at T2 towards end of maturity. Glen Ora Late, Powell Summer and Witkrans had the best colour development in relation to the other selections. Powell Summer had on average the highest Brix (11.7°) and acid (1.0%) levels relative to the other selections. Glen Ora Late had the second best average Brix levels (11.4°) and good acid (1.1%), with Autumn Gold and Lane Late following closely with average Brix of 11.0° and acid of 0.94%. The remaining selections had an average Brix of higher than 10.5°. Of these, Cambria performed the best with an average Brix of 10.9° although acid levels were low (average 0.84%). This was followed by Witkrans (average Brix 10.6° and acid 0.91%) and then Summer Gold (average Brix 10.5° and acid 0.96%).

Conclusion

Of the early to mid maturing navels, Lina (TC) was first to mature followed by Fukumoto (TC), Newhall (TC), Tulegold (TC) and then Washington (SC). The late navels extend the season with Cambria maturing first, followed by Witkrans, Autumn Gold, Summer Gold, Lane Late, Powell Summer and lastly Glen Ora Late. Colour development and internal quality of early to mid navels was acceptable with better acids and improved colour development. The late navels had better colour development and internal qualities. Yields improved for most navels.

Table 5.5.17.3. Internal fruit quality data for early and mid Navel selections from the Addo (Penhill) region of the Sundays River Valley during the 2012 season.

Selection	Root-stock	Date harvested	Count	Juice %	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Fukumoto	CC	03-Mar-12	56	49.3	9.5	1.13	8.4	0	5-6
Fukumoto	CC	12-Apr-12	56	49.6	9.9	1.27	7.8	0	6
Fukumoto	TC	03-Mar-12	56	50.2	9.6	1.28	7.5	0	6-7
Fukumoto	TC	12-Apr-12	56	51.4	10.0	1.17	8.5	0	5-6
Fukumoto	TC	03-May-12	56	51.7	10.9	1.02	10.7	0	1-2
Fukumoto	TC	24-May-12	56	52.0	11.5	0.98	11.7	0	1
Fukumoto	TC	04-Jun-12	56	51.2	12.1	0.98	12.3	0	1
Lina	CC	03-Mar-12	72	53.0	10.7	1.14	9.4	0	6-7
Lina	CC	12-Apr-12	64	51.7	10.1	1.15	8.8	0	5
Lina	TC	03-Mar-12	64	53.0	10.1	1.10	9.2	0	6-7
Lina	TC	12-Apr-12	56	52.9	9.8	1.10	8.9	0	7
Lina	TC	03-May-12	56	54.6	10.2	0.85	12.0	0	2-3
Lina	TC	24-May-12	56	51.6	10.9	0.78	14.0	0	1
Lina	TC	04-Jun-12	56	53.4	11.1	0.77	14.4	0	1
Newhall	CC	03-Mar-12	64	48.7	10.0	1.18	8.5	0	6-7
Newhall	CC	12-Apr-12	56	50.3	10.0	1.11	9.0	0	6
Newhall	TC	03-Mar-12	64	53.2	9.4	1.28	7.3	0	7
Newhall	TC	12-Apr-12	56	50.3	9.1	1.19	7.6	0	7
Newhall	TC	03-May-12	56	54.4	10.9	1.03	10.6	0	2-3
Newhall	TC	24-May-12	56	52.6	10.6	0.87	12.2	0	1
Newhall	TC	04-Jun-12	56	52.9	10.3	0.80	12.9	0	1
Palmer	CC	12-Apr-12	72	48.2	11.0	1.69	6.5	0	7
Palmer	CC	03-Mar-12	72	48.1	10.9	1.66	6.6	0	7-8
Palmer	SC	03-Mar-12	72	49.1	9.3	1.29	7.2	0	7-8
Palmer	SC	12-Apr-12	64	49.1	9.0	1.15	7.8	0	7-8
Palmer	SC	03-May-12	56	52.0	10.2	0.93	11.0	0	5-6

Palmer	SC	24-May-12	56	50.9	10.0	0.86	11.6	0	3
Palmer	SC	04-Jun-12	56	50.7	10.8	0.83	13.0	0	1-2
Tulegold	TC	03-Mar-12	64	49.1	9.0	1.07	8.4	0	7
Tulegold	TC	12-Apr-12	64	50.3	9.3	1.07	8.7	0	7
Tulegold	TC	03-May-12	64	52.9	10.1	1.14	8.9	0	2-3
Tulegold	TC	04-Jun-12	56	48.1	10.0	0.83	12.0	0	1
Tulegold	TC	24-May-12	64	53.1	10.0	0.81	12.3	0	1
Washington	SC	03-Mar-12	64	51.9	9.4	1.47	6.4	0	7-8
Washington	SC	03-May-12	64	53.3	10.5	0.98	10.7	0	5-6
Washington	SC	24-May-12	56	51.7	10.1	0.97	10.4	0	4-5
Washington	SC	04-Jun-12	64	53.0	10.8	0.87	12.4	0	1-2

Table 5.5.17.4. Internal fruit quality data for late Navel selections from the Kirkwood (Dunbrody) region of the Sundays River Valley during the 2012 season.

Selection	Root-stock	Date harvested	Count	Juice %	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Autumn Gold	CC	04-Jun-12	56	51.0	11.0	0.99	11.1	0	2-3
Autumn Gold	CC	03-Jul-12	56	51.7	10.9	0.88	12.4	0	1-2
Cambria	RL	04-Jun-12	56	52.5	11.2	0.95	11.8	0	4-5
Cambria	RL	03-Jul-12	56	51.6	10.1	0.79	12.8	0	2-3
Cambria	RL	31-Jul-12	56	53.1	11.4	0.79	14.4	0	1
Glen Ora Late	RL	04-Jun-12	56	54.0	11.3	1.27	8.9	0	2
Glen Ora Late	RL	03-Jul-12	56	51.6	11.1	1.02	10.9	0	1
Glen Ora Late	RL	31-Jul-12	56	53.3	11.9	1.02	11.7	0	1
Lane Late	CC	04-Jun-12	64	54.7	11.3	0.98	11.5	0	1-2
Lane Late	CC	03-Jul-12	56	53.3	10.7	0.89	12.0	0	1
Powell Summer	CC	04-Jun-12	56	50.6	10.7	0.97	11.0	0	2
Powell Summer	CC	03-Jul-12	56	53.3	12.6	1.12	11.3	0	1
Summer Gold	CC	04-Jun-12	56	54.1	10.6	1.03	10.3	0	3
Summer Gold	CC	03-Jul-12	56	53.0	10.4	0.89	11.7	0	2
Witkrans	CC	04-Jun-12	56	56.1	10.5	0.96	10.9	0	1-2
Witkrans	CC	03-Jul-12	56	53.1	10.6	0.86	12.3	0	1

5.5.18 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Navel oranges in a cold production region (Western Cape)

Project 998D by J. Joubert, R. Fenwick and S. Meeding (CRI)

Opsomming

Opbrengs op die jong bome was beter as gemiddeld vir die tweede seisoen wat hulle vrugte produseer. Suurvlakke het toegeneem en Brix was so hoog as 14 gewees. Die seisoen begin met Lina nawel, die seleksie het goeie kleur ontwikkeling en hoë Brix vlakke gelewer. Santa Catarina 3 het gevolg met groot vruggrootte en goeie interne kwaliteit. Witkrans was eerste om ryp te word van die later seleksies, met medium vruggrootte en hoë Brix vlakke. Santa Catarina 1 was volgende om ryp te word, met groot vruggrootte en goeie interne kwaliteit in vergelyking met die ander seleksies. Letaba en Krajewski Early was volgende, glad nie vroeg vir hierdie seisoen, toekoms evaluasies sal piek rypwordings tyd bepaal vir hierdie twee seleksies, maar hou in gedagte hierdie was slegs die tweede oes op die bome. Glen Ora Late en Coetzee Late was saam gereed om geoes te word, die twee seleksies wat laaste ryp geword het vir hierdie proef, met Coetzee Late wat die laagste Brix opgelewer het in vergelyking met die ander seleksies, maar nog steeds aan die minimum uitvoer standarde voldoen het. Die oes proses moet nie langer as 2 tot 3 weke wees nie, dit verseker goeie interne gehalte en voorkom skil probleme.

Summary

Yields on the trial trees were better than average since it was now the second crop for these trees. Acid levels increased and Brix levels were as high as 14. Lina was the first to mature and had high Brix levels and good colour development, fruit size was medium. Santa Catarina 3 followed with large fruit size and good internal quality. Witkrans was the first to mature of the later selections with high Brix levels and medium fruit size. Santa Catarina 1 was next to mature with large fruit size and good internal quality compared to the other selections. Letaba and Krajewski Early matured next, not being that early this season. Future evaluations will determine the peak maturity time for these two selections because this was only the second crop on the trees. Glen Ora Late and Coetzee Late matured at the same time, being the two latest selections in this trial, with Coetzee Late having the lowest Brix compared to the other selections, but still complying with the minimum export standards. Picking periods should not be longer than 3 - 4 weeks to maintain a good internal quality standard and to avoid rind disorders.

Objectives

- To select Navel cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the cultivar characteristics of new Navel cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Navel selections from the Citrusdal region of the Western Cape. The following early to mid maturing selections were evaluated: Coetzee Late, Glen Ora Late, Krajewski Early, Letaba Early, Lina, Santa Catarina 1, Santa Catarina 3, and Witkrans.

Table 5.5.18.1. List of navel selections evaluated in the Citrusdal (Patrysburg) region of the Western Cape during the 2012 season.

Selection	Rootstock	Planted
Coetzee Late	Carrizo	2009
Glen Ora Late	Carrizo	2009
Krajewski Early	Carrizo	2009
Letaba Early	Carrizo	2009
Lina	Carrizo	2009
Santa Catarina 1	Carrizo	2009
Santa Catarina 3	Carrizo	2009
Witkrans	Carrizo	2009

Results and discussion

These results are from an experimental site in the Citrusdal area on Carrizo citrange rootstock.

According to the results in Tables 5.5.18.2, Lina was the earliest selection to mature. This was followed by Santa Catarina 3 and then Witkrans. Letaba Early followed with good internal quality and high Brix levels (13.5°). Santa Catarina 1 matured slightly before Krajewski Early, and future evaluations will determine how early this selection will be, because results for this trial indicated that Krajewski was the third last selection to mature, followed by Coetzee and Glen Ora Late. Glen Ora Late ended the navel season at this trial site. The two early selections matured after the middle of the navel range planted at the trial site. This may be attributed to tree age, since it is the second crop on these trees. Another contributing factor is the unusually high Brix and lower acid levels. Krajewski Early had the highest average Brix levels (14.4°). Witkrans and Letaba Early had similar Brix levels (avg 14°). Glen Ora Late, Coetzee Late and Santa Catarina 3 had the largest fruit size (count 56), one count size smaller compared to 2011, followed by Letaba Early (count 56/64) and Witkrans (count 64/72) with Krajewski Early having the smallest fruit size (count 105). The colour development for this season was excellent with all the selections peaking at T1 when the last evaluation was completed. Yields were average for the early navels as it was the second crop on these trees.

Conclusion

Lina matured first, followed by Santa Catarina 3, Witkrans, Santa Catarina 1, Letaba Early, Krajewski Early, Coetzee Late and Glen Ora Late at the end. Krajewski Early had the smallest fruit size and Glen Ora Late as well as Santa Catarina 3 had the largest fruit size. The young trees had average yields and internal quality was better compared to 2011. The Brix on young trees was high and peaked at 14 on two of the selections.

Table 5.5.18.2. Internal fruit quality data for Navel selections from the Citrusdal region (Patrysburg) of the Western Cape during the 2012 season.

Selection	Root-stock	Date harvested	Count	Juice %	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Coetzee Late	CC	19-Jun-12	56	53.6	11.5	1.23	9.3	0	1
Coetzee Late	CC	31-Jul-12	56	54.2	11.8	0.99	11.9	0	1
Glen Ora Late	CC	19-Jun-12	56	53.3	11.6	1.21	9.6	0	1
Glen Ora Late	CC	31-Jul-12	56	57.8	13.2	1.12	11.8	0	1
Krajewski early	CC	19-Jun-12	105	46.7	14.1	1.47	9.6	0	1
Krajewski early	CC	31-Jul-12	105	52.8	14.7	1.25	11.8	0	1
Letaba early	CC	19-Jun-12	64	51.9	13.1	1.22	10.7	0	1
Letaba early	CC	31-Jul-12	56	51.1	13.9	1.20	11.6	0	1
Lina	CC	19-Jun-12	64	52.1	13.1	0.93	14.1	0	1
Santa Catarina 1	CC	19-Jun	56	50.0	13.2	1.08	12.2	0	1
Santa Catarina 3	CC	19-Jun-12	56	52.9	12.7	0.91	14.0	0	1
Santa Catarina 3	CC	31-Jul-12	56	55.0	13.3	0.82	16.2	0	1
Witkrans	CC	19-Jun-12	72	54.2	14.0	1.13	12.4	0	1
Witkrans	CC	31-Jul-12	64	55.1	13.8	0.93	14.8	0	1

5.5.19 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Experimental Navel oranges in a cold production region (Sundays River Valley) Project 1001A by J. Joubert, R. Fenwick and S. Meeding (CRI)

Opsomming

Habata Early was die vroegste seleksie om ryp te word. Interne gehalte was goed, met 'n diep oranje-rooi skil kleur. Opbrengs was gemiddeld, maar kan toegeskryf word aan die toestand van die bome. Fukumoto was volgende as kontrole met goeie interne kwaliteit en opbrengs. Palmer het opgevolg ook as kontrole. Die EH Navel volg, met beter opbrengste, diep oranje-rooi skilkleur en aanvaarbare Brix vlakke, maar lae suurvlaeke. Volgende was die 99 Navel. Hierdie seleksie het goeie eksterne kleur ontwikkeling gehad met sagte vesel, swak opbrengs, lae Brix en hoë suur vlakke. HE Late was die laaste seleksie van die seisoen met goeie opbrengs en die beste interne gehalte van al die seleksies. Die oes periode moet nie langer as 2 tot 3 weke wees nie om goeie interne kwaliteit te verseker met minimum skil probleme.

Summary

Habata Early was first to mature with good internal quality and deep orange rind but average yield, mostly attributed to poor tree health. This was followed by Fukumoto as a control with good internal quality and yield. Then Palmer followed, also as a control. EH Navel was next to mature, which had good yields, deep red-orange rind and acceptable Brix but poor acid levels. 99 Navel was next, which had 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.

Objectives

- To select Navel cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new Navel cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Navel selections from various regions of the Sundays River Valley. The following selections were evaluated: HE Late, Habata Early, 99 Navel, EH Navel and their respective control cultivars, Palmer and Fukumoto.

Table 5.5.19.1. List of Navel selections evaluated at various sites in the Eastern Cape during the 2012 season.

Selection	Rootstock	Planted
Habata Early	Rough Lemon	Unknown
EH Navel	Carrizo	Unknown
EH Navel	Troyer	Unknown
EH Navel	Swingle	Unknown
Palmer	Rough Lemon	Unknown
99 Navel	Rough Lemon	Unknown
HE Late	Carrizo	Unknown

Results and discussion

These results are from commercial orchards in the area on various rootstocks.

When the ratio between sugar and acid is 10:1, the fruit is considered to be at peak maturity. A ratio of 9:1 is considered to be the build-up towards peak maturity of 10:1. After reaching the peak, the ratio increases to 11:1, after which it is considered overmature. This process from start to the end of the peak is approximately three weeks long. Fruit harvested before and after this period would result in greater instances of quality and rind issues.

According to Tables 5.5.19.2, Habata Early matures first, followed by Fukukmoto, and then EH Navel (TC) and Palmer at the same time, followed by EH Navel (SC), then 99 Navel and lastly HE Late. Fruit size was similar in all selections (count 56) except for Habata Early, EH Navel (CC) and 99 Navel with the smallest fruit size (count 72). Habata Early and 99 navel had the poorest yields. Habata Early tree is not in very good condition and 99 Navel appeared to have gone into an alternate bearing cycle due to a heavy crop in the previous season. HE Late, Palmer and EH Navel had similar good yields. HE Late had on average the best internal quality (Brix 12.5° and acid 1.0%), followed by Fukumoto (Brix 11.5° and acid 1.1%). Habata Early followed with good Brix (10.7°) but lower acid (0.88%). This was followed by Palmer (Brix 10.1° and acid 1.1%), then EH Navel (Brix 10.0° and acid 1.1%). The 99 Navel had the poorest Brix levels (9.7°) but highest acid levels (1.3%). Colour development was best on the EH Navel (SC), EH Navel (TC) and 99 Navel which had fully coloured up when internally mature (T1). Fukumoto developed up to T2 colour with peak maturity and HE Late averaged between T2 & T3. All other selections had poor colour development and ranged between T4 to T6 at time of peak maturity. EH Navel has the deepest rind colour (red-orange) and easily stands out when compared to the other selections. Habata Early has similar colour but not as intense. The 99 Navel has the softest rag of all the selections and no navel end, but thickest rind.

Conclusion

Habata Early matures first followed by Fukumoto, Palmer, then EH Navel (TC) and (SC), 99 Navel and lastly HE Late. Yields were best on HE Late, Palmer and EH Navel. Habata Early and 99 Navel had the poorest yields. 99 Navel had the lowest Brix levels but highest acid levels.

Table 5.5.19.2. Internal fruit quality data for Experimental Navel selections from the Sundays River Valley region of the Eastern Cape during the 2012 season

Selection	Root-stock	Date harvested	Count	Juice %	Brix °	Acid (%)	Ratio	Avg. seed	Colour
EH Navel	CC	03-Mar-12	64	47.2	9.2	1.36	6.8	0	6-7
EH Navel	CC	12-Apr-12	72	47.3	9.5	1.25	7.6	0	5-6
EH Navel	SC	03-Mar-12	72	47.1	9.1	1.56	5.8	0	7
EH Navel	SC	12-Apr-12	64	47.8	9.2	1.26	7.3	0	7
EH Navel	SC	03-May-12	64	51.9	9.8	1.13	8.7	0	4
EH Navel	SC	24-May-12	56	49.9	10.4	1.02	10.2	0	1
EH Navel	SC	04-Jun-12	56	49.7	10.6	0.98	10.8	0	1
EH Navel	TC	03-Mar-12	64	47.5	9.3	1.24	7.5	0	7
EH Navel	TC	12-Apr-12	64	49.3	9.2	1.24	7.4	0	6
EH Navel	TC	03-May-12	56	49.7	9.7	1.06	9.2	0	3
EH Navel	TC	24-May-12	56	49.3	10.0	0.95	10.5	0	1
EH Navel	TC	04-Jun-12	64	48.1	10.4	0.90	11.6	0	1
99	RL	03-Mar-12	88	52.0	9.1	1.61	5.7	0	7
99	RL	12-Apr-12	125	49.5	9.3	1.70	5.5	0.1	6-7
99	RL	03-May-12	72	51.7	10.0	1.21	8.3	0	5
99	RL	24-May-12	72	48.8	9.8	1.01	9.7	0	1
99	RL	04-Jun-12	72	48.6	10.1	1.07	9.4	0	1
Palmer	RL	03-Mar-12	64	50.0	9.0	1.38	6.5	0	7
Palmer	RL	12-Apr-12	56	47.9	9.5	1.19	8.0	0	7-8
Palmer	RL	03-May-12	56	49.5	10.2	1.01	10.1	0	6
Palmer	RL	24-May-12	56	49.8	10.4	0.95	10.9	0	2-3
Palmer	RL	04-Jun-12	56	51.4	11.5	0.85	13.5	0	1
Habata Early	RL	12-Apr-12	72	49.3	10.2	0.98	10.4	0	6
Habata Early	RL	03-May-12	72	52.5	11.2	0.78	14.4	0	1-2
Fukumoto		12-Apr-12	56	44.1	11.0	1.13	9.7	0	4-5
Fukumoto		03-May-12	56	47.9	12.0	1.05	11.4	0	1
H E Late	CC	04-Jun-12	72	56.0	12.4	1.06	11.7	0	2-3
H E Late	CC	03-Jul-12	64	53.8	12.7	1.11	11.4	0	1
H E Late	CC	31-Jul-12	56	50.0	12.5	0.93	13.4	0	1

5.5.20 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Experimental Navel oranges in a cold production region (Gamtoos River Valley)
Project 1001B by J. Joubert, R. Fenwick and S. Meeding (CRI)

Opsomming

Die vroegste nawel seleksie in Suid Afrika vir die afgelope vyf jaar is Patensie Early. Interne gehalte sowel as kleur ontwikkeling was nie goed vir die 2012 seisoen nie. EDP1 en EDP Dogter bome was volgende, met uitstekende kleur ontwikkeling, goeie opbrengs en goeie vruggrootte. Fischer nawel is volgende met baie goeie opbrengs, vruggrootte, baie goeie interne gehalte en goeie eksterne kleur. Nawel-ente is totaal geslote. EDP 2 word volgende ryp met groot vruggrootte en goeie interne kwaliteit. Cambria en Lazy Boy was volgende, waar Lazy Boy een van die beste interne kwaliteite vir hierdie proef ontwikkel het. Suitangi was volgende om ryp te word met gladde skil en een van die beste interne kwaliteite van al die seleksies, alhoewel opbrengs effens laer en vruggrootte effens kleiner was. KS nawel is die laaste seleksie en het goeie opbrengs, goeie vruggrootte, gladde skil en goeie interne kwaliteit. Die oes periode moet nie langer as 2 tot 3 weke wees nie om goeie interne kwaliteit te verseker sonder skil probleme.

Summary

Patensie Early is the earliest maturing navel selection in South Africa and has maintained its earliness for five years. Internal qualities as well as colour development was not good this season. EDP1 and EDP Daughter trees mature next with excellent colour development, good yields and fruit size. Fisher navel is next to mature and has very good yields, fruit size, good internal quality and good colour. Navel ends are completely closed. EDP 2 matures next with large fruit size and good internal quality. Cambria and Lazy Boy were next, with Lazy Boy having one of the best internal qualities for this trial. Suitangi is the next to mature and also has a smooth rind, but has one of the best internal qualities of all selections, however, yields are slightly lower and fruit size slightly smaller. KS Navel is the latest selection and has very good yields, good fruit size, smooth rind and good internal quality. Picking periods should not be longer than 3 - 4 weeks to maintain a good internal quality standard and to avoid rind disorders.

Objectives

- To select Navel cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new Navel cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Navel selections from various regions of the Gamtoos River Valley. The following selections were evaluated: Patensie Early, Fischer, EDP 1&2, KS navel, Suitangi, Lazy Boy their respective control cultivars Cambria.

Table 5.5.20.1. List of Mandarin hybrid selections evaluated at various sites in the Eastern Cape area during the 2012 season.

Selection	Rootstock	Planted
Patensie Early	Rough Lemon	Old
Fischer	Rough Lemon	Old
EDP1	Rough Lemon	Young
Cambria	Rough Lemon	Old
KS Navel	Rough Lemon	Young
Suitangi	Rough Lemon	Old2
Lazy Boy	Rough Lemon	Young
Washington	Rough Lemon	Control

Results and discussion

These results are from commercial orchards in the area on various rootstocks. The exact age of the selections are not known but have been indicated as either young or old trees.

According to the results shown in Tables 5.5.20.2, Patensie Early is by far the earliest new selection, but with the first evaluation the internal quality was average with low Brix (8,6°) and acids (0.9%). Future evaluations will determine precise maturity time, for the 2011 season the selection matured end of February. This is followed by EDP1, EDP Daughter, Fischer navel, EDP2, Cambria as well as Lazy Boy, Suitangi, and KS Navel. Suitangi were previously recorded as very late but have now fallen in the mid late-maturing category. This may be as a result of the unusually low acid levels but high Brix levels which are usually associated with the selection. The maturity of Lazy Boy has remained in the indicated region since it was discovered, but the daughter trees seem earlier, future evaluations will determine maturity status (young tree age scenario). Lazy Boy, EDP1/2/daughter, Washington and Fischer had the largest fruit size (count 56) followed by Suitangi, Cambria and KS Navel (count 64), with Patensie Early having the smallest fruit size (count 88). Lazy Boy and Suitangi had the best colour development being fully coloured at maturity (T1). This was followed by EDP 1, 2 and Daughter, as well as Fischer and Washington (T2-3). Patensie Early had poor colour development (T5). On average, Lazy Boy had the highest Brix levels (12.8°) with good acid (average 0.80%). This was followed by Suitangi (Brix 12.4°) with an acid level of average 0.88%, a substantial decrease compared to the 2011 season (acid 1.34%). KS Navel also had good internal quality (average Brix 10.9 and acid 0.87%). EDP2 also had good Brix levels (10.8°) but slightly higher acid levels than KS Navel (0.92%). EDP1 and Fischer navel had low but acceptable average Brix levels (10.3°) with low acid levels (average

0.87%). Patensie Early had the lowest Brix levels (8.6°), as well as low acids (0.9%). KS Navel, Cambria and Fischer had the best yields, followed by EDP1 and Suitangi. Lazy Boy had slightly lower yields than the other selections but trees are young. EDP1 has a completely closed navel end and is easily mistaken for a Valencia. The rinds for Cambria, Suitangi and KS Navel are smoother than the other selections.

Conclusion

Patensie Early is the earliest navel selection. This is followed by EDP1, EDP Daughter, Fischer, EDP 2, Cambria and Lazy Boy and Suitangi. KS Navel is the latest navel selection. Yields were best in KS Navel, Cambria and Fischer. Lazy Boy had average yields. Lazy Boy had the best internal quality and Cambria had the lowest Brix levels, but EDP1 the lowest acid levels.

Table 5.5.20.2. Internal fruit quality data for Experimental Navel selections from the Gamtoos River Valley region of the Eastern Cape during the 2012 season.

Selection	Root-stock	Date harvested	Count	Juice %	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Lazy Boy	RL	05-Jul-12	56	48.1	12.7	0.87	14.6	0	1-2
Lazy Boy	RL	01-Aug-12	64	48.1	13.0	0.87	14.9	0	1
Lazy Boy	RL	20-Aug-12	56	45.6	12.7	0.80	15.9	0	1
Lazy Boy (Daughter)	RL	20-Aug-12	56	46.5	11.7	0.63	18.6	0	1
Suitangi	RL	05-Jul-12	64	51.9	11.7	0.94	12.4	0	1
Suitangi	RL	01-Aug-12	64	53.8	12.6	0.90	14.0	0	1
Suitangi	RL	20-Aug-12	64	55.6	12.9	0.80	16.1	0	1
Cambria	RL	05-Jun-12	72	52.3	10.0	0.88	11.4	0	3-4
Cambria	RL	05-Jul-12	64	51.9	9.8	0.83	11.8	0	1-2
KS Navel	RL	05-Jun-12	64	51.7	11.1	0.95	11.7	0	1-2
KS Navel	RL	05-Jul-12	64	47.8	10.6	0.79	13.4	0	1
EDP Daughter	RL	09-May-12	56	50.9	9.2	0.90	10.2	0	3
EDP Daughter	RL	23-May-12	56	47.9	9.2	0.84	11.0	0	1
EDP Daughter	RL	05-Jun-12	56	51.2	10.9	0.81	13.5	0	1
EDP1	RL	09-May-12	56	49.8	10.0	0.80	12.5	0	2-3
EDP1	RL	23-May-12	56	52.7	10.3	0.89	11.6	0	1
EDP1	RL	05-Jun-12	56	51.7	10.6	0.68	15.6	0	1
EDP2	RL	09-May-12	56	53.0	10.2	1.04	9.8	0	2
EDP2	RL	23-May-12	56	51.0	11.1	0.87	12.8	0	1
EDP2	RL	05-Jun-12	56	53.1	11.2	0.86	13.0	0	1
Fischer	RL	11-Apr-12	56	49.1	10.8	1.06	10.2	0	6-7
Fischer	RL	09-May-12	64	52.5	9.6	0.91	10.5	0	2-3
Fischer	RL	23-May-12	56	50.5	10.1	0.94	10.7	0	1
Fischer	RL	05-Jun-12	56	50.5	10.9	0.88	12.4	0	1
Washington	RL	09-May-12	56	51.6	10.2	1.13	9.0	0	2-3
Washington	RL	23-May-12	56	48.8	10.2	0.97	10.5	0	1
Patensie Early	RL	11-Apr-12	88	51.0	8.6	0.90	9.6	0	5

5.5.21 **PROGRESS REPORT: Establishment of a molecular citrus genotype reference database for citrus cultivar verification within the Citrus Improvement Scheme**
Project by A. Severn-Ellis, *et al.* ARC-ITSC

Summary

Citrus accessions entering the Virus Free Nucleus Block at the ARC's Institute for Tropical and Subtropical Crops have been characterised based on important morphological or agronomical features. These defining morphological or agricultural characteristics are not continuously expressed within the potted greenhouse environment. It is therefore not always possible to check or verify the trueness-to-type of an accession which may prevent the detection of misidentifications or duplicates. A large number of microsatellite (SSR) markers have been developed for Mandarin genotyping. In the pilot study conducted, 71 SSR primer pairs were screened and a set of 13 SSR markers was selected and assessed for their ability to distinguish between 32 randomly selected Mandarin accessions. 26 microsatellite (SSR) markers were finally selected based on their ability to differentiate between all Mandarin hybrid-, Clementine- and Satsuma cultivars, level of polymorphism and transferability across all citrus cultivars and genetically related citrus species. PCR amplification, visualisation and documentation of DNA fragments generated for the Mandarin hybrid-, Clementine-, Satsuma-, lemon-, lime-, grapefruit-, pummelo-, diverse citrus and rootstock cultivars as per CIS list has been completed. An Excel database has been created and the capturing of fragment sizes in the database has commenced. Although genetic differences were detected between most of the cultivars using the selected SSR markers, limited genetic variation was however detected between a selected group of sweet orange cultivars sampled. Additional marker systems such as sequence related amplified polymorphism or SRAP markers may provide the supplementary genetic information required to distinguish between these closely related cultivars. The use of SRAP markers to distinguish between the sweet orange cultivars in addition to the current set of SSR markers will be investigated.

Opsomming

Sitrus kultivars wat opgeneem word in die Virusvrye van die LNR se Instituut vir Tropiese en Subtropiese Gewasse word normaalweg gekarakteriseer op grond van morfologiese en agronomiese eienskappe. Hierdie eienskappe word is nie noodwendige altyd sigbaar in die glashuis nie waar die kultivars in potte groei nie. Dit is daarom nie altyd moonlik om die tipe-egtheid of identiteit van hierdie kultivars te bepaal nie wat kan veroorsaak dat verkeerdlik geïdentifiseerde of duplisering van kultivars ongemerk kan plaasvind. 'n Groot aantal mikro-stateliet (SSR) merkers is reeds ontwikkel vir die tipering van Manderyne op genetiese vlak. In 'n voorafgaande loodsproef in 71 SSR merker kombinasies getoets en daarna is 13 SSR merker kombinasies geselekteer vir hul vermoë om tussen 32 spesifieke Manderyn kultivars te onderskei. 'n Finale groep van 26 SSR merker kombinasies is egter uiteindelik geselekteer op grond van hul vermoë om te onderskei tussen alle Manderyn-hibried-, Clementine- en Satsuma kultivars; hul ordraagbaarheid ten opsigte van alle ander sitrus kultivars en verwante spesies. PKR-amplifisering, visualisering, en dokumentasie van DNA fragmente is uitgevoer vir alle Manderyn-hibried-, Clementine- Satsuma, suurlemoen, lemmetjie, pomelo, wortelstok asook diverse ander sitrus kultivars soos opgevat in die CIS kultivar lys. 'n Excel databasis is saamgestel waarin die DNA fragment resultate vir elke merker kombinasie sal vervat word. Die vervatting van die data het dan ook reeds begin. Genetiese verskille het voorgekom tussen meeste van die sitrus kultivars getoets met die geselekteerde SSR merkers, maar beperkte genetiese variasie is egter waargeneem by die soet lemoene. Addisionele merker kombinasies soos byvoorbeeld volgorde gebonde amplifisering polimorfisme- of sogenaamde SRAP merkers kan verdere genetiese inligting verskaf wat dit mag moontlik maak om te onderskei tussen die soet lemoen kultivars asook ander naverwante sitrus kultivars. Die gebruik van die SRAP merkers tesame met die geselekteerde SSR merkers om kan te onderskei tussen die soet lemoen kultivars sal sovoorts ondersoek word.

5.6 Climatic Regions of Southern Africa and cultivars being evaluated

CLIMATIC REGION	AREA	PLACE	CULTIVARS	
Hot-Dry	Limpopo	Tshipise	Grapefruit	
			Valencias	
			Mandarin Hybrids (Late)	
		Musina	Grapefruit	
			Valencias	
			Mandarin Hybrids (Late)	
		Letsitele	Grapefruit	
			Valencias	
			Mandarin Hybrids (Late)	
		Hoedspruit	Grapefruit	
			Valencias	
			Mandarin Hybrids (Late)	
Hot-Humid	Mpumalanga	Malelane	Grapefruit	
			Valencias	
			Mandarin Hybrids (Late)	
		Komatipoort	Grapefruit	
			Valencias	
			Mandarin Hybrids (Late)	
	KwaZulu-Natal	Pongola	Grapefruit	
			Valencias	
			Mandarin Hybrids (Late)	
		Nkwaleni	Grapefruit	
			Valencias	
			Mandarin Hybrids (Late)	
	Swaziland	Lowveld	Grapefruit	
			Valencias	
			Mandarin Hybrids (Late)	
Mozambique	Southern	Grapefruit		
		Valencias		
		Mandarin Hybrids (Late)		
Intermediate	Limpopo	Tom Burke	Navels (Mid/Late)	
			Valencias	
			Mandarin Hybrids (Mid/Late)	
			Lemons	
		Letaba	Navels (Mid/Late)	
			Valencias	
			Mandarin Hybrids (Mid/Late)	
			Lemons	
		Levubu	Navels (Mid/Late)	
			Valencias	
			Mandarin Hybrids (Mid/Late)	
			Lemons	
		Marble Hall	Marble Hall	Navels (Mid/Late)

	Mpumalanga	Nelspruit	Valencias
			Mandarin Hybrids (Mid/Late)
			Lemons
			Navels (Mid/Late)
		Karino	Valencias
			Mandarin Hybrids (Mid/Late)
			Lemons
			Navels (Mid/Late)
		Hazyview	Valencias
			Mandarin Hybrids (Mid/Late)
			Lemons
			Navels (Mid/Late)
	Schagen	Valencias	
		Mandarin Hybrids (Mid/Late)	
		Lemons	
		Navels (Mid/Late)	
Swaziland	Ngonini	Navels (Mid/Late)	
		Valencias	
		Mandarin Hybrids (Mid/Late)	
		Lemons	
Cold/Coastal	Eastern Cape	East Cape Midlands	Midseasons
			Navels/Valencias
			Mandarin Hybrids/Satsumas
		Gamtoos River Valley	Lemons
			Mandarin Hybrids
			Navels
	Satsumas/Clementines		
	Sundays River Valley	Lemons	
		Mandarin Hybrids	
		Navels/Valencias	
	KwaZulu-Natal	Richmond	Lemons
			Navels
		Ixopo/Umzimkhulu	Lemons
			Navels
	Western Cape	Knysna	Lemons
			Mandarin Hybrids
Heidelberg		Navels	
		Mandarin Hybrids	
		Lemons	
Paarl		Navels	
		Mandarin Hybrids	
		Satsumas/Clementines	
Wolseley	Navels		
	Mandarin Hybrids		

		Citrusdal	Satsumas/Clementines
			Navels/Valencias
			Mandarin Hybrids
		Clanwilliam	Lemons
			Navels/Valencias
			Mandarin Hybrids
		Swellendam	Lemons
			Navels/Valencias
			Mandarin Hybrids
			Satsumas
		Robertson	Navels/Valencias
			Mandarin Hybrids/Satsumas
Lemons			
Cool-Inland	North-West	Rustenburg	Navels (Mid)
			Navels (Late)
			Mandarin Hybrids
	Limpopo	Zebediela	Navels (Mid)
			Navels (Late)
			Mandarin Hybrids
		Mokopane	Navels (Mid)
			Navels (Late)
			Mandarin Hybrids
		Burgersfort	Navels (Mid)
			Navels (Late)
			Mandarin Hybrids
	Ohrigstad	Navels (Mid)	
		Navels (Late)	
		Mandarin Hybrids	
Mpumalanga	Ngodwana/Schoemanskloof	Navels (Mid)	
		Navels (Late)	
		Mandarin Hybrids	
Semi-Desert	Northern Cape	Kakamas/Blouputs	Navels (Late)
			Valencias
			Grapefruit
			Mandarin Hybrids (Late)
		Groblershoop/Upington	Navels (Late)
			Valencias
			Grapefruit
			Mandarin Hybrids (Late)
		Vaalharts	Midseasons
			Navels (Late)
			Valencias
			Mandarin Hybrids (Late)

5.7 Approximate maturity periods

Approximate Maturity Periods in the Cape region of South Africa

	March				April				May				June				July				Aug				Sept			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Early Clementine (Exp)			■	■	■																							
Oronules			■	■	■	■																						
Marisol					■	■	■																					
Early Oroval							■	■	■																			
SRA63								■	■	■																		
Oroval									■	■	■																	
Nules									▨	▨	▨	▨	▨	▨														

Exp = Experimental Cultivar

■	Solid blocks indicate average maturity periods for the area overall
▨	Striped blocks indicate variation due to microclimates

Approximate Maturity Periods in the Northern region of South Africa

	March				April				May				June				July				Aug				Sept			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Star Ruby																												
Marsh / Nartia																												
Jackson																												
Ray Ruby																												
Henderson																												
Rosé																												
Flamingo																												
Star Ruby late																												

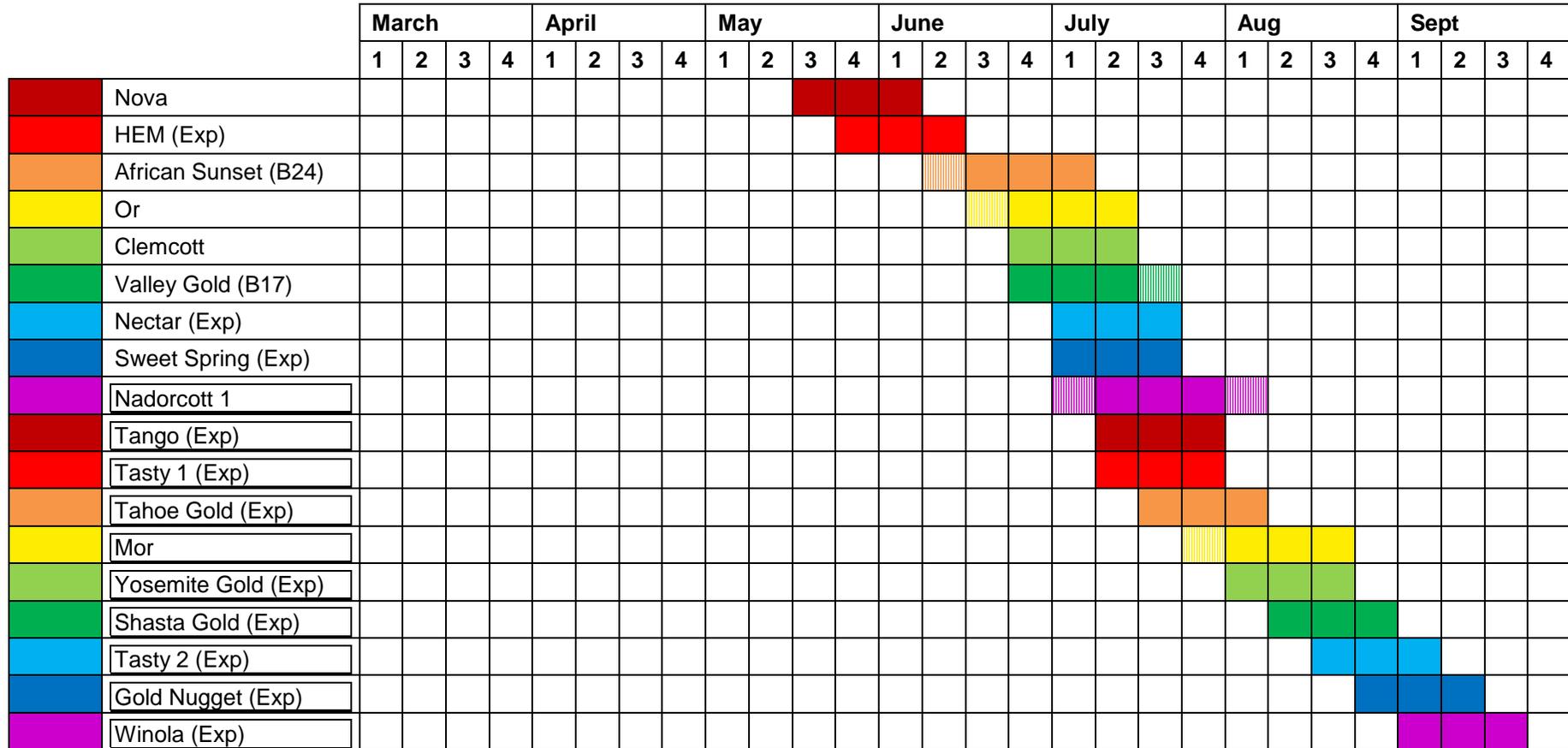
Exp = Experimental Cultivar

Approximate Maturity Periods in the Cape region of South Africa

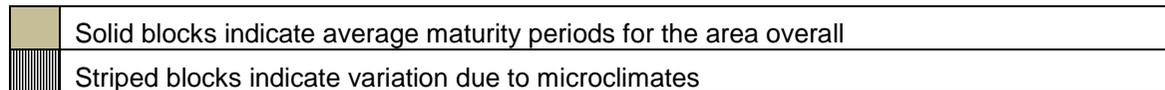
		March				April				May				June				July				Aug			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	Eureka																								
	Eureka SL (Exp)																								
	Genoa																								
	Lisbon																								
	Limoneira																								

Exp = Experimental Cultivar

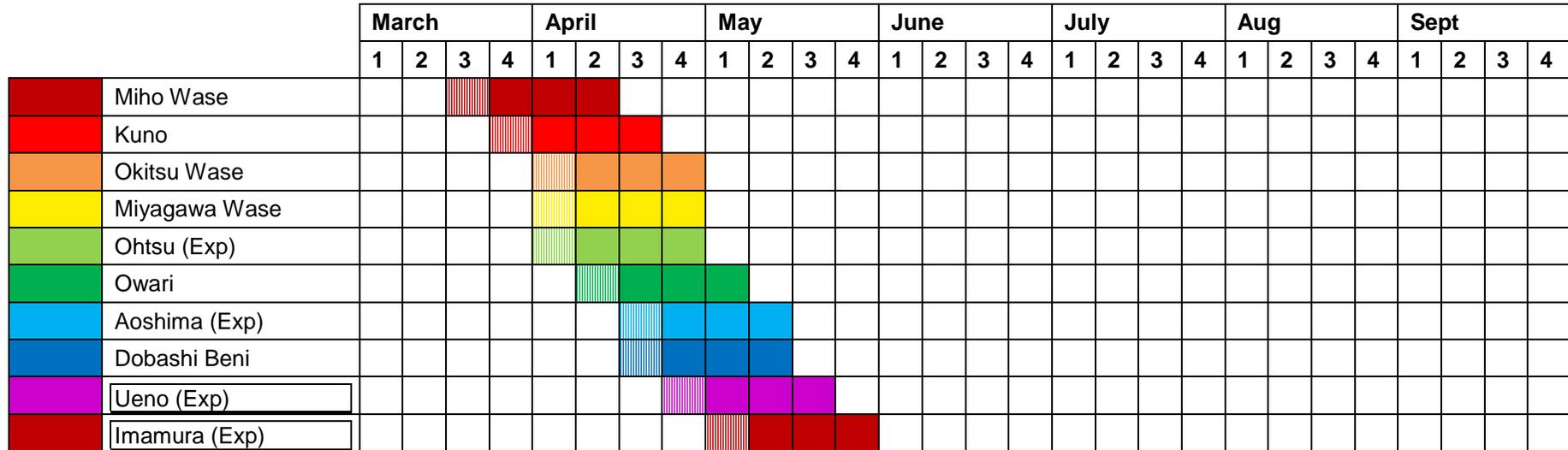
Approximate Maturity Periods in the Cape region of South Africa



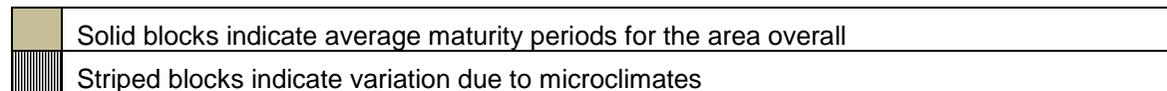
Exp = Experimental Cultivar



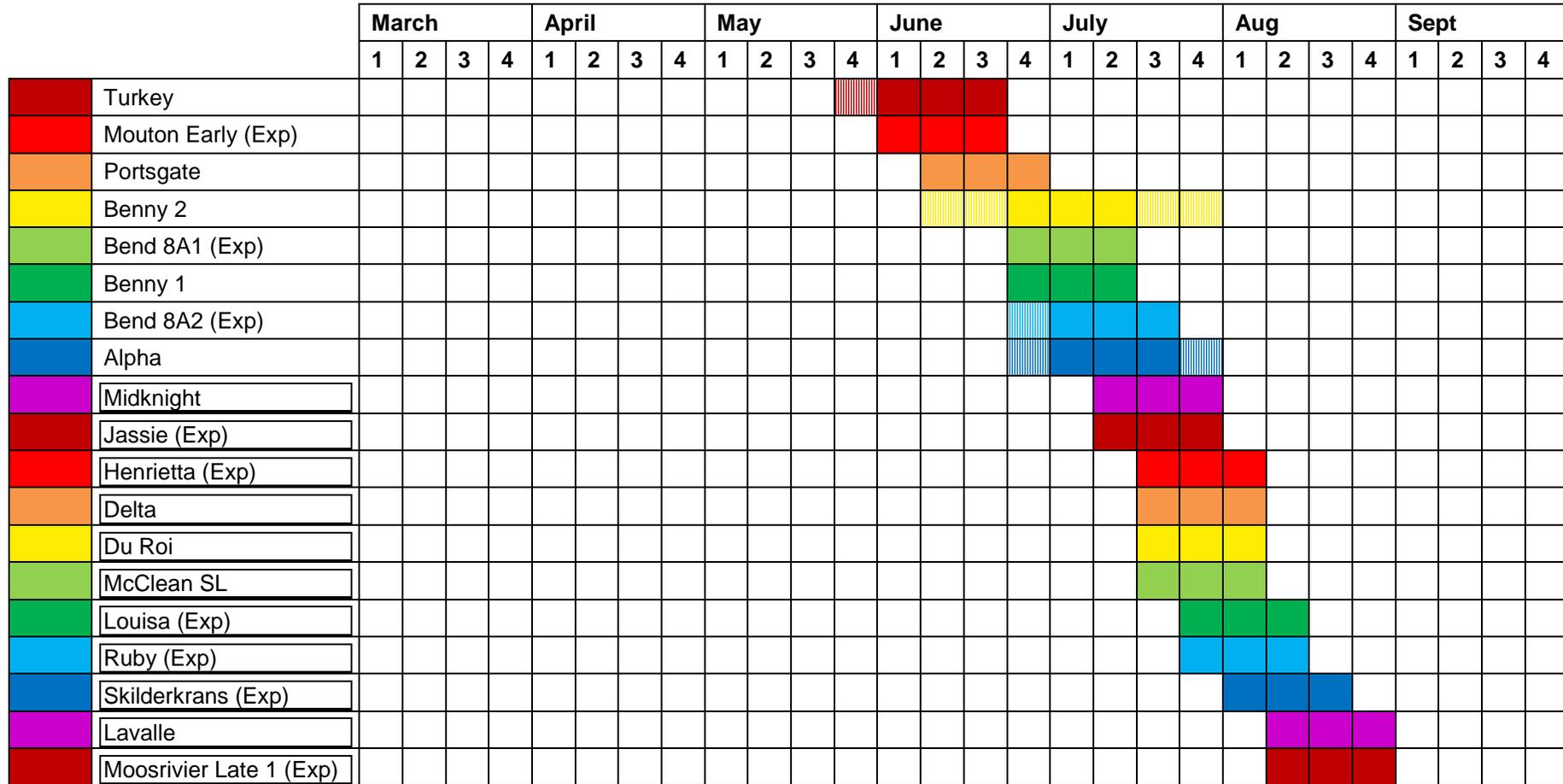
Approximate Maturity Periods in the Cape region of South Africa



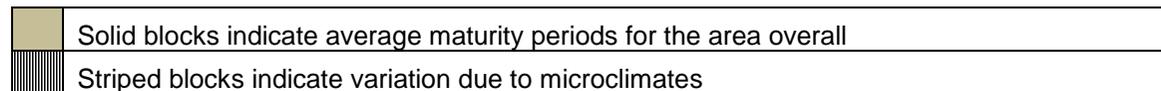
Exp = Experimental Cultivar



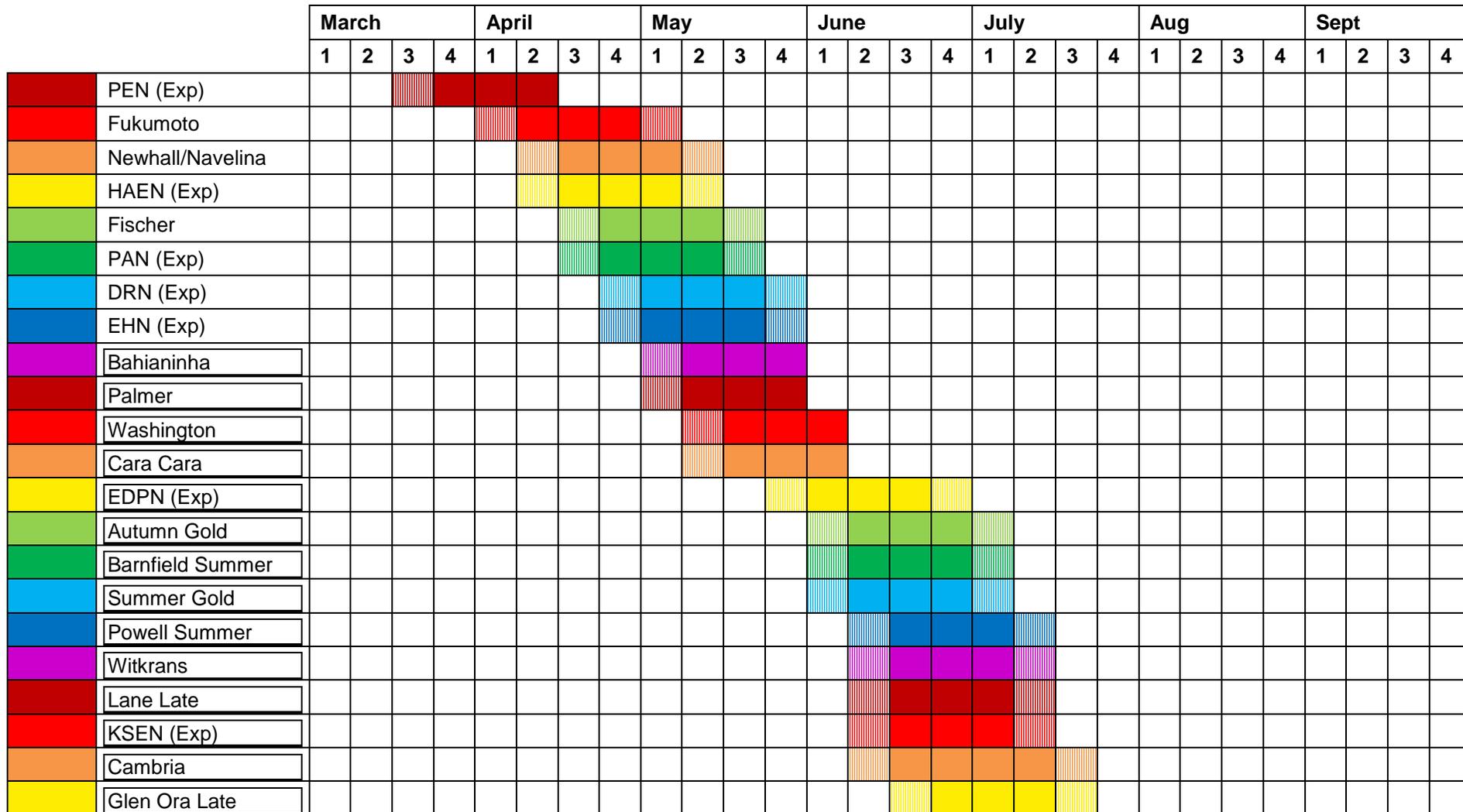
Approximate Maturity Periods in the Northern region of South Africa



Exp = Experimental Cultivar



Approximate Maturity Periods in the Cape region of South Africa



6 CITRUS IMPROVEMENT SCHEME (CIS)

By M.M.N. du Toit, P.H. Fourie, M. le Roux, L. Olivier, S.P. van Vuuren, J.H.J. Breytenbach and G. Cook (CRI)

6.1 Budwood

This report summarises the seasonal supply of budwood from May 2012 to April 2013. A total of 3,207,039 buds were supplied by the Citrus Foundation Block (CFB) and authorised for cutting in nurseries. This is 41,587 more buds than in the same period of 2012 and 86,377 more buds than in the same period of 2011 (Tables 6.1.1 and 6.1.2). Eureka is still the most popular variety. Star Ruby, the most popular variety in 2009, has decreased in popularity and currently lies in the 14th position (Table 6.1.3). During this period 8,250 buds were exported to neighbouring countries (Table 6.1.1.c). Mandarin (28.73%) was the most popular citrus type, followed by lemons (22.46%), Valencia's (20.92%) and navels (18.09%) (Table 6.1.1.d). The Top 30 varieties are listed in the report and comprised 92.67% of total number of buds supplied. During the 2012-2013 season, the proportion of CIS certified budwood that were supplied by means of authorised cutting in certified nurseries (40.8%) were markedly higher than in 2011-12 (25.1%) and 2010-11 (21.4%) seasons. CFB's inability to timeously supply >80% of demand was ascribed to the unprecedented demand for Eureka and other lemon varieties, as well as the high demand for recently introduced privately managed late navel and mandarin varieties. This also indicates the recent change in budwood supply patterns from gradual increased demand pattern to more peaked demand patterns. New initiatives at CFB will attempt to better predict these peaks and also to reduce response times to meet demand from 2-3 years to 1-2 years.

6.2 Seed

In this period, 3,073 litres of seed were supplied locally (Table 6.2.1.b) and 372 litres of seed were exported (Table 6.2.1.c). Carrizo Citrange remains the most popular rootstock (48.89%), followed by Swingle citrumelo (12.62%), C35 citrange (11.51%) and Cairn Rough Lemon (10.48%).

6.3 Production

With multiplication trees in production, the CFB presently carries a potential budwood stock of 6.5 million buds per season of 288 varieties. As the top 30 varieties comprise 93% of demand, multiplication tree stocks are being managed in order for CFB to be timeously able to supply demand of the sought-after varieties.

Greenhouse 5 was erected in 2011 and 312 replacement mother trees were established here. These will be used for evaluation purposes and not as mother trees. Following the comprehensive mother block indexing and the 2012 variety evaluations at the CFB, 390 mother trees of 65 different varieties were made from budwood cut from the best established mother trees. 19 varieties pre-immunised with GFMS12 and 6 newly released varieties were budded on 560 seedlings before the winter.

During September, 17 new varieties were received from the ARC and CRI STG facilities and established at the CFB. In March 2013, 6 newly released varieties were received from CRI and 4 from the ARC and established at the CFB. Another 8,656 seedlings in GH5 and were budded with varieties in high demand. In Greenhouse 1, 7,392 expired increase trees were replaced with seedlings and were budded with varieties in high demand during January 2013. Greenhouse 4 was enlarged with four bays for the establishment of another 9,353 increase trees. Two existing tunnels were converted with double layer plastic for better isolation and will be heated in the winter to fast-track seedling growth and budwood production.

6.4 Tree Certification

There were 896,001 trees certified during April to March 2013. This is 1,011,094 less trees than in the same period of 2012 and 1,097,793 less than in 2011 (Table 6.4.1). Most of the pending application forms was finalised and a large number of overdue tree certification application forms were received during the last quarter of the certification period and are being processed.

6.5 Nursery Registration

Twenty-five nurseries were visited during the May and November 2012 audits. Two new nurseries were certified and two are still in the process of applying for certification. Another two nurseries, that have temporally lost their certification status due to high *Phytophthora* infestation, have improved their systems and were again certified. The increase of *Phytophthora* infestation in certain nurseries is of concern and additional support is given to assist these nurseries to correct the problem.

6.6 Statutory Improvement Scheme

The proposed Scheme document was submitted to the Registrar and further feedback is expected. A discussion document stating the merits and demerits of a statutory scheme is being compiled, which will be circulated to all stakeholders and workshopped to address concerns raised by certain parties.

6.7 Protective zone surrounding the Citrus Foundation Block

The legislation, declaring a radius of 5km around the CFB as a citrus free area, has been published in the Government Gazette on 21 January 2011. Orders to remove all citrus trees were issued by DAFF and feedback were received that some residents have removed their citrus trees, while other refused. DAFF has made follow-up visits to owners refusing to remove trees, and is currently seeking legal advice to ensure that the remainder of the trees are removed.

6.8 Establish and maintain a virus-free gene source at CRI: Project 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 18 new selections were submitted for STG and a further 40 submissions, from previous years, are at various stages in the process before release. Fifteen of the latter are being indexed to ensure virus-free status. 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 of each variety is maintained in an insect-free tunnel at CRI. Nine new cultivars and selections were supplied to the CFB in this report period and added to the CRI gene source, which now comprises 270 cultivars and selections. Erection of a new tunnel for housing the nucleus block is nearing completion. This facility was funded by SACNA and CRI.

6.9 Diagnostic services for graft transmissible diseases: Project 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 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 biological evaluation of CTV severity in 174 mother trees was initiated this year and will be completed in March 2013. Viroid indexing of selected CFB multiplication blocks was finalised.

SITRUSVERBETERINGSKEMA (SVS)

Deur M.M.N. du Toit, P.H. Fourie, M. le Roux, L. Olivier, S.P. van Vuuren, J.H.J. Breytenbach en G. Cook (CRI)

6.1 Okuleerhout

Hierdie verslag is opsommend van die seisoenale okuleerhout verskaffing. 'n Totaal van 3,207,039 ogies is deur die Sitrus Grondvesblok (SGB) verskaf en goedgekeur vir sny in kwekerie. Dit is 41,587 meer ogies as in dieselfde periode van 2012 en 86,377 meer ogies as in dieselfde periode van 2011 (Tabel 6.1.1 en 6.1.2). Eureka is steeds die gewildste kultivar. Star Ruby, was die gewildste in 2009, maar het afgeneem in gewildheid en is tans in die 14^{de} posisie (Tabel 6.1.3). Gedurende hierdie periode is 8,250 ogies na die naburige lande uitgevoer (Tabel 6.1.1.c). Mandaryne (28.73%) was die gewildste sitrus tipe, gevolg deur suurlemoene (22.46%), Valencia's (20.92%) en nawels (18.09%) (Tabel 6.1.1.d). Die Top 30 variëteite verteenwoordig 92.67% van die totale okuleerhout verskaffing.

6.2 Saad

Gedurende Mei 2012 tot April 2013, was daar 3,073 liter saad plaaslik verskaf (Tabel 6.2.1.b) en 372 liter saad was uitgevoer (Tabel 6.2.1.c). Carrizo Citrange bly die gewildste onderstam (48.89%), gevolg deur by Swingle citrumelo (12.62%), C35 citrange (11.51%) en Cairn Growweskil Suurlemoen (10.48%).

6.3 Produksie

Met die vermeerderingsbome tans in produksie, het die SGB 'n potensiële okuleerhout voorraad van 6,5 miljoen ogies, van 288 variëteite, per jaar. Omdat die Top 30 variëteite 93% van die aanvraag

verteenwoordig, word die voorraad diensooreenkomstig bestuur. Sodoende sal die SGB in staat wees om aan die aanvraag van die gesogste variëteite te voldoen.

Kweekhuis 5 is in 2011 opgerig en 312 vervangingsmoederbome is hier gevestig. Hierdie sal vir evalueringdoeleindes gebruik word en sal nie as moederbome dien nie. Na aanleiding van die omvattende herindeksering van die moederbome en die 2012 kultivar evaluering by die SGB, is 390 nuwe moederbome van 65 verskillende variëteite gemaak. Okuleerhout van die beste bestaande moederbome is gebruik. Negentien variëteite, wat met GFMS12 gepreïmmuniseer is, en 6 nuut-vrygestelde variëteite is voor die winter op 560 saailinge geokuleer.

Gedurende September is 17 nuwe variëteite van die LNR en CRI se GPE fasiliteite ontvang en by die SGB gevestig.

In Maart 2013 is ses nuwe vrygestelde variëteite van CRI en 4 van die LNR ontvang en by die SGB gevestig. Daar is ook 8,656 saailinge in Kweekhuis 5 met variëteite wat in hoë aanvraag is, geokuleer. In Kweekhuis 1 is 7,392 verstreke vermeerderingsbome met saailinge vervang, wat daarna in Januarie 2013 met hoë aanvraag variëteite geokuleer is. Kweekhuis 4 is met 'n addisionele vier koepels vergroot, wat nog 9,353 vermeerderingsbome sal kan huisves. Twee bestaande tonnells is omskep met 'n dubbele laag plastiek wat verbeterde isolasie verseker en in die winter verhit sal word. Die doelwit is om die saailing en okuleerhout produksie te versnel.

6.4 Boom Sertifisering

Daar is 896,001 bome gesertifiseer gedurende April tot Maart 2013. Dit is 1,011,094 minder bome as in dieselfde periode van 2012 en 1,097,793 minder as in 2011 (Tabel 7.4.1). 'n Groot hoeveelheid boom sertifikaat aansoeke is in die laaste kwartaal ontvang en sal in die volgende kwartaal geprosesseer word.

6.5 Kwekery Registrasie

Vyf-en-twintig kwekerye is gedurende Mei en November 2012 oudits besoek. Twee nuwe kwekerye is gesertifiseer, terwyl 'n ander twee nog in die proses is om aansoek om sertifisering te doen. Twee kwekerye wat tydelik hul sertifisering, weens die hoë *Phytophthora* besmetting verloor het, het hul sisteme verbeter en is toe weer gesertifiseer. Die toename van *Phytophthora* besmetting is sekere kwekerye is rede tot kommer en addisionele ondersteuning word aan hierdie kwekerye gebied, om sodoende die probleem reg te stel.

6.6 Statutêre Verbeteringskema

Die voorgestelde Skema dokument is aan die Registrateur voorgelê en verdere terugvoering word verwag. 'n Besprekingsdokument wat die voordele en nadele uiteensit, word saamgestel en sal aan die belanghebbendes gestuur word. Werkswinkels sal gehou word om die bekommernisse van sekere partye aan te spreek.

6.7 Buffer sone rondom die Sitrus Grondvesblok

Die wetgewing wat die area binne 'n 5-km radius rondom die SGB as 'n sitrus-vrye area verklaar, is op 21 Januarie 2011 in die Skaatskoerant gepubliseer. Bevele om alle sitrus in hierdie sone te verwyder, is deur DAFF uitgereik en terugvoering is ontvang dat sommige inwoners hul bome verwyder het, terwyl ander geweier het. DAFF het opvolgbesoeke by die inwoners wat weier om hul bome te verwyder afgelê en is tans besig om regsadvies in te win om te verseker dat die oorblywende bome ook verwyder sal word.

6.8 Vestiging en onderhoud van 'n virus-vrye genebron by CRI: Projek 790

Groei-puntenting (GPE) word gebruik om sitrus materiaal te reinig van ent-oordraagbare patogene voor toevoeging tot die Sitrusverbeteringskema se genebron. Gedurende die jaar is 18 nuwe seleksies vir GPE ingedien en 'n verdere 40 seleksies van vorige introduksies is in verskeie fases voor vrystelling. Vyftien van laasgenoemde word reeds geïndekseer om virusvrye status te bevestig. Virusvrye materiaal word met 'n toepaslike *Citrus tristeza virus* bron gepreïmmuniseer voordat dit aan die Sitrus Grondvesblok (SGB) buite Uitenhage vrygestel word. Virusvrye boompies van verskillende variëteite en seleksies word as 'n genebron in 'n insek-vrye tunnel by CRI bewaar. Nege nuwe seleksies van 2012 is aan die SGB voorsien en die is by die CRI genebron gevoeg, wat tans uit 270 variëteite en seleksies bestaan. Oprigting van 'n nuwe tunnel om die genebron te huisves, is bykans afgehandel. Hierdie fasiliteit is deur SACNA en CRI befonds.

6.9 Diagnostiese dienste vir ent-oordraagbare siektes: Projek 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. Die skema behels beide die eliminerings van patogene en die onderhou en verspreiding van gesonde voortplantingsmateriaal. Diagnose van entoordraagbare 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 aktiwiteite van die SVS. Die moederbome by die Sitrus Grondvesblok word op 'n rotasie basis, elke tweede jaar ge-herindekseer om te bepaal of enige strawwe CTV rasse, of enige viroïede (CVd), in die moedermateriaal voorkom. Die biologiese evaluasie van die CTV virulensie in 174 moederbome is gedurende die jaar begin en behoort in Maart 2013 voltooi te wees. Viroïed indeksering van spesifieke SGB vermeerderingsblokke is ook gefinaliseer.

Table 6.1.1. CIS budwood supplied during the period May to April: 2011-2013.

a) Summary of Budwood Supplied:

Area	2011	2012	2013	Total
South Africa	3 099 862	3 138 402	3 198 789	9 437 053
Exports	20 800	27 050	8 250	56 100
	3 120 662	3 165 452	3 207 039	9 493 153

b) CIS budwood supplied in South Africa:

Area	2011	2012	2013	Total
Eastern Cape	677 878	611 175	640 907	1 929 960
Gauteng		100		100
KwaZulu-Natal	31 200	34 300	41 800	107 300
Limpopo	1 175 617	1 259 732	1 268 530	3 703 879
Mpumalanga	273 195	260 583	223 085	756 863
North-West Province	84 500	246 194	121 000	451 694
Northern Cape	212 800	141 465	149 669	503 934
Western Cape	644 672	584 853	753 798	1 983 323
	3 099 862	3 138 402	3 198 789	9 437 053

c) CIS budwood exported

Area	2011	2012	2013	Total
Namibia	4 500	14 150		18 650
Other African States			8 100	8 100
Swaziland	4 300			4 300
Zimbabwe	12 000	12 900	150	25 050
	20 800	27 050	8 250	56 100

d) CIS budwood supplied per variety type:

Variety type	2011	%	2012	%	2013	%
Clementine	79 897	2.56%	115 947	3.66%	128 700	4.01%
Ellendale	3 900	0.12%	1 540	0.05%	1 800	0.06%
Grapefruit	150 495	4.82%	24 385	0.77%	87 670	2.73%
Kumquat	7 900	0.25%	10 550	0.33%	7 710	0.24%
Lemon	694 814	22.26%	681 002	21.51%	720 418	22.46%
Lime	23 800	0.76%	47 600	1.50%	37 170	1.16%
Mandarin	674 896	21.63%	829 289	26.20%	921 457	28.73%
Midseason	862	0.03%	735	0.02%	9 340	0.29%
Navel	602 653	19.31%	643 722	20.34%	580 017	18.09%
Ornamental	2 732	0.09%	1 500	0.05%	4 580	0.14%
Pummelo	2 230	0.07%	850	0.03%	850	0.03%
Rootstock	130	0.00%	20	0.00%		0.00%
Satsuma	155 145	4.97%	159 696	5.04%	36 270	1.13%
Seville	3 532	0.11%	1 000	0.03%	220	0.01%
Valencia	717 676	23.00%	647 616	20.46%	670 837	20.92%
	3 120 662	100.00%	3 165 452	100.00%	3 207 039	100.00%

Table 6.1.2. CIS budwood supplied during the period May to April: 2011-2013.

Variety	Year	Eastern Cape	Gauteng	KwaZulu-Natal	Limpopo	Mpumalanga	Namibia	North-West Province	Northern Cape	Other African States	Swaziland	Western Cape	Zimbabwe	Total
Clementine	2011	8 250		500	15 500	1 500		1 000				53 147		79 897
	2012	4 600			64 710	1 068		2 900	4 300			38 369		115 947
	2013	2 600		500	10 000	600			7 000			108 000		128 700
Ellendale	2011											3 900		3 900
	2012					20			920			600		1 540
	2013								300			1 500		1 800
Grapefruit	2011	3 000		400	27 705	58 560		1 700	51 400		4 300	1 430	2 000	150 495
	2012	800		1 000	8 150	10 130	500	1 300	20			1 585	900	24 385
	2013	1 100		2 000	54 660	27 120				1 000		1 790		87 670
Kumquat	2011	200		600		4 300		1 600				1 200		7 900
	2012	150		600	1 800	2 000		3 000	500			2 500		10 550
	2013	500		3 000	200	200		500	10			3 300		7 710
Lemon	2011	285 169		2 000	244 900	19 385		13 600	49 620			80 140		694 814
	2012	202 882	100	8 000	191 050	57 740	100	106 900	500			113 730		681 002
	2013	190 428		7 000	325 780	17 905		47 700	41 500	150		89 955		720 418
Lime	2011	200		700	7 000	4 200		4 000				7 700		23 800
	2012	2 050			10 700	5 100	50	19 500				10 200		47 600
	2013	5 560		500	10 200	2 860		9 000	550			8 500		37 170
Mandarin	2011	95 673		4 500	226 650	43 915	1 500	12 500	17 620			272 538		674 896
	2012	155 014		6 000	270 100	29 796	7 000	33 900	63 600			263 879		829 289
	2013	200 752		500	344 860	39 395		26 100	40 700	1 150		268 000		921 457
Midseason	2011	715				40						107		862
	2012							350				385		735
	2013											9 340		9 340
Navel	2011	127 560		14 200	259 352	53 705	1 500	22 300	34 840			89 196		602 653
	2012	156 259		9 700	248 000	80 919	4 500	55 044	26 075			63 225		643 722
	2013	178 244		11 500	95 240	55 660		18 200	28 540	2 800		189 833		580 017
Ornamental	2011			1 200	350			200	500			482		2 732
	2012								1 000			500		1 500
	2013				3 100			400				1 030	50	4 580
Pummelo	2011				2 000	160						70		2 230
	2012					650						200		850
	2013				600				50			200		850
Rootstock	2011	80				50								130
	2012					20								20

Satsuma	2011	38 850		3 400	20 400	9 000		3 000	8 000			72 495		155 145
	2012	37 968		6 000	56 000	4 933		2 000	2 000			50 795		159 696
	2013	4 700		12 000	3 500	1 660						14 410		36 270
Seville	2011			200								3 332		3 532
	2012											1 000		1 000
	2013											120	100	220
Valencia	2011	118 181		3 500	371 760	78 380	1 500	24 600	50 820			58 935	10 000	717 676
	2012	51 452		3 000	409 222	68 207	2 000	21 300	42 550			37 885	12 000	647 616
	2013	57 023		4 800	420 390	77 685		19 100	31 019	3 000		57 820		670 837
		1 929 960	100	107 300	3 703 879	756 863	18 650	451 694	503 934	8 100	4 300	1 983 323	25 050	9 493 153

2011	3 120 662
2012	3 165 452
2013	3 207 039
Total	9 493 153

Table 6.1.3. Top 30 varieties supplied from CFB and authorised for cutting in nurseries (BCIN) during the period May to April: 2011-2013.

2011					2012					2013				
Variety	CFB	BCIN	Total	%	Variety	CFB	BCIN	Total	%	Variety	CFB	BCIN	Total	%
Eureka	158 695	365 933	524 628	16.81%	Eureka	230 800	238 329	469 129	14.82%	Eureka	283 273	263 185	546 458	17.04%
Midnight	338 470	5 000	343 470	11.01%	Nadorcott 1	145 423	120 618	266 041	8.40%	Or 4	50 800	194 950	245 750	7.66%
Nova	253 339		253 339	8.12%	Midnight	179 801		179 801	5.68%	Midnight	189 672	35 025	224 697	7.01%
Nadorcott 1	124 434	44 000	168 434	5.40%	Nova	177 628		177 628	5.61%	Late Valencia	123 790	71 000	194 790	6.07%
Bahianinha	164 215		164 215	5.26%	Chislett M7	58 000	112 849	170 849	5.40%	Chislett M7	45 530	121 404	166 934	5.21%
Star Ruby	131 920		131 920	4.23%	Late Valencia	144 098	10 000	154 098	4.87%	ARCCIT 1614	37 000	116 750	153 750	4.79%
Miho Wase	111 000		111 000	3.56%	Bahianinha	131 020		131 020	4.14%	Nadorcott 1	95 221	55 932	151 153	4.71%
Late Val.	110 645		110 645	3.55%	Or 4	43 765	79 120	122 885	3.88%	Nova	120 959	2 000	122 959	3.83%
Palmer	100 295		100 295	3.21%	Lisbon	88 418	17 500	105 918	3.35%	Tango	45 451	76 359	121 810	3.80%
Lisbon	68 980	25 051	94 031	3.01%	Benny 2	100 772		100 772	3.18%	Carninka Late	25 502	71 605	97 107	3.03%
Mor 26	76 626		76 626	2.46%	Sonet	94 070		94 070	2.97%	Witkrans 3	41 290	54 610	95 900	2.99%
Washington	67 742		67 742	2.17%	Limoneira 8A	50 170	42 000	92 170	2.91%	Lisbon	70 330	21 000	91 330	2.85%
Delta	60 751		60 751	1.95%	Nules	75 250		75 250	2.38%	Clemenluz	6 250	79 300	85 550	2.67%
Benny 2	56 535		56 535	1.81%	Delta	66 785		66 785	2.11%	Star Ruby	79 760		79 760	2.49%
Gusocora (G5)	44 850	10 000	54 850	1.76%	Washington	66 356		66 356	2.10%	Benny 2	57 571	8 000	65 571	2.04%
Nova ARC	49 020		49 020	1.57%	Palmer	60 720		60 720	1.92%	Gusocora	29 282	31 500	60 782	1.90%
Chislett M7	18 860	27 702	46 562	1.49%	Miho Wase	55 178		55 178	1.74%	Cambria 4	23 868	35 263	59 131	1.84%
ARCCIT 3212	43 576		43 576	1.40%	Mor 26	54 378		54 378	1.72%	Cambria 3	32 337	23 558	55 895	1.74%
Nules	39 500	3 300	42 800	1.37%	Cambria 3	39 615	11 510	51 125	1.62%	Limoneira 8A	44 540		44 540	1.39%
Genoa	15 815	26 000	41 815	1.34%	Bearss	43 950	3 000	46 950	1.48%	Delta	43 042		43 042	1.34%
Cambria 3	41 500		41 500	1.33%	Empress	45 500	1 000	46 500	1.47%	Genoa	37 340		37 340	1.16%
Lina	39 199		39 199	1.26%	ARCCIT1614	23 485	21 950	45 435	1.44%	Bearss	36 770		36 770	1.15%
Limoneira8A	30 160	3 500	33 660	1.08%	Gusocora (G5)	39 775		39 775	1.26%	Mor 26	33 870		33 870	1.06%
Sonet	21 620	11 800	33 420	1.07%	Cambria 4	22 700	13 000	35 700	1.13%	Empress	26 410	5 500	31 910	0.99%
Lavalle	26 725	5 050	31 775	1.02%	Witkrans 3	9 475	23 794	33 269	1.05%	Nules	28 820		28 820	0.90%

Empress	26 400	3 000	29 400	0.94%	Tango	23 060		23 060	0.73%	Lavalle 2	22 000		22 000	0.69%
Fischer	28 720		28 720	0.92%	Midknight1 (115)	19 450		19 450	0.61%	Bahianinha	20 940		20 940	0.65%
Cambria 4	24 038		24 038	0.77%	Star Ruby	19 210		19 210	0.61%	Miho Wase	17 770	1 000	18 770	0.59%
Bearss	22 400		22 400	0.72%	Lavalle 2	18 865		18 865	0.60%	Washington	15 407	3 000	18 407	0.57%
Lane Late	19 207		19 207	0.62%	Carninka Late	9 215	8 500	17 715	0.56%	Palmer	16 270		16 270	0.51%
Top 30	2315237	530 336	2 845 573	91.18%	Top 30	2136932	703 170	2 840 102	89.72%	Top 30	1701065	1270941	2 972 006	92.67%
Total	2452726	667 936	3 120 662	100.00 %	Total	2370960	794 492	3 165 452	100.00%	Total	1906108	1300931	3 207 039	100.00%

Table 6.2.1. Rootstock seed supplied during the period: May to April 2011-2013.

a) Summary of seed supplied:

Area	2011	2012	2013	Total
South Africa	3 083	3 069	3 073	9 225
Exports	1 152	575	372	1 971
	4 235	3 644	3 445	11 196

b) Seed supplied in South Africa:

Area	2011	2012	2013	Total
Eastern Cape	215	459	337	983
KwaZulu-Natal	9	14	14	29
Limpopo	1 952	1 986	1 833	5 759
Mpumalanga	48	45	11	104
North-West Province	237	52	98	387
Northern Cape	18	58	75	151
Western Cape	604	455	706	37
	3 083	3 069	3 073	7 450

c) Seed exported:

Area	2011	2012	2013	Total
Australia/NZ		135	72	207
Caribbean	10	8		18
Europe		72	100	172
Far East	1 016			1 016
Mozambique		3		3
Namibia	1	8		9
Other African States	70	148	176	267
South America	20	201	21	242
Zimbabwe	34		2	1 764
	1 152	575	372	3 698

d) Seed supplied per variety:

Variety	2011	%	2012	%	2013	%
C35 Citrange	458	10.82%	531	14.57%	396	8.12%
Carrizo Citrange	1 744	41.19%	1 507	41.36%	1 684	50.76%
Cleopatra Mandarin		0.00%	25	0.69%	5	0.15%
Flying Dragon	2	0.05%	208	5.71%	227	6.84%
Minneola X Trifoliolate	125	2.95%	81	2.22%	73	2.22%
Rough Lemon	337	7.95%	243	6.67%	361	10.88%
Rough Lemon (Schaub)	54	1.28%	60	1.65%	17	0.51%
Rough Lemon (Van Staden)			14	0.38%		
Sunki Beneke	2	0.05%		0.00%		0.00%
Swingle Citrumelo	724	17.10%	516	14.16%	435	13.10%
Troyer Citrange	471	11.12%	146	4.01%	75	2.26%
Volkameriana	65	1.54%	64	1.76%	57	1.72%
X639	233	5.50%	234	6.42%	78	2.35%
Yuma Citrange	20	0.47%	15	0.41%	36	1.09%
	4 235	100.00%	3 644	100.00%	3 445	100.00%

Table 6.4.1. Tree certification: April to March 2011-2013.

Variety	Year	Angola	Botswana	Eastern Cape	Gauteng	KwaZulu - Natal	Limpopo	Mozambique	Mpumalanga	Namibia	North-West Province	Northern Cape	Swaziland	Western Cape	Zimbabwe	Total
Clementine	2013			1 200	5 560		18 899		2 102	560				22 212		50 533
	2012			9 751			5 440		13 190					14 396		42 777
	2011			500			1 500			2 870				5 670		10 540
Ellendale	2013									270						270
	2012													10		10
	2011													200		200
Grapefruit	2013			570		5 500	9 730		1 420	20						17 240
	2012			8 662		12 000	23 976		17 919		10	40 017	3 000	5 925		111 509
	2011		50	23 555		9 900	123 994		50 438	225	150	79 669	45 528	1 472		334 981
Pummelo	2013										6					6
	2012						603		10							613
	2011						1 400		4 502	111						6 013
Kumquat	2013						30		100					200		330
	2012						200									200
Lemon	2013	1 500		120 417		6 684	11 038		25 555	1 050	5 400			21 808		193 452
	2012		50	130 438		19 750	168 281		61 295	4 602		5 837	2 900	56 035		449 188
	2011		10	106 519		900	91 441		50 888	2 979	25 700	3 955		14 660		297 052
Lime	2013						6 516		2 000					1 045		9 561
	2012						7 082		6	3				1 190		8 281
	2011				400		6 210		555	2 285			1 500	1 800		12 750
Mandarin Hybrid	2013		500	20 479		4 000	25 983		22 219	40	21 430	2 424		89 235		186 310
	2012			77			80		96	1	27	13	1	99		398

			700	560			227		716	160	372	791	700	367		593
	2011		760	81 397	1 500		41 171		29 824	4 015	38 053	28 835	10 000	54 514		290 069
Midseason	2013			340												340
Navel	2013	1 100		53 689	50	4 500	17 597		92 294	4 565	7 750		7 500	20 890		209 935
	2012		1 600	100 310	50	4 600	112 643		68 008	3 302	5 894	7 531		31 652		335 590
	2011	10 000	890	165 301		940	128 932		110 204	7 625	26 970	10 655		24 770		486 287
Ornamental	2012					240										240
Satsuma	2013	1 930		4 859	230		398		1 720					10 214		19 351
	2012			29 251			11 660		14 905		3 050			4 555		63 421
	2011			19 457			29 292		13 198			1 030		11 007	6 610	81 224
Seville	2012												170		170	
Valencia	2013		1 500	23 671	4 560	9 950	126 639		31 112	310	300	300		10 331		208 673
	2012		1 800	40 346	80	5 150	310 913		87 047	6 105	8 701	3 391		32 970		496 503
	2011	10 000	925	47 989	500		221 823	3 250	110 951	5 869	6 103	24 328	3 520	39 220	200	474 678

Totals: 2011 = 1 993 794; 2012 = 1 907 095; 2013 = 896 001

6.8 Establish and maintain a virus-free gene source at CRI

Project 790 (2005 - 2025) by S.P. van Vuuren, G. Cook and J.H.J. Breytenbach, (CRI)

Introduction

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 to 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 source plant are budded on a standard rootstock in the glasshouse. After bud grown and maturation (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 tip, containing the leaf 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 starts 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, the graft is closed by a plastic bag for 8 days. Once the graft has sufficiently grown, buds for indexing are taken from this material.

Virus indexing: Elimination of graft transmissible pathogens is established by indexing the STG material on sensitive biological indicators as described by van Vuuren and Collins (1990). Biological indexing results are thereafter 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"> Receive and maintain new selections/cultivars. 	Ongoing: 40 brought forward from previous year; 18 new selections received in current year.
<ul style="list-style-type: none"> Do shoot tip grafting (STG) of new selections/cultivars and index for graft transmissible diseases, to ensure they are virus-free. 	Ongoing: 214 STGs, 32 successful micro-grafts.
<ul style="list-style-type: none"> Maintain the virus-free nucleus block in an insect-free tunnel. 	Ongoing: currently 270 cultivars and selections.
<ul style="list-style-type: none"> Establish a pre-immunised source of the new selection/cultivar with a suitable CTV cross-protection source and supply budwood to the CFB. 	Ongoing: 9 additions supplied to CFB.
<ul style="list-style-type: none"> Re-index the virus-free selection every three years. 	Ongoing: Partly PCR'd for CVd, CPsV and Greening

STG:

The STG procedure was initiated at CRI in 2004 and the existing facilities completed in 2005. The introductions for STG and subsequent releases to the CFB from 2008 to date are summarised in Table 6.8.1. Eighteen new selections of three cultivar groups were submitted for STG in the current year and 40 brought forward from the previous year. During this report period a total of 214 STGs were done on these introductions, including failed grafts. Of these, 32 were successfully micro-grafted.

Fifteen of the successful STGs have been indexed biologically while the remainder are duplicate ex-plants or micro-grafts which are still too small for indexing. Eleven of the 15 successful STGs indexed negative for CTV, ASGV and CVd by biological indexing, and four tested positive for CVd (Table 6.8.2). Twenty STG's were biologically indexed for CPsV and CID and tested negative (Table 6.8.3). On average it takes 24 to 30 months to obtain a virus-free STG, which includes the scheduled indexing to confirm the virus-free status of the cultivar. However, delays can occur with elimination of some pathogens. 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.

Confirmation of biological indexing by PCR on a number of STG submissions is reflected in Table 6.8.4. Nine 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 ²	STG introductions and releases 2008 to 2012 ¹															
	2008			2009			2010			2011			2012			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	2	0	1	0	1	0	0	1	5	1	5
G	2	0	1	1	0	0	1	0	1	0	0	0	0	0	0	0
L	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0
Mi	7	0	2	5	0	4	1	0	2	1	0	0	1	0	0	1
Ma	1	1	1	1	1	0	2	3	2	3	0	2	1	0	1	0
N	25 [*]	4	1	20 ^{**}	6	7	19	6	3	22	11	4	29	10	5	34
R	2	0	1	1 ^{***}	0	0	0	0	0	0	0	0	0	0	0	0
V	6	1	0	7 ^{***}	2	1	6	0	0	6	2	2	6	2	2	6
Or	6	0	4	2	0	0	2	0	1	1	0	0	1	0	0	1
Rs	0	1	0	1	0	0	1	0	1	0	1	0	1	1	0	2
Total	52	8	11	41	9	15	32	10	10	34	14	8	40	18	9	49

¹ Bf = Brought forward from previous year; Balance = Balance for the current reporting year.

² 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
Navel	9	6	3
Midseason	-	-	-
Mandarin	1	1	-
Valencia	4	3	1
Clementine	1	1	-
Grapefruit	-	-	-
Lemon	-	-	-
Ornamental citrus	-	-	-
Rootstock	-	-	-
Total	15	11	4

Table 6.8.3. STG submissions indexed biologically for CPsV and CID.

Variety Group	Number of plants	Negative	Positive
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	-	-	-
Total	20	20	

Table 6.8.4. STG plants indexed by PCR for CVd, ASGV and CPsV and Greening.

Cultivars	CVd	ASGV	CPsV	Greening
Navel	6	6	13	8
Midseason	-	-	3	-
Valencia	2	3	8	2
Reticulata	-	-	1	1
Mandarin	1	1	4	1
Grapefruit	-	-	1	-
Clementine	1	1	2	-
Lemon	-	-	1	-
Ornamental	-	-	-	-
Rootstock	-	-	-	-
Total	10	11	33	12

Maintaining the virus-free gene source:

The numbers of selections maintained at CRI are listed per cultivar/variety group in Table 6.8.5. Nine 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.

Erection of a new tunnel for housing the nucleus block is nearing completion. This facility was funded by SACNA and CRI. The transfer of the gene source to the new glasshouse is expected to be finalised by end June 2013.

Table 6.8.5. The number of accessions per cultivar/variety group maintained at the CRI nucleus block.

Variety Group	No. of selections maintained at CRI
Clementine	24
Diverse (Citron, Sour orange, etc.)	2
Ellendale	4
Grapefruit	18
Kumquat	1
Lemon	20
Lime	4
Mandarin	5
Midseason	27
Navel	55
Ornamnetal	4
Pummelo	7
Reticulata	33
Rootstock	21
Satsuma	8
Valencia	45
Total	270

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.
- Nine new selections were added to the gene source and also released to the CFB.
- Eighteen 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 2014 and Jan-Mar 2015

- 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

Project 796 (2005 - 2025) by S.P. van Vuuren, G. Cook, J.H.J. Breytenbach, (CRI) and B.Q. Manicom (ARC-ITSC)

Introduction

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.

efficient. If add some soap (5% Tween) it improves extraction and spreading when sprayed. *Tephrosia* pesticide could be commercialized.

S1-19 **S. Okweche et al.** Comparative efficacy of pesticidal plant products and carbofuran in the management of maize stem borers in Nigeria.

Tephrosia vogelii widely used in Africa as is neem. *Gmelina arborea* also used in trial. As effective as carbofuran.

S1-20 **Pofu et al.** A ground leaching technology for application of bio-nematicides from indigenous plants in small holder farming systems of South Africa.

Use fruits of *Cucumis* spp. (spiky cucumbers). Also using rootstock for watermelon to provide resistance. Ground leaching of powdered product on soil around plant. Requires irrigation. Dry *C. myriocarpus* fruit for 72 h at 52°C, then grind up. Material is active for 56 d. Reduced nematodes by 92% in root and 86% in the soil. Almost as good as aldicarb. Did not affect pH of soil but improved EC.

S1-23 **Nampeera et al.** Potential of crude leaf extracts of *Cupressus lusitanica*, *Nicotiana tabacum*, *Azadirachta indica* and *Lantana camara* for control of sweet potato weevil.

Bores inside the stems and damages the tubers. Used most susceptible variety. 2 kg plant leaves pounded with 250 ml water. One kg used per plot and poured at base of plant.

Mexican cedar and tobacco most effective. Neem and Lantana not very effective.

Extra talk: **Bagarama.** Soil organic amendments reduce root-knot nematode infestations in tomato in Tanzania.

Goat manure and bat manure controlled nematodes but not cow manure. Tobacco leaves had similar controlling effect.

S2-P **Steve Belmain.** Optimisation of pesticidal plants for post-harvest systems.

On-farm storage in rural Africa is generally not insect proof. If could store grain for a while it could be sold for a better price. Grain borers mainly rely on aggregation pheromones to find a host that is already infested. The first beetle almost finds the host by chance. Important to replicate lab work in the field. Safety: tirucallicine in *Euphorbia tirucalli*. Ascaridine, nicotine. Everything can be poisonous at the wrong dosage. Paracelsus 1536 said "Poison is in everything and no thing is without poison. The dosage makes it either a poison or a remedy." Sampling bias may lead to wrong conclusions, e.g. wrong time of year, wrong species/chemotype, wrong concentration. *Securidaca longepedunculata* African violet tree has many traditional uses, winged samara like *Tipuana*. Methanol extract gives good control of *Sitrophilus* on maize. But root bark is what is used so it is not sustainable. Has a distinct menthol smell due to methyl salicylate that provides bioactivity. Other bioactive chemicals are saponins which are soluble in water so can be extracted that way. It requires less material but is more labour intensive so is not popular. Usage is therefore not always correlated with best efficacy. In some parts of Africa the same plant does not have saponins and is not toxic. Need to know if polar or non-polar solvents are required. When and where to collect material. Get the taxonomy right. How toxic is acceptable? Think of new application methods, e.g. push-pull technique. Indigenous knowledge is not held equally but needs to be protected to ensure that it is not lost. Need new registration process for low dosages of chemicals that have been used for generations. *Tephrosia* grows rapidly and is easy to grow but others are very slow. Soaps can also bind to active ingredients and may cancel the effect so again you need to know the chemistry.

S2-1 **Sarah Arnold et al.** Intraspecific variation in the response of *Callosobruchus maculatus* to methyl salicylate, a botanically-derived repellent.

Securidaca longepedunculata root powder and methanol extract repel *Sitrophilus zeamais*. Methyl salicylate is responsible for this behaviour. Occurs in many plants. Tried against *C. maculatus* which is dimorphic with one morph being more active than another. Used EthoVision motion-tracking software. Air goes through charcoal filter before going through odour source. At 1 mg/ml females definitely repelled but not males. Active beetles are not repelled as much by methyl salicylate as inactive forms.

S2-2 **Serame et al.** Chemical composition and insecticidal effect of essential oils of some aromatic plants of Burkina Faso.

Lippia multiflora one of plants used for essential oils. Also used as a tea and in traditional medicine. *Cymbopogon schoenanthus*, *Cymbopogon giganteus*, *Laggera aurita* has some healing properties. *Laggera oloptera* has pinene and limonene main components. *Anona senegalensis*, *Ocimum basilicum* for repelling mosquitoes and very repellent to weevils.

S2-3 **Kitonde et al.** Phytochemical and utilization of *Vernonia glabra* in the management of food spoilage and poisoning in Kenya.

Toxins from *Aspergillus niger* can cause kidney failure and liver cancer. *V. glabra* evaluated for its effect against *A. niger*, *S. aureus* and *E. coli*. Leaves and flowers showed most microbicidal activity based on inhibition zones. Could be used to treat food to prevent people from getting sick.

S2-7 **Asogwa et al.** Evaluation of ethanolic plant extracts for protection of *Cola nitida* against Kola weevils in storage.

Used *Cedrela odorata*, *Khaya*, *Chromolena odorata* and other plant extracts at 4 concentrations. Soaked nuts in extracts. All extracts generally suppressed oviposition and emergence from nuts.

Extra talk **Akhatar et al.** Toxicity and feeding deterrent effects of a pea extract and protein-rich pea flour.

Pea flour was found to be toxic to various stored-product beetle pests. High concentrations would have to be used and it leaves a visible residue and odour. A peptide and soyasaponin had a synergistic effect in extracts from the flower and were much more effective than either alone. Methanol, isopropyl alcohol or ethanol extracts could be used. Lab screening on *Trichoplusia ni* using 0.25% to 2% on leaf disks. Use scanning digitometer to quantify how much of the leaf disks had been eaten. Not seen to be all that active in deterring feeding but extract is much more toxic as contact insecticide than crude powder. Spider mites seem to be more susceptible than *T. ni* as are stored product beetles.

Keynote - **Ahmed Hassanali**. Bioprospecting for phytochemicals or control of vectors of animal and human diseases: building on ethno-practices.

Mosquito repellents for *Anopheles gambiae*: *Corymbia citriodora* burning of leaves gives 51.3 % repellency but thermal fumigation (heating on clay plate) increased to 74%. *Ocimum suave* gave 53% repellency with thermal fumigation. Potted plants like *Lantana camara* caused 33% repellency just in room. Could be taken outside during the day and brought inside in the evening. *Coryza newii* has distinct odour and essential oil gives knockdown of mosquitoes. Monoterpenes perillaldehyde and perillyl alcohol plus geraniol. Being investigated now for fumigation properties. About 8 essential oils have repellency that is greater than DEET but most too volatile. Put in oil base at about 2-5% essential oil and heat from below with candle or paraffin lantern. *Ocimum kilimandharicum* being developed in postharvest protection of maize and legumes, mosquito repellent and flowers are very attractive to bees. It has about 8% camphor. Now being grown in fields in Kakamega area of Kenya and in Tanzania. Have equipment to produce own oil and products being sold locally. Looking at terpenoids with low volatility for postharvest use and personal repellents, e.g. p-Menthane diol from *Corymbia* is effective for personal protection against mosquitoes. Mentane diol at 8% gave 100% protection after 8 h equivalent to 20% DEET. Product called Mozigone. Found how to convert citronella from grasses to Mentane diol as available in large quantities. Two other sesquiterpenoids with low volatility also being investigated. Tick *Rhipicephalus appendiculatus* (brown ear tick) is vector of East Coast Fever and kills calves. *Tagetes minuta* and *Tithonia diversifolia* used to treat whole animal but only lasts 24 h. Anal odour repels ticks towards head and ear odour attracts ticks. Evaluated essential oils of above plants at 10% in Vaseline in ears and got significant repellency for 2 weeks. Using push-pull techniques on each cow with possible trap with acaricide. Using similar approach for tsetse with odours from hosts and non-hosts.

S2-24 **Mwangi et al.** Activity of extractives from *Albizia anthelmintica* and *Teclea trichocarpa* as biorational alternatives to control the maize weevil (*Sitophilus zeamais*)

Albizia used as grain protectant. *Teclea* bark antifeedant for *Spodoptera exempta* (African army worm). Extracts from powdered leaves using hexane, chloroform, ethyl acetate and methanol. Brine shrimp used as an indicator of toxicity. Triterpenoids from hexane extract and alkaloids with other solvents. *Teclea* extracts similar activity to Actellic super standard.

S2-25 **Ogemah**. The feeding deterrence effect of neem oil on the larger grain borer *Prostephanus truncatus* (Horn).

Neem oil used at 0.5 to 4%. Measured live weight of beetles after being offered treated maize. First instar more susceptible, 2nd instar only susceptible to 2 and 4%, 3rd instar not susceptible. Feeding deterrence on grains was better than on flour. High rate is effective.

S2-26 **Bett**. Insecticidal activity of two Kenyan plants against *Tribolium castaneum* and *Acanthoscelides obtectus*.

Lab studies with *Eucalyptus saligna*, *Cupressus lusitanica* against above beetles. Evaluated essential oils as fumigants with up to 20 uL/L air. Better results against *A. obtectus*. Similar results to those others have found with other Lamiaceae spp.

S2-27 **Ng'eno**. Phytochemical studies of some indigenous plants as grain protectants against *Sitophilus zeamais*.

Investigated *Lippida kituiensis*, *Plectranthus sylvestris* and *Chenopodium chenopoides*. *Plectranthus* leaves contains globulol, caryophyllene. Acetone extracts were placed on half a filter paper which when dry was placed in a petri dish with half of an untreated disk. Then observed where the beetles spent their time. Also mortality tested with grain treated with extracts and effect determined after various periods. *Lippida* was as effective as pyrethrum when used at 1%. Others caused little mortality, including *Ricinus communis*. Camphor, limonene and eugenol considered to contribute most to mortality.

S2-29 **Deng et al.** Toxic and repellent properties of *Cupressus lusitanica* and *Eucalyptus saligna* leaf essential oils against *Callosobruchus chinensis* and *Sitophilus zeamais*.

Fumigation and repellent effects evaluated. *C. chinensis* more susceptible to fumigant at high dosages than other beetle after 7 d evaluation. Only *Eucalyptus* was effective in topical applications. Repellency in petri dishes not convincing for *C. chinensis*. A bit better for *Sitophilus*.

S2-30 **Kenneth Wilson et al.** Can the biofuel crop *Jatropha curcas* be used as a locally

Market for biofuel has collapsed in Zambia so search for alternative uses. Realism versus control in designing experiments is a challenge to get good data. Need to try to conduct research at all levels (lab, field and farm). Green living movement has been doing farm work. Treatments included 2% *Jatropha* powder and 5% *Jatropha* solution as a dip. Dried the leaves over several weeks then crushed into a fine powder. This was mixed with the maize. For solution, soaked with water overnight then dipped maize in mixture, then left to dry in the shade until dry. Then put in bags and stacked with replicates together. Samples taken at monthly intervals after treatment. Powder was acting as a slight attractant. Soaking the seed in water for a minute increased the number of beetles. Wet *Jatropha* treatment was even worse for large number of insects and damage to seeds. Wet seeds were lighter. Perhaps *Jatropha* soln could be used as an attractant in push-pull. Perhaps water is not suitable for extracting the insecticidal components. Not much oil in the dried leaves so not responsible for attraction of treatments.

S3-16 **Murungi.** Chemical composition of essential oil of *Solanum sarrachoides* and its bioactivity against *Tetranychus evansii*.

Solanum sarrachoides known as hairy African nightshade and can be eaten but not preferred due to pubescent leaves. The latter may confer resistance to *T. evansii*. Major components in leaves are camphor, monoterpenes and fatty acids. Made serial dilutions in 5% DMSO in water. Leaf oil effective at 100 ug/ml due to monoterpenes. Fatty acids don't contribute much to mortality.

S3-P **Eustace Kiarii.** Status of organic agriculture and use of pesticidal plants by organic farmers in Kenya. CEO of Kenya Organic Agriculture Network (Sponsor).

Have a system where consumers can order organic fruit and veg in a basket that is delivered directly to their home. *Lantana camara* leaves are being used against weevils as a postharvest treatment.

S3-1 **Saskia Vermeylen.** The poisonous and the sensuous in Chibobo's community lab: Lessons learned from a farmers'-led biopesticidal experiment in Zambia.

Jatropha introduced on a large scale in Zambia about 10 years ago. Asked farmers to keep diaries about farm management. Meetings with extension worker to discuss strategies with growers. Have been using various botanical extracts or powders in their agriculture. Challenge will now be to share new knowledge with others.

S3-3 **Matasyah.** Natural botanical larvicides for the control of the malaria vector *Anopheles gambiae*.

Climate change may result in malaria appearing in parts of Europe. Product Carplarvex is a larvicide. Float a mesh-topped container with larvae and treated water on a water source for trial purposes. Alkaloids from *Zanthoxylum lemairei* as larvicides and *Z. leprierii*. Fungus *Podospora* sp. in *Laggera alata* (Asteraceae) has anthraquinone derivatives that toxic to *Anopheles*. However, some say it is a relative of aflatoxins and cannot be put in water sources.

S3-4 **Munyua and Wagara.** Potential and uncertainties in utilization of pesticidal plants in pest management among small-scale farmers in Nakuru, Kenya.

Some growers use stinging nettle with Omo for blight on tomatoes. *Tagetes minuta* used for spider mites, *Capsicum* spp. for various insects, etc. even though not found to be very effective. Info was not obtained from a reliable source. Trial and error approach to using pesticidal plants. Lack of info on dosages and possible side effects.

S3-5 **Chikwanda et al.** The effectiveness of *Sphenostylis erecta* in controlling blue tick (*Boophilus decoloratus*) infestation.

Sphenostylis erecta is a legume used as food, fish poison and dye. Has a bulbous root that is used as the acaricide. For lab tests put bulb extract in olive oil on filter paper in petri dishes and exposed ticks for

different times. Over 72 h, 10% conc caused 55% mortality and higher mortalities were obtained with higher dosage. 100% conc equivalent to Amitraz and caused 100% mortality after 3 d. Other published work on *Lippia javanica* for use in the same way.

S3-7 Jambo and Magreta. Effects of land ownership on land investments: a case of Malawi.

90% of Malawian population are small-holder farmers. Agriculture is declining due to climate and erosion. Land ownership security has no effect on control approaches used. Need to get farmers to implement known knowledge.

S3-8 Mdangi. Farmers' knowledge and perceptions on the use of pesticidal plants for rodent control in Maize farming systems in Tanzania.

Commercial rodenticides have non-target effects. After drought, rats are biggest threat to grain farmers. Farmers were willing to use pesticidal plants but only 10% are using them.

Keynote Monique Simmonds. Plant compounds in pest control: challenges and opportunities.

Since 1985 have been working on 20 000 diff. species of plants and 15 000 insect-plant interactions. Around 2000 many of the companies interested merged so fewer companies were involved. Around this time more work on authentication started too. Work on chemosystematics and authentication has been more valuable than random screening which has produced very little. Work has been influenced by the new phylogenies found with molecular work. About 28 300 spp. of plants have pesticidal activity. Only 460 of these with solid evidence. Certain families more likely to have them than others. The "older" plants have more pesticidal activity e.g. Ginkgo. Legumes have a lot. Info on the pesticidal plants is in public domain. Types of IP involved: Patents, Know how (trade secret), Copyright, Database rights, Design rights and Trademarks. Copyrights and Database rights vary from country to country. Information should be retained in the country where it originated so that other usage is secondary. Convention on Biological Diversity has had a large impact on terms used. Access to genetic resources – Article 15. Had various guidelines since then e.g. Bonn Guidelines on how to share info. Delhi Declaration 21 Jan 2005 very important for neem. Have to disclose country of origin of plant, not where the plant is actually growing. Aichi Nagoya Japan Summit on biodiversity 18-29 Oct 2010 on benefit sharing and launch of economics of ecosystems and biodiversity www.cbd.int/doc/press/2010/. Example of getting a product to market: Dan Janzen in 1980 sent some seeds from Costa Rica to Kew because were not being eaten by insects. Found DMDP was responsible. Asked another group BTG if they would investigate it and declined. Then included in an abstract so went into public domain. Then found to have nematocidal properties so patented. Made collaborative agreements with Costa Rica and permitted them to use it as a nematocide. Developed into a revenue sharing agreement. Then found DMDP in blue bells and Hyacinths. Could use discarded material from flower industry. Licence agreements then assigned to INBio and used for control of nematodes in bananas and coffee. Formulation is important as it is water-based. 50% of royalties going back to Costa Rica. Industry is now starting to protect biodiversity. Every year plants are being misidentified. In China 596 plant species officially used in medication, water soluble and taken by mouth; 267 of these with pesticidal activity. Traditional Chinese Medicine (TCM) in Chinese Pharmacopoeia sometimes with different names. Names can be wrong in publications e.g. 215 records in Genbank but 75% incorrect for one particular species. www.gp-tcm.org. Need to know what is a quality extract and what compounds are responsible. In medicines plants are often combined together to help penetration of membranes or uptake. Less likely to be a single chemical that most effective. Lessons learnt: solid evidence that have a lead. Check plant and review literatures (look for 3 asterisks). Talk with someone with knowledge about IP regulations. Make collaborations early. Involve industry to contribute to formulation. Involve growers of plants and formulators. Quirks: Darwin 1876 reported ivy rootlet secretes yellowish matter while climbing a surface. Identified as nanoparticles by Zhang in 2008. Difficult to work on South African plants because information is not accessible. If make a new chemical from a plant chemical the plant chemical needs to be acknowledged but can patent the new chemical. Would have to get benefit back to original country.

S4-P Opendor Koul. Botanical insecticides: An Asian perspective.

We concentrate on structure, complexity and toxicity. Koul 2003 in Recent Trends In Chemistry shows lists of plant family names with pesticidal properties. Writing book on Naturally occurring insecticidal toxins. 650 known compounds that are purely insecticidal 1160 compounds considered to be antifeedants. Natural chemicals that are currently marketed are 89. Toosendanin from *Melia toosendan* (synonym for *M. azedarach*) from bark extract. Has Chinese patent. *Sophor flavescens* has sesquiterpene polyester and an alkaloid plus plant volatile oil sold as BBM and patented. Chinese registration procedure has some exemptions for full analysis of chemicals. Registered products with cold pressed neem oil in USA for bed bugs. In India only Azadirachtin and Pyrethrum registered botanical pesticides. No Chinese products registered there. Have found other limonoids besides azadirachtin in neem. *Entandrophragma candolei* also have limonoids but no dominant toxin. Sugar esters have been studied in India and glycol diesters which effective against various pests such as whiteflies, mealybugs. Have also looked at effect of essential

oils in preventing oviposition behaviour. Cyclotides from Violaceae, Rubiaceae and Cucurbitaceae are also insecticidal. Ecwin producing botanical pesticides in South Korea. Specific constraints in Asia: 1. Selection of ideal plants. Should be perennial, wide distribution, should have additional uses. 2. Should be systematic studies. Many different approaches to antifeedant studies. 3. Patents cannot protect traditional knowledge in the Asian-Pacific region. 4. Reasonable regulation processes are required to assist registration.

S4-1 Nyalala, Petersen and Grout. How growing conditions and developmental stage of *Gynandropsis gynandra* influence emission of potential miticidal leaf volatiles.

Reduces thrips damage in beans. when intercropped in roses it reduces spider mites. Emits bioactive acetonitrile (methyl cyanide) and bioactive volatiles against spider mites. Did some work in greenhouses in Denmark and Edgerton Univ. In both places under greenhouse conditions methyl cyanide was the main chemical produced but quantity can vary with temperature. Various isothiocyanates are also produced when the plant is quite old. Also some aldehydes like hexanal that had an effect on spider mites that were being produced by old plants.

S4-4 Zida et al. Seed treatment with a binary pesticide and aqueous extract of *Eclipta alba* (Asteraceae) for improving sorghum yield in Burkina Faso.

Aqueous extract from leaves and stems. Plant material dried then crushed to powder which mixed with water. Used at 6-25% to treat sorghum seed. There was a fungicidal effect but in the field there were no significant differences in yield.

S5-PL Chege. Intellectual property issues in the research and development of innovations.

Churchill: "the empires of the mind are the empires of the future". Everything we do is knowledge driven and based on intellectual creation. Patents are used to protect inventions of a technical nature. Phases of innovation: 1. Research phase, 2. Design and development phase, 3. Commercialisation phase. In 1 need to have material transfer agreements, collaboration agreements and institutional IP policies. In 2 could involve development of prototypes and private companies. Phase 3 requires market testing and collaboration with private companies. Patent will usually last 20 years. Don't undervalue or underestimate your work at the initial stages.

S5-1 Sola et al. Botanical pesticides production, trade and regulatory mechanisms in sub-Saharan Africa; making a case for plant pesticide products.

Neem and *Tagetes minuta* have been the most common sources in the past. *Tephrosia* and *Securidaca* are widely used but no commercial products. Kenya only supplying 20% of pyrethrum these days. Also grown in Tanzania and Burundi. For pyrethrum production, leaves are picked, dried and powdered. Then processed overseas. Often a limitation by the growing of plants for the pesticide extraction.

S5-2 Severine. Governance structures for bridging the access and utilization gap of bio-pesticides produced by different farmers in Tanzania.

75% of pesticide products imported into Tanzania are reported to be fake. Government structures do not address commercialisation of biopesticide pesticides.

S5-5 Magreta et al. Economic evaluation of optimized pest management options on bean cropping systems in southern Africa.

Using *Vernonia amygdalina* and *Tephrosia vogelli* treatments. Tv at 2 and 5% was giving best marginal rate of return. Strong smell when pounding is not appreciated and blockages when spraying require fine filtering or stocking.

S5-6 Omolo et al. Bioprospecting for phytochemical control agents of the stored product pest, *Prostephanus truncatus* from some western Kenya flora.

Larger grain borer (nickname Osama Bin Laden). Labiatae, Verbenaceae, Euphorbiaceae and Compositae. Extracted essential oils by steam distillation. *Conyza newii*, *Plectranthus marruboides* and *Tetradenia reparia* were good fumigants.

S5-7 Innocent. Translating science into community life: an experience at the institute of traditional medicine.

Annona squamosa and *Annona senegalensis* bark and stem extracts are effective against mosquito larvae.

S6-P Muthoka. Conservation and propagation of pesticidal plants for sustainable use.

www.centerforplantconservation.org has some info about some of the plants being used.

S6-1 Mulanda et al. Prospects for a rapid *in vitro* regeneration system for propagation of the pesticidal tree *Melia volkensii*.

If could clone plants would have a more consistent pesticidal product. Callus induction from zygotic embryos. Whole process took about 105 d to viable plant.

S6-? **Njogu et al.** Use of detached leaf bioassay to evaluate host plant chick pea susceptibility to *Helicoverpa armigera*.

Exposed different chick pea genotypes to neonate larva and larger larvae collected from the field. No varieties were completely resistant but some resulted in lower weight larvae. Four genotypes should be avoided because of high susceptibility.

7 INTERNATIONAL VISITS

7.1 REPORT ON THE XII INTERNATIONAL CITRUS CONGRESS IN VALENCIA, SPAIN 18-23 NOVEMBER 2012

SUMMARY (Tim Grout)

The International Society of Citriculture's congress was held in Spain in November 2012 and being relatively close to South Africa, CRI sent a large contingent of research and extension staff to benefit from this important scientific congress that takes place once every four years. Fourteen presentations were made by people that were either funded directly by CRI or were conducting research on CRI-funded projects. CRI delegates also participated in various technical tours before, during and after the congress to learn about the latest techniques and equipment being used in the industry. Apart from the obvious benefit of conversing with top researchers in their field and making new contacts some of the highlights of the conference were as follows. A comprehensive workshop and several individual papers on citrus greening and vector control from several parts of the world; global approaches to MRL regulatory issues and novel IPM approaches to reduce dependence on pesticides; further benefits from using plant growth regulators; some further progress in reducing postharvest rind disorders and waste; several papers on calcium-nitrogen fertilisation; various new approaches to fruit fly management and updates on pests in other parts of the world, including the news that our psyllids are now called trioizids and the Asian psyllid is a liviid; several papers were presented on citrus tristeza virus, detection of viroids and citrus improvement schemes; citrus black spot was very topical with it receiving most of the attention in the fungal diseases session; the latest trends in cultivars and rootstocks were also discussed with the Australian navels losing popularity. In most fields of citrus research this was a very worthwhile conference and the knowledge gained will benefit our research for years to come.

OPSOMMING

Die Internasionale Sitruskongres is in Spanje in November 2012 gehou en omdat dit relatief naby aan Suid-Afrika is, het CRI 'n groot groep navorsers en voorligtings-beamptes gestuur om voordeel te trek uit die belangrike wetenskaplike kongres wat slegs elke vier jaar gehou word. Veertien referate is deur persone gelewer wat óf direk óf indirek deur CRI befonds word, óf wat navorsing op CRI-befondsde projekte doen. CRI-afgevaardigdes het ook in verskeie tegniese toere vóór, tydens en ná die kongres deelgeneem om van die nuutste tegnieke en toerusting wat in die bedryf gebruik word, te leer. Afsien van die vanselfsprekende voordeel van gesprekke met top navorsers in hul velde en die opbou van nuwe kontakte, is van die hoogtepunte van die kongres as volg: 'n Omvattende werkwinkel en verskeie individuele referate oor sitrusvergroening en vektorbeheer van verskeie dele van die wêreld, globale benaderings tot MRL regulatoriese aspekte en nuwe GPB benaderings om die afhanklikheid van plaagdoders te verminder, verdere voordele van die gebruik van plantgroeireguleerders, verdere vordering in die vermindering van na-oes skilprobleme en bederf, verskeie referate oor kalsium-stikstofbemesting, verskeie nuwe benaderings tot vrugtevliegbestuur en opdaterings oor peste in ander dele van die wêreld; insluitend die nuus dat ons psilla nou "trioizids" genoem word en die Asiatiese psilla nou 'n "liviid" is; verskeie referate is oor sitrus tristeza virus aangebied, opsporing van viroïede en sitrusverbeteringskemas, sitrus swartvlek was ook baie aktueel en in die sessie oor swamsiektes is die meeste aandag hieraan gewy, die nuutste tendense in kultivars en onderstokke is ook bespreek, met die Australiese nawels wat gewildheid verloor. In die meeste velde van sitrusnavorsing was dit die moeite werd om die kongres by te woon en die kennis wat verkry is, sal tot voordeel van ons navorsing in die jare vorentoe wees.

PLENARY TALKS

The Spanish citrus industry

Luis Navarro (Instituto Valenciano de Investigaciones Agrarias (IVIA), Spain)

An historical overview of the Spanish citrus industry was given. Prior to the introduction of a statutory citrus improvement scheme and mandatory pathogen elimination through shoot tip grafting (STG) of all cultivars, the Spanish citrus industry had very few clean cultivars, generally low production and poor economy. Navarro reported that STG saved the Spanish citrus industry. Similar to the South African Citrus Improvement Scheme, the Spanish Citrus Variety Improvement Programme has the following objectives: provide the industry with pathogen-free cultivars by cleaning existing cultivars and cleaning all cultivars prior to introduction; maintaining a pathogen-free germplasm bank of all cultivars; and managing of a certification scheme. IVIA maintains the germplasm bank as well as the mother trees of all commercial cultivars, while the nurseries maintain their own multiplication trees. However, these are regularly re-indexed using biological indexing as well as complementary serological and molecular detection techniques. Spanish nurseries cultivate trees in open-ground or containers. [Comment: the rapid and wide spread of

Phytophthora diseases in Spain indicates that the production of citrus trees in open-ground nurseries is not advisable].

Spain has 330,000 ha citrus planted, of which 48% is oranges, 35% soft citrus and 16% lemons; 50% of the 6.5 million tonne production is exported, 20% consumed locally, and 18% processed. From 2007-2011 the most popular sweet orange cultivars were Navelina, Lane Late, Powell, Chislett and Salustiana. For soft citrus the most popular cultivars were Clemenruby, Clemenules, Hernandina, Oronules and Orogrande clementine; Nova, Murcott, Ortanique, Safor and Nadorcott mandarin; and Iwasaki, Okitsu and Owari Satsuma. Verna, Fino, Eureka lemon and Bears lime were the most popular lemon/lime cultivars. The most popular rootstock cultivars are Troyer and Carizzo Citranges (56%), Macrophylla (20%), Cleopatra mandarin (6%), Volckameriana (6%), C-35 (5%), Swingle (5%), Sour orange (1%) and a new cultivar FA-5 (1%).

The highlighted problems of the Spanish industry were low prices, high production cost (especially labour), small size of plantings (too many small operators) and over production of some cultivars.

The importance of citrus for the juice and beverage industry

Ademerval Garcia (Grove 2 Glass Trading GmbH, a 'The Coca-Cola Company' subsidiary, Switzerland)

Currently focused on sustainability but soon will change to metabolic living. G2G = Grove to Glass juice procurement for Coca-Cola. Coca-Cola is the largest juice company in the world. Project Nurture in Kenya and Uganda with Bill and Melinda Gates gets mangoes from all over the place. Now making a blend of mango and granadilla. This approach can be done anywhere. Kenya uses 50 000 farmers. Orange juice for consumers has become cheaper relative to inflation. Costs going up and there is no growth in Florida and Brazil. New areas of production will therefore emerge. Cross functional science to be encouraged. Coke is funding research on HLB. They move in direction that the consumer directs. They don't PROMOTE science but USE science. Therefore they won't look at GM fruit yet.

Food safety, social compliance and sustainability, in relation to commercial fruit and veg strategies with special reference to citrus

Gé Happe (European Sourcing Director Ahold, The Netherlands)

There are over 3000 stores in the Ahold group. They advocate a better place to shop with great quality and shopping ease. They have 280 000 employees and have several social programmes that all products are part of. Their company is based on 6 strategic pillars. One of these is responsible retailing. All products they sell are below the MRL levels. They cut the MRLs in half to prevent any exceedances. Water issues are becoming more important. Need continual innovation. Taste, health and convenience are the 3 big trends.

The experience of huanglongbing control in Brazil

Antonio J. Ayres (Fundo de Defesa da Citricultura (FUNDECITRUS), Brazil)

Brazil supplies 70% of the world's orange juice. 628 000 ha are planted to citrus in Sao Paulo state and 78% without irrigation; 95% sweet oranges, most important variety Pera. Citrus provides 230000 direct jobs. Brazil faces the "Big 5" of citrus diseases, namely Canker (1.4% of blocks), Citrus Variegated Chlorosis (CVC; 38%), Citrus Black Spot (51%), Leprosis and HLB. In 2003 no formal report of HLB in S. America. Diaphorina citri present since 1942. Found in Murraya exotica in 2005, both Lam and Las. Found another phytoplasma causing HLB symptoms but not Liberibacter – possibly from ground cover crop, *Crotalaria juncea*. Initially Lam was dominant but by 2007 Las became dominant and now Lam has almost disappeared. As an HLB control measure, tree removal of infected trees was mandatory, although growers did not necessarily approve. Fundecitrus trained 8000 inspectors for HLB who inspected for CVC, Canker and HLB. In 8 years they have removed at least 20 million trees. In Sao Paulo state, HLB incidence spread from 3.4% in 2004 to 64% of citrus blocks in 2012 (mean of 6.9% infected trees). Incidence on smaller farms is higher than on large farms where management is better and there is an area-wide control effect. To date, HLB is successfully controlled using the following area-wide 3-pronged strategy: 1) produce citrus trees only in covered nurseries, 2) reduce inoculum by removal of infected trees, and 3) vector control. CVC, which is also insect-vectored, forced them to cover nurseries before HLB (2003). For improved inspection, they introduced platform inspection to look at tops of trees where most symptoms are. Systemic insecticides are used in nurseries. On bearing trees, mostly contact products are used. In orchard need to spray in dry weather as systemics don't work well without irrigation. Distance from groves without control measures is important. If growers work together they can do area-wide control. Management costs from \$240 to \$1040 per ha per annum. Need intense efforts on borders of orchards to control the vector. Recommend removal of whole farm if high infection, not just block as young trees will be close to infected trees in other blocks. Releases of *Tamarixia radiata* are assisting vector control in urban areas or abandoned orchards. More emphasis on HLB management than nutritional approach but the latter is being investigated. In 2 years,

HLB incidence where no control but nutritional sprays has doubled. Research priorities: Improve inspection (pink glasses), systemic insecticides, low volume applications, EPF, pheromones? or attractants, resistant trees (GM?). If no GM, will need area-wide control. The most important lessons learnt were start control early while disease incidence is still low, implement area-wide control using the 3-pronged approach, and maintain effective quarantine measures (they suspect HLB was introduced via illegally imported budwood from China, while Canker was similarly introduced from Japan).

Area-wide pest management (replaced planned presentation)

Jorge Hendrichs (Joint FAO/IAEA Division), Insect Pest Control Section, Austria

Knipling advocated preventive, area-wide approach for pest control. Area-wide addresses both space and time. Boll weevil is being eradicated in the US using traps and insecticide. Now only present in Texas and New Mexico. Rinderpest finally eradicated in the world in 2011. Monitoring required in area-wide approaches and sanitation must be done diligently.

WORKSHOPS

W01 'HLB Control' (Hennie le Roux)

This workshop was convened by Prof J.M. Bové and M Rogers from CREC in Florida. The goal of the workshop was to answer the questions: Can HLB be controlled, how and when?

The following points were made:

- It was estimated in 2012 that, in Florida, Huanglongbing (HLB) has resulted in the loss of \$1.3 billion in revenue to growers and \$3.63 billion in economic activity. The State's commercial citrus acreage has shrunk to 531,493 acres, a 28% decline from 748,555 acres. Also in São Paulo State, Brazil, HLB has taken a heavy toll. Since 2004, 18 million HLB-affected trees (~10%), representing a value of \$216 million at \$12 a tree, have been eliminated.
- There is little evidence of genetic resistance to HLB in citrus. Apparently, citrus has had only a recent association with liberibacters, an association too short to have built up resistance to the bacterium. Hence, according to general consensus, resistance to HLB must be obtained by engineering, using genes with anti-liberibacter and/or anti-psyllid activity. However, such HLB-resistant cultivars will probably not become available to the growers for several years.
- HLB outbreak in Argentina: In June 2012, a positive detection of HLB was confirmed in a backyard tree in the Northern Misiones Province across the border from Brazil. Since then, 5 surveys were carried out in the area surrounding the focus with the detection of 15 positive trees, all in backyards. In all cases, the trees were eradicated by the owners. The psyllid population in this area is very low and all PCR samples of the vector are negative. At present (November 2012), HLB has not been detected in commercial groves.
- California: The first Asian citrus psyllid (ACP) was detected in southern California in 2008 and has since become widespread in, but is limited to, southern California and mainly in residential areas. In March 2012 one backyard tree tested positive for *Candidatus Liberibacter asiaticus*. To maximize efforts while using minimal resources, California has implemented a "Risk-Based Residential Survey" devised by Drs. Timothy Gottwald and Weiqi Luo. A Texas risk-based survey has also been deployed and an Arizona survey will be deployed in early 2013.
- Texas: The Asian citrus psyllid, was first reported in Texas in 2001. Its potential as an economic pest was underestimated, and limited to no attention was paid to this pest. HLB was detected in two adjacent commercial groves in January 2012. A rapid response program including roguing and destruction of all known HLB-infected trees, and an aggressive psyllid control program in the two groves and all commercial and residential citrus within a one-mile radius is being implemented. Such measures have thus far limited the spread of the disease as no additional tissue detection has been made in the ongoing detection surveys.

LONG-TERM CONTROL SYSTEMS (ASIAN HLB and AFRICAN HLB):

- BREEDING FOR RESISTANCE/TOLERANCE TO HLB WITHIN CITRUS. Development of citrus cultivars resistant to HLB is the best long-term control solution for endemic diseases such as HLB. Compared to other tested cultivars in experiments outside the US, lower susceptibility to HLB associated with CLAs, has been reported for limes, pummelos, lemons, some mandarin types (e.g. 'Ladu' and 'Som Pan' in Thailand) and various non-cultivated Citrus or related species.

Because of continued evidence of HLB resistance in *C. trifoliata*, several trials are now underway using diverse trifoliates and their hybrids, including some advanced material with near commercial fruit quality. The hope is that molecular markers can be identified to facilitate introgression of resistance through conventional breeding and/or genes can be used to generate HLB-resistant standard cultivars using transgenic methods.

PRODUCTION OF GENETICALLY MODIFIED CITRUS (GMC) TREES.

I. Anti-liberibacter genes. Ed Stover, USDA-ARS, USHRL, 2001 S. Rock Rd., Ft. Pierce, FL, USA. Since no strong HLB resistance has been found in cultivated citrus scion varieties, transgenic citrus may provide the best opportunity for developing HLB-immune citrus scions. Transgenic strategies also should confer HLB resistance while maintaining known desirable traits of existing cultivars. Numerous approaches are being used.

II. Antimicrobial peptides (AMPs) and defensins, systemic acquired resistance (SAR) related genes, and a phage gene disrupting outer membranes of gram-negative bacteria (Gabriel personal comm.) are among the HLB-directed transgenics with greatest progress to date since these approaches are not dependent on in-depth knowledge of the HLB-pathosystem. AMPs disrupt bacterial membrane integrity, but do not damage plant cells, and confer some resistance to a wide array of disease-producing pathogens.

III. Anti-psyllid genes. W. O. Dawson, UF, CREC, Lake Alfred, FL, USA. There are two different methods that are being considered to control psyllids by producing anti-psyllid products in plants, specifically the phloem, for uptake by psyllids while feeding.

a) Antimicrobials to prevent endophytic bacteria in psyllids. One approach is to allow feeding psyllids to take up antimicrobials, probably antibacterial peptides, from phloem as they are feeding to kill endophytic bacteria that reside in psyllids. It has been shown that aphids, which are relatives of psyllids, need endophytic bacteria for production of necessary amino acids and other precursors for the survival of the aphid. This approach is based on the assumption that psyllids also need endophytic bacteria for their survival. Plant-produced antimicrobials that accumulate in the phloem would be sucked up by the psyllid. This could be at any stage of the insect. However, Nymphs that grow rapidly and take up large amounts of phloem sap would likely be more susceptible.

b) RNAi molecules to suppress psyllid enzymes. Gene silencing by RNA interference (RNAi) is an innate defense mechanism of eukaryotic organisms. Double-stranded regions of RNAs are cleaved by a double-strand specific ribonuclease (dicer) into small RNAs that associate with the argonaute complex to target specific RNAs for degradation. If the target is a messenger RNA, production of the associated gene product is prevented. Thus, the objective of this strategy is to target psyllid enzymes that are necessary for its survival. Since psyllids suck up sap from plant phloem, this allows the targeting of psyllid enzymes by producing RNAi molecules in citrus phloem. Again, nymphs that take up large amounts of phloem sap would likely be the most susceptible target. The good news is that several labs are obtaining encouraging results using this approach. For instance, Nabil Killiny's laboratory has selected the abnormal disk wing gene (*awd*). Psyllid nymph-instars that acquired *awd*-dsRNA had diminished development and survival. Moreover, knockdown of *awd* gene expression was observed through malformation of adult wings.

IV. CTV gene vector. W. O. Dawson, UF, CREC, Lake Alfred, FL, USA.

There are two major methods to express foreign genes in citrus trees. One is by transformation, which is insertion of the foreign gene into the nuclear DNA. The advantage of this approach is that it is generally considered to be permanent. Another approach is the use of virus-based vectors. This procedure is not permanent because eventually the virus loses the foreign sequences.

Citrus tristeza virus (CTV) is an endemic virus in most citrus industries. Although some isolates of the virus cause serious economic losses, many isolates cause little damage. By recombinant DNA technologies, CTV can be engineered into a transient expression vector to produce foreign proteins or RNAs in citrus trees. CTV is a phloem-limited virus, and this is an advantage when trying to control the bacterium that causes HLB because it also is limited to phloem. Additionally, the psyllid vector feeds on citrus phloem. The most remarkable feature of the CTV vector is that it is much more stable than other virus-based vectors so far examined. The CTV vector is capable of production of foreign gene products for years. The vector makes large amounts of foreign gene products in cells adjacent to sieve elements of the phloem.

The initial use of the CTV vector was in screening foreign genes for activity against HLB or the psyllid vector. The vector, which can be transmitted to other citrus plants of different varieties and ages by grafting is a much faster screening tool. However, because HLB has spread much faster than expected in Florida and because the majority of citrus is for juice, the time required to get resistant or tolerant trees in production is critical. Since juice processing plants require minimal

amounts of fruit for processing to remain open and the fear is that production will start declining, it is possible that processing plants will have to close unless developments are made to stabilize fruit production. Although it is likely that transgenic citrus trees will be the long-term answer to citrus production, there is concern that they will not be available in adequate numbers in time to save the industry. For those reasons, the CTV vector is being considered as a temporary measure to protect trees until transgenic trees become available. The advantage of the CTV vector with an anti-HLB gene over transgenic plants is that the vector can be deployed sooner. Another advantage of the CTV vector is that if effective anti-HLB genes or anti-psyllid genes or RNAi molecules can be found, the vector could be used to treat trees in the field that are already infected with HLB.

V. Guava Effect: Production of GMC trees that repel psyllids. Berta Alquezar, IVIA, Moncada, Valencia, Spain.

It has become known in Vietnam that guava grown in proximity to or intercropped with citrus has a repellent effect against the Asian citrus psyllid. This effect is likely due to volatiles produced from the guava leaves, because the protective effect is present all year round. The repellent effect of guava leaf volatiles on *Diaphorina citri* was confirmed employing a Y-tube olfactometer. Genes responsible for the biosynthesis of these volatiles were cloned from citrus and *Arabidopsis* and the functional activity of encoded proteins were confirmed by *in vitro* functional assays. Transgenic sweet orange plants carrying some of these constructions have been generated and are already available in the greenhouse to be further tested.

SHORT-TERM CONTROL SYSTEMS (AFRICAN HLB)

H.F. le Roux, hlr@cri.co.za, S.P. van Vuuren, M.C. Pretorius (CRI), C.H. Buitendag, Pest & Disease Consultants (PSK), South Africa.

- An overview of the way in which African greening was controlled and how the southern African citrus industry coped with Greening was given.

SHORT-TERM CONTROL SYSTEMS (ASIAN HLB)

The three-pronged system (TPS) for HLB management is based on three measures: (i) surveys for identification and removal of HLB-affected trees to reduce sources of inoculum, (ii) use of healthy plants grown in covered, insect proof nurseries for resets and (iii) insecticide applications against the Asian citrus psyllid (ACP) vector.

The TPS is a preventive control system, preventing trees from becoming infected with *Ca. L. asiaticus* through transmission by the Asian Citrus Psyllid (ACP). It was applied in SPS immediately after HLB was detected in March 2004, a time when HLB-incidence in the farms was still low. Practically all the many large farms (≥ 600 ha or 300,000 trees) used the TPS. From 2004 until now (November 2012), the TPS has been constantly improved and adapted to changing situations. The need to control psyllids and to remove HLB-affected trees has never been questioned. The major difficulty for well-managed farms was the presence of neighboring farms with no or poor HLB-management. The TPS has made it possible to keep the HLB-incidence below $\sim 1\%$ affected trees a year, meaning that $\sim 98\%$ trees are uninfected and healthy, thus providing a very low HLB environment. The acreage of TPS-managed farms with low ($\leq 1\%$) HLB-incidence amounts to $\sim 200,000$ hectares or one third (1/3) of the total SPS citrus acreage. SPS is the only region in the world where the TPS has been so successful on a large scale.

The successful results obtained with the TPS have changed the perspectives of the SPS citrus industry. Until it was demonstrated in 2010 that HLB management by the TPS was successful, it was believed that the TPS was only a short-term solution to keep the citrus industry alive until such time that HLB-resistant, transgenic cultivars would hopefully become available. Today, SPS has not only the transgenic, long-term solution available, but also a second option for its citrus industry: regular, non-transgenic orchards kept at very low HLB-incidence by TPS-management. This second option will also offer most favorable conditions for the development of the young, transgenic orchards, as these will benefit from the low HLB-environment provided by the TPS.

The TPS has also been used successfully in Southern Florida on some large farms, but many growers were reluctant to remove HLB-affected trees. Growers have turned to enhanced nutritional programmes and by 2012, the HLB-incidence in most farms was too high for the TPS to work. Florida probably has only one long-term option: HLB-resistant, transgenic trees.

ENHANCED NUTRITIONAL PROGRAMMES (ENP)

ENPs have been applied when HLB-incidence was already high because growers had been reluctant to remove HLB-affected trees even though they applied insecticides to control psyllids. In orchards where ENPs are applied, the trees look better, but in spite of psyllid control, the percentage of HLB-infected trees increases rapidly and soon, in a few years, 100% of trees are infected (HLB-incidence: 100%).

Even if productivity is increased, area-wide applications of ENPs result in HLB-saturated citrus environments and high inoculum pressure, which most likely hinder, today, the development of new, young orchards and might even jeopardize, tomorrow, the establishment of orchards with genetically modified citrus. ENPs are not substitutes for TPS!

W04 'new Perspectives in Pest Control'

Convener: A. Urbaneja (Instituto Valenciano Investigaciones Agrarias (IVIA), Spain
aurbaneja@ivia.es)

Josep Jacas. Construction of artificial infrastructures: nesting boxes for insectivorous birds? Don't know the impact on pests and natural enemies. Alternative foods for natural enemies? Cover crops, flower strips etc but little information on specific plants. Ant control – how best to do it? Avoid dust and pesticide side-effects. Difficult to quantify any increase in natural enemies from supplementary food.

Vicente Navarro Llopis. Semiochemicals. Different types of pheromones. Must be careful of different release rates – not in specifications. Attract & kill, Attract & infect, Attract & sterilize. Mating disruption uses low pheromone quantity and may result in other pests becoming dominant. In Spain the government pays for mass trapping with BioLure. Trimethylamine is the best amine for fruit flies.

Jorge Hendrichs. Fruit fly management. *B. zonata* is in Libya and the Middle East. Debate about *B. dorsalis* complex. MAT with Splat on telephone posts. *Anastrepha* not as aggressive as *Bactrocera*. Need multiple approaches for control. Trend towards solid lures (rather than liquids) that can now be combined for monitoring purposes. Mexico is rearing 50 million parasitoids per week. Hormonal supplements make males mature faster. Generic dose of 150 Gy for all fruit flies.

José Inquierdo. Probably less money going into chemical development in the future. IPM definition differs from place to place and crop to crop. Different application technologies. Clients in some countries demand mixtures of good IPM chemicals to have a broader spectrum.

Victor: Oxylin pathway and SA-JA crosstalk in citrus: Change away from sour orange rootstocks due to CTV susceptibility to other rootstocks has increased populations of *Tetranychus urticae*. Tolerance to *T. urticae* associated with increased SA and JA pathways and induced by oxylin.

Beth Grafton-Cardwell. Invasive pests require broad spectrum pesticides. We have very high expectations of new insecticides. Still need old chemistry with known MRLs to take care of invasives. Releasing of natural enemies against alien insects is being frustrated by conservative approaches. Leafminer pheromone highly attractive with potential for attract and kill but pheromone for peelminer does not work in A&K because of variability during the year. How do we respond to invasives? Must detect and prevent establishment. J. Millar creating portable GC to work out pheromones of expected future pests. Respond to invasions: olfactory research. How to respond to pests that have established? Nupsyllid – is a molecular approach that will cause fatal mutations or prevention of transmission of bacteria. RNA interference technology that interferes with critical genes – silence genes that control various critical processes.

Marketing of GM technology will need to be very carefully done – e.g. Oxitec.

W05 'Dwarf Citrus trees in High-Density Plantings'

Convener: K. D. Bowman (U. S. Horticultural Research Laboratory, USA;
Kim.Bowman@ars.usda.gov)

Presentations in this workshop were largely on the development of rootstocks with dwarfing characteristics. Kim Bowman highlighted a promising USDA rootstock US-897 (Cleopatra x Flying Dragon) based on 23 years of trial work. It reduced tree size by 50%, showed no graft incompatibility, but yielded smaller fruit. Jude Grosser and Fred Gmitter presented Changsha X50-7 as a cold-hardy and Phytophthora tolerant rootstock. Mike Roose mentioned the dwarfing rootstock Bitters, which is an open-access rootstock in California. Discussion of high-density plantings (800 to 900 trees/ha) concluded that based on the high capital layout, it is not economically viable at more dense plantings.

W08 'Global Citrus Industry Collaboration on MRL Regulatory Issues'

Convener J. R. Cranney, Jr. (California Citrus Quality Council, USA; jcranney@calcitrusquality.org)

Jim Cranney attends annual Codex mtg as a representative of ISC. 40% of Californian citrus revenue comes from exports. Primarily goes to Asia so need fungicides for long shipment. More small countries are wanting to exercise their sovereignty and consumer demands which results in more regulation and residue testing. Korea and Japan share knowledge on exceedances. Migration from Codex: Korea, Hong Kong and China. Likely that they will have different MRLs. Codex is coordinating global joint reviews – may help get MRLs sooner. The EU can influence the Codex process. Propose collaboration on Codex, country-specific MRL issues, EU import tolerances, EU food retailers policies, Japan food additive process. We could write joint or separate letters to policy makers. More MRLs strengthen IPM programmes and provide potentially faster access to chemicals.

Nikki Johnson (NZ) said that Australia accepts NZ MRLs but not necessarily Codex. Difficult to figure out how the Codex system works. Codex MRLs = WTO Trading standard. NZ levels are based on GAP but Codex standards are focused on what is safe in food. Local use MRL may therefore be different to what is used on imports which may follow Codex. Some countries use Codex levels for their domestic use and for import levels. In NZ there is a lack of funding for research on specific pests. Data protection laws are very weak in NZ so new chemicals may only have 5 years of exclusive use. OECD drives a lot of NZ regulation. Residue data must match the intended use. Feb 2012 in Rome had Global Minor Use Summit. Two of 5 key outcomes were: Coordination and Collaboration, and Residue and MRL setting.

Andrew Harty (Aus) 5 points: Loss of MRLs due to revision of older chemistry. Delays in establishing MRLs for new chemistry. Import compliance. Erratic recognition of Codex MRLs. Analytical methodology. EU moving to hazard-based rather than risk-based regulatory system. Nobody wants to support reregistration of old datapacks. Results of reTests are sometimes different. Get MRL breaches even though following GAP.

Vaughan Hattingsh on European retailer demands. Workshop convened by Freshfel. Problematic demands from retailers: Limiting residue levels to a proportion of an MRL. Unrealistically limiting the number of AI residues. Using an acute reference dose as a residue tolerance. Compliance is a guarantee that GAP has not been followed. Process undermines IPM and drives use of preventive measures before petal fall. Under-dosing drives resistance development. Postharvest decay is difficult to manage. Neutralises the value of billions of Euros on PPP development. Introduces heightened compliance unpredictability. Increased costs of testing against a moving target. Undermines the confidence in science-based legal MRLs. Creates consumer perception that fresh produce is risky. Jeopardises market access by undermining ability to control market access pests. Need to communicate through the value chain to redirect before major damage is done. Need to revert to GAP-based reliable residue assurance system.

MRLs in Chile MRL expertise required. New AIs sometimes not registered fast enough in some countries. Supermarket challenges. Pressure from NGOs against residues. Other non-pesticide problems like Morpholine and Quats.

Discussion

The NGOs are problematic in the EU system. Need a consumer body with an interest in agriculture to start some counter measures against the private standards. NGOs are not interested in engaging discussions on these issues. Ted Baxter agrees that we can't work with NGOs but could form your own NGO that would have more influence than the existing NGOs. There has been an attempt to do this in California.

There was a lot of agreement in trying to resolve these issues in the future but a representative group is required so they are asking for contacts.

W11 'Quarantine Security for Tephritid Fruit Fly Pests in Citrus'

Convener: N. J. Liquido (USDA-APHIS-PPQ, Centre for Plant Health Science and Technology, Plant Epidemiology and Risk Analysis Laboratory, USA; Nicanor.J.Liquido@aphis.usda.gov)

Alternatives to Probit 9 may be suitable for hosts with natural low infestation rates or where the pest population density is very low, due perhaps to MAT or other field control methods. Canada, USA, Australia and NZ are discussing alternatives to Probit 9. Probit 9 requirement of approx. 100 000 without survivors. But Japan, Australia and NZ accept Probit 8.72 based on sample size. What about using a diagnostic dose approach?

B. tryoni does not seem to follow a linear relationship between mortality and cold treatment.

Landolt et al. 1984 equation based on Poisson distribution. Considers probability of having at least one or more mating pairs per shipment. Simplified by Baker et al 1990. Vail et al 1993 took this further so you can work out the required quarantine treatment mortality. NZ have a term "maximum pest limit". RSM30 requires 99.99% efficacy on non-host which is below Probit 9. Liquido et al. 1997 equation 2 looks at probability of establishment.

There is also the category of "Conditional non-host". Avocado only changed to this status from 2011. Different varieties can also be important.

Should we standardise the quarantine treatments? Perhaps the IPPC could become involved. The probability value cannot be a moving target. Need to determine what is an acceptable probability level.

ORAL PRESENTATIONS AND POSTERS

Session 05: Biotechnology

Rodriguez, A. et al. Limonene accounts for 90% of terpenoids in citrus. Limonene attracts fruit flies and is required for fungal infection. Fruit without limonene had less fungal infection.

Poster highlights

Juárez and Navarro presented the applications of *in vitro* shoot-tip grafting, which apart from virus elimination can also be used to regenerate irradiated plants, haploids, tetraploids and transgenic plants.

Soler et al. from IVIA demonstrated that transgenic Mexican lime plants expressing the gene *p23* from *Citrus tristeza virus* (CTV) under the control of a phloem-specific promoter display symptoms closely similar to those accompanying virus infection.

Muniz et al. from Sao Paulo University evaluated 60 several transgenic Valencia and Hamlin lines containing coat CTV protein or conserved genes and identified several promising lines that will be evaluated further.

Session 06: Fruit physiology (Paul Cronje, Tim Grout)

Peréz, M.C. et al. Brassinosteroid increased yield 38% but girdling still better at 54%.

Tadeo, F.R. et al. Various groups of genes are associated with fruit abscission.

Fanciullino, A.L. et al. Sugars are precursors to carotenoids in fruit. If modify photosynthesis and therefore sugars, can affect carotenoid levels. Carbohydrate control over carotenoid build up in citrus is conditional on fruit ontogeny: The starvation of fruit of carbohydrate during the first growth stage lead to an increase in carotenoid accumulation. This result is contradictory to what is currently thought regarding colour formation and the positive relationship between carbohydrate content and carotenoid syntheses.

Xu, J. et al. Different regulation mechanisms occur in different cultivars. No lycopene is found in the leaves. Not sure if carotenoid accumulation has a direct effect or side effect.

Gambetta, G. et al. Lower GA levels result in chloroplasts being converted to chromoplasts. Endogenous factors affecting fruit colour development in navel sweet orange: Rind colour is positively associated with endogenous ABA and CHOs and negatively with N. However the mode of action and the interaction with other plant growth regulators such as GA still remains unclear. The role of norflurazone is also not clear.

Poster highlights

Gravina, A. et al. Cross-pollination and control of seed formation in Afourer mandarin. In net isolated trees, all fruit were seedless (0.001 to 0.04 seed per fruit) compared with open pollinated trees (3.2 to 4 to seeds per fruit). Valencia orange pollen resulted in high seed production in Afourer (2.6 seeds per fruit) compared with Afourer (0.2) and Nules Clementine (0.4) pollen. Three applications of GA₃ + CuSO₄ (50 + 125 mg/L) at 25-55% open flowers reduced seed number per fruit (4.6 to 3.3) and increased seedless fruit percentage (19 to 33%) in open pollinated trees and reduced the average number of seed.

Ikoma, Y. et al. The effect of combined spraying of gibberellin and prohydrojasmin on peel puffing in Satsuma mandarin. The increase in temperature during fruit development is thought to increase peel puffing.

The application of GA and PDJ before colour break reduced the incidence of puffing and the optimum concentration is 1 and 25 mg/L for GA and PDJ respectively.

Session 07: Regulation of growth and development (Paul Cronje)

Goldschmidt E.E. Citrus developmental research: a historic, conceptual perspective. An overview of his research was given and the complete paper will be very interesting. In short he think that with currently knowledge and research tools (molecular) we should focus again on integrating what we know about mineral nutrients, plant growth regulators and sink source balance on solving the remaining mysteries in citrus production.

Marinez-Fuentes, A. et al. The effect of paclobutrazol and fruit on flowering and carbohydrate accumulation in roots branches and leaves of alternate bearing Clementine mandarin. In the current study the application of PCB could not explain changes in carbohydrate levels in the leaves or roots, and further research will be conducted in this area.

Tumminelli R. Foliar application effect of the 3,5,6-TPA on Tarocco red orange yield and fruit size in 'on years' of eastern Sicily orchard. TPA application (30 g.a./ha) at 10 mm fruit size reduced the total yield and increased yield of commercial valuable large fruit in the "on year. In addition the total yield was increased and commercial yield decreased in the "off year", indicating the possible usefulness of this PGR in crop load manipulations in alternate bearing cycles

Session 09: Postharvest physiology and pathology (Paul Cronje, Arno Erasmus, Ncumisa Njombolwana and Paul Fourie)

Alferez, F. et al. It was previously found in plants that the PLA2 gene was induced by blue light. Used different wavelength LED devices at 40 umol per s per sq. m for different periods of time then inoculated with spores. Blue light reduced infection by *P. digitatum* but not other colours. Red light inhibited PLA and promoted infection. Octanol increased 6X after blue light treatment. If apply octanol to the fruit you can also reduce *Penicillium* growth but it is difficult to find a dosage that is not detrimental to the peel.

Cronje P. Could ethylene influence Peteca spot incidence in lemon fruit? The use of ethephon prior to harvest of peteca prone fruit was shown to reduce the incidence. This, as well as the efficacy of AVG to reduce PS incidence indicate that the endogenous ethylene metabolism could be involved in determining the fruit susceptibility to this disorder.

Alos, E. Delay in color break in 'Tardivo' late ripening mandarin mutant, is related to a defective ethylene response. Late mandarin colour development is not fully elucidated and is of high economic importance. The study on this cultivar indicate that it have an altered ethylene-induced gene expression, displaying a transient response to ethylene that is unable to sustain it subsequently, leading to a weak colour development. Therefore this cultivar will not react favourably on degreening treatments.

Romero P. et al. Postharvest water stress leading to peel disorders in citrus fruit involves regulation of phospholipase by ABA. The experiment determines that the involvement of ABA in regulating the phospholipase activation in the pathway and the response to water stress occurs upstream by activation of PLA2.

Torres-Leal G.J. et al. Stem end rind breakdown of citrus fruit a new postharvest physiological disorder of lemon fruit in Tucuman (Arg). Fruit with a sunken brown spots around the stem end, later becoming darker were seen in Tucuman in 2008 and 2012. The disorder is more prevalent in thin rind fruit from high humid high rainfall areas that were under water stress. Postharvest treatments as the control of SERB do also reduce the severity of this disorder.

From a postharvest pathology perspective this session did not deliver much value for applied research, as it was overwhelmed by molecular biology.

Hongye Li from China presented a talk on the resistance genes involved in imazalil resistance in *Penicillium digitatum*. This is important work, especially for the resistance assay presently being developed in a CRI-funded project.

Peter Taverner from Australia shared their strategy for sour rot control where they have no registered fungicides (for export) against this disease. They rely on combining GRAS (generally regarded as safe) chemicals with fungicides and/or sanitisers for control, but have no clear protocol.

Parra, J.P. et al. from Citrosol presented work showing that potassium sorbate applied through wax increased fruit weight loss and did not contribute to disease control.

The following posters were of interest to the postharvest plant pathologists:

Effectiveness of the fungicide application system in the control of *Penicillium digitatum* in orange in Tucuman, Argentina by Torres-Leal, G.J. et al. where dip application was shown to be more effective than a rotating spray application.

Resistant strains to postharvest fungicides in Algarve citrus companies. Packhouse sampling and *in vivo* assays by Salazar, M. et al.: A survey was done over seven seasons in 14 packhouses in the Algarve region, Portugal. The incidence of resistance to thiabendazole (TBZ) and imazalil (IMZ) increased over the years and was higher for TBZ (24%) than IMZ (11%). Strains were tested on fruit to determine the impact, but result varied and no clear conclusion could be made. This work shows that quantifying resistance in packhouse populations is complex and more work needs to be done on this topic.

Influence of paraben concentration on the development of Green and Blue molds on 'Valencia' orange fruit by Moscoso-Ramirez, P.a., et al.: Three paraben sodium salts (sodium-methyl, -ethyl and -propyl) were evaluated for *Penicillium* mould control. All the actives showed promise and more work will be done.

Optimal concentration of inoculum, types of injuries and control of *Penicillium digitatum* in orange fruit by Gonza'lez-Fierro, P., et al.: Nine different inoculum concentrations were tested on three different wounds for infection. Any inoculum concentration of $\geq 6 \times 10^5$ spores/mL gave 100% infection; the lower concentrations of 1×10^4 and 3×10^4 spores/mL did not show symptoms. Wounds that did not penetrate the albedo did not show infection.

Session 10: Watering and nutrition (Teunis Vahrmeijer)

Water use in citrus increasing

Water scarcity and the increase in water use efficiency were important themes at the conference. Research results on the determination of evapotranspiration from sap flow and micrometeorological techniques were presented as a foundation for the better understanding of citrus water use. These techniques are currently state of the art in the research on tree water use and allow us to differentiate between the transpiration and evaporation components of evapotranspiration under commercial orchard conditions (Consoli, S et al.). The effect of partial root-zone drying (PRD) (Ortega, J et al.), sub surface drip and deficit irrigation strategies on increasing water use efficiency (WUE) (Stagno, F et al.) of citrus was evaluated and the results were presented. The results indicate that deficit irrigation and the sub-surface irrigation system can both be used as measures for increasing plant WUE regardless of the rootstock employed. Contrary to previous evidence obtained in more arid environmental conditions, PRD did not result in any gain in plant performance when compared to similar watering regimes.

In another approach a model was developed in Sao Paulo (Brazil) to use low temperature (<19°C) and soil water content as a flowering management system. Medium to low drought stress conditions were used to reduce the amount of flowers on the trees. The model is currently used in more than 12 000 ha and resulted in an increase in income of approximately \$ 1300 per hectare (Albrigo L.G et al.).

Nutrition

Experiments for verifying and updating nutrient guidelines are very expensive because it requires several years of field experiments in mature bearing trees. Therefore, the Israeli guidelines for citrus leaf mineral status were updated using: i) a 10 year leaf mineral database and ii) a database that summarised yield and leaf mineral concentration collected from 122 orchards over a seven year period. Results indicated that the optimal leaf mineral concentration for grapefruit is 1.7-2.1% dry weight (DW) of N, 0.08-0.010% DW of P, 0.37-0.48% DW of K, and 0.33-0.45% DW of Mg. For orange trees the optimal leaf mineral concentration is 1.9-2.3% DW of N, 0.11-0.14% DW of P, 0.80-1.00% DW of K, and 0.19-0.26% DW of Mg. For easy peelers trees the optimal leaf mineral concentration is 2.0-2.4% DW of N, 0.09-0.12% DW of P, 0.55-0.69% DW of K, and 0.19-0.26% DW of Mg. Maintaining the leaf within these ranges will allow for a maximal yield of 110-120 tha^{-1} for grapefruits, 65-70 tha^{-1} for oranges, and 60-70 tha^{-1} for easy peelers (Raveh, E.).

Research on calcium-nitrogen fertilisation was also prominent at the conference. Results indicated that the yield and Ca-content of the leaves for calcium nitrate were higher than for ammonium nitrate applications (Quiñones, A. et al.). Other important conclusions indicated: (i) an interdependence between N and Ca-absorption, (ii) a decrease in $\text{N-NO}_3^-/\text{N-NH}_4^+$ ratio in the soil solution and in the sap extracts with increased ammonium nitrate application rates, which negatively affected Ca-absorption and its concentration in the sap

extract and leaves, (iii) maximum fruit yield was attained with N-NO₃⁻/N-NH₄⁺ ratios of 25 in the wet bulb solution and seven in the sap extract, and (iv) unbalanced absorption of cations and anions caused by excessive N-NH₄⁺ absorption, which consequently resulted in a decrease in the pH of the soil solution (Quaggio J.A.).

Poster highlights

Emerson et al. presented mechanical and chemical methods to recover plugged irrigation drippers. Both methods gave similar results, but a combination of mechanical impact treatment (wood stick) and chemical (600 ppm hydrogen peroxide for 1 hour) gave best results.

Session 11: Cultural practices and mechanization (Paul Fourie)

M. Zekri from Florida presented a paper on “Performance of citrus nursery trees and two-year older trees after transplantation in the field” where different ages of nursery trees were planted different sized pots. After 6 years in the field, all treatments were equal in terms of size and quality, indicating that the trees planted when smaller/younger will catch up to older/bigger trees.

De Lima from Brazil presented “Effect of inarched, two-rootstock trees on development and yield of ‘Valencia’ orange orchards”. Inarching is used to manage Citrus Sudden Death, but is expensive in the field. In his CitroLima nursery he started producing inarched trees with a combination of a vigorous rootstock (required for establishment, but susceptible to Sudden Death) and a disease resistant rootstock with good quality attributes. In trials, he demonstrated that trees with the combination rootstocks grew slower than those on the vigorous-alone rootstock, but that the latter rootstock got Phytophthora, while the other did not. It was 10-15% more expensive to produce inarched nursery trees.

Carrol Lovatt presented “Morphological and yield characteristics of ‘Washington’ navel orange and ‘Tahiti’ lime trees produced with buds from floral versus vegetative mother shoots”. The hypothesis was that buds from vegetative shoots produced more vigorous trees with juvenile characteristics. In trials, floral buds had higher yields, but after 4 years no significant difference was observed between trees made with buds from floral or vegetative shoots.

Roka FM. Evolution of citrus mechanical harvesting in Florida – Lessons for the future
HLB has resulted in a decline in effectiveness of using mechanical harvest due to the reduction in tree performance. However, the cost benefit still remains high for mechanical harvest.

Poster highlights

Garrán and co-workers presented FruTIC, which is an information and communication tool for integrated citrus health management in Argentina. This platform tracks phenology, weather and pest and disease pressure.

The costs of organic citrus production in Portugal were determined by Reis and co-workers. After 1 season, organic production cost was more expensive than conventional (3980 vs. 3636 Euro/ha) and yield was lower in organic production (20 vs 21.2 t/ha). A premium on organic fruit would be required to make organic production economically feasible.

Session 12: Citrus HLB and other bacterial diseases (Paul Fourie)

Duan, Y.P. et al. Full genome sequence of *Liberibacter asiaticus* (Las) has been published (1.268 million bp). Different phenotypes are visible in HLB-affected Periwinkle. Different frequencies of different sequences expressed in 16S; at least 9 variants of Las. Certain genotypes were never found in psyllid. Multiple phages and prophages were found in Las; possible respond to stress. To do with adaptation to diversity of host. Some Florida isolates are closely related to Indian ones but others are related to Chinese. Certain prophages are not found in psyllids and not found in citrus but are present in Periwinkle.

Cambra presented a new 25 min detection tool for Las: tissue print/squash followed by rtPCR (www.plantprint.net), which was 100% effective in symptomatic trees and 74% effective in asymptomatic leaves from infected trees. Referred to seed transmission of Las.

Cen presented a new vector of HLB, pomelo psyllid *Cacopsylla citrisuga*, was found at higher elevation (1000 m) plantings in Yunnan province, China. It appears to be limited to *Citrus* and *Poncirus trifoliata* and was not found on *Murraya*.

Bowman, K.D. and Albrecht, U. Plants were grafted with infected material. Benecke and Carrizo had lower levels of infection. Benecke had a lower titre than Cleopatra mandarin. Benecke also showed no leaf symptoms, but symptoms in Cleopatra were high. Cleopatra stem diameter did not increase as fast relative to control. In field Volkameriana and Cleopatra had more symptoms. With Valencia scions, all showed decline after 9 -11 years and no rootstocks resistant.

Dutt, M. et al. Screening of citrus clones for resistance to canker and Las. CEME antimicrobial peptide gives a high degree of resistance but still a few lesions.

Luque-Williams M.J. The latest Strathmore find of *Diaphorina citri* (ACP) was in a commercial orchard whereas S. Calif. finds were mostly home gardens. In June 2011, numbers started increasing exponentially. In March 2012 Las was found in a residential area in California for the first time. They suspect it to be Pummelo probably imported illegally from China, but the tree had been regrafted 23 times by a “knowledgeable” Chinese gentleman. Following this HLB find, they immediately implemented the following steps: 1) quarantine 5-mile radius surrounding site with door-to-door ACP/HLB surveys; 2) sprayed 870 properties with cyfluthrin and imidacloprid drench; 3) conducted a spoke survey on a 10 mile radius (within each spoke, surveyed 25 properties per sq. mile); 4) did a trace-back, trace-forward analysis to determine the origin of the incursion (suspected to be illegally imported budwood from China); 5) eradicated the positive trees (bagged, double-bagged all plant material including roots, and psyllids, and destroyed); 6) survey neighbourhood for 2 years: Zone 1 - within 500 m all hosts bi-monthly, Zone 2 – all hosts in next 500 m three times per year, Zone 3 – 50% of hosts twice a year. To date, 3490 ACP and 3566 plant samples within 1.2 km radius from the infected plant all tested negative by PCR analysis. In California, they have tested a total of 11,164 plant and >20,000 ACP samples by means of PCR; all negative, except for the one positive find.

Poster highlights

Jim Graham's group at University of Florida demonstrated the significance of early root infection in HLB disease. Apart from feeder root loss and subsequent effects such as yield loss, roots appeared to be the first major source of Las replication and main reservoir and source of Las to new flush.

Batista and co-workers did a spatial analysis of HLB epidemics and demonstrated the fast spread of the disease, as well as the importance of primary infection from external sources (*i.e.* the very important need for effective vector control).

For faster rapid detection of HLB, Fink and co-workers identified HLB infection specific volatiles in asymptomatic trees and developed portable sensors to detect these in orchards.

Carvalho and co-workers studied the effects of pre-inoculation of trees with virus and viroid combination on HLB symptoms. Mild strains of CTV appeared to reduce infection levels, while severe CTV strains or combinations with viroids (exocortis or cachexia) intensified the disease.

Lopes and co-workers ascribed lower incidence and less rapid spread of Las in warmer regions of Sao Paulo state to the influence of higher temperatures on the survival and multiplication of Las in leaves and subsequent transmission by the vector.

Renato Bassanezi presented the seasonal detection of HLB symptomatic trees and ACP in Sao Paulo state. HLB symptomatic trees were detected in all months, but 79% was detected in February to August and less than 10% in October to December. ACP was also detected in all months, but peaks of detection were found in October and December; 80% were detected from August to January, during which control should be intensified.

An interesting project “Unforbidden Fruits” in California aims to prevent citrus smuggling by introducing varieties that are culturally significant to ethnic communities in the improvement scheme. These include curry leaf (frequently used in Indian cooking), bael (leaf used in Hindu rituals) and own-rooted Etrog (fruit used in Jewish rituals).

Salas and co-workers presented research on the influence of different copper spray volumes and doses on canker control. Following 3 seasons trials, generally no significant difference was observed between treatments in low-pressure years, but when canker incidence was severe, high dose copper (15 kg/ha) sprayed at medium (5000 L/ha) and low (2000 L/ha) improved control compared with the traditional high volume (10,000 L/ha) sprays.

Several other posters were presented on Citrus Canker, which are not reported on here.

Session 13: Fruit flies (Tim Grout and Sean Moore)

Hendrichs, J. Fruit fly spread around the world with fruit carried by ships for scurvy. There is a need to integrate methods of control that are more sustainable and acceptable. With SIT, not introducing exotic species. \$200-300 per million flies from large factories. Must integrate SIT with sanitation and other treatments. Very good management is required. Need area-wide approach. Guatemala producing 2000 million flies per week. California, since 1994 started SIT for Medfly in the whole LA basin. Eradicated in whole area in 1997 and continued preventive releases since then. This has been cheaper than spray eradications. Also SIT programmes in Valencia and Croatia (Neretva River Valley). If using SIT for permanent suppression there are more opportunities for companies to be established to do this. IPPC categorised sterile insects as beneficial organisms in 2005.

Juan-Blasco M. et al. Valencia factory for SIT is producing 400 million flies per week. Can successfully evaluate success of SIT releases using the sperm ID method.

Ortego, F. et al. Generated resistant strains of Medfly to malathion, cyhalothrin and spinosad with different modes of action. Malathion usage stopped in 2009. Resistant allele to Malathion is widespread in Spain but not found in other countries. Further selection for malathion resistance led to 100% heterozygous due to duplication of the resistant gene. With spinosad have nicotinic receptor as mode of action. Spanish field populations not very susceptible to cyhalothrin but are generally susceptible to spinosad at the moment.

Papadopoulos, N.T. et al. Eggs survive in all parts of the fruit but larvae do not survive in flavedo of sweet orange or lemon, but do in bitter orange. Larval developmental time affected by acidity and TSS. Limonene lower in lemon than other citrus types but higher pinenes. Lemon oils less toxic as pinenes are less toxic than limonene. No. of oil glands, volume of oil glands and quantity of oil in rind reduces fecundity by up to 91%. Citrus oils do stimulate oviposition - mainly due to limonene. Males are attracted to citrus oils. Linalool has the opposite effect.

Liquido, N.J. & Griffin, R.L. Probit 9 is not a dogma e.g. Australia, New Zealand and Japan accept probit 8.72. Vapour heat treatments should achieve a pulp temperature of 43°C.

Poster highlights

The Oxitec GM approach to ensuring male only fruit fly for SIT was outlined in poster S13P02 by Slade et al. Bayer CropScience had a poster on their Decis trap for fruit fly control. This is a disposable plastic trap coated internally with Decis and costing about 3 Euro each. It is recommended at 50 per ha (Wirtz, K. et al. S13P06). A mass-trapping comparison of Ceratrap against Tripack in Tunisia gave similar results but in a navel orchard 12% of fruit were still damaged so this control level would not be adequate for us (Hafsi A. et al. S13P10). Magnet MED is Suterra's approach to mass-trapping for Medfly and is also recommended at 50-75/ha. This technique employs a BioLure Unipack which is covered by a sticky white cardboard exterior. The flies get stuck on the cardboard so no pesticide is necessary and the lure is supposed to last 6 months. The limited surface area of the trap may be a problem if there are high numbers of flies (Colas, C. et al. S13P12). Magnet MED at 50/ha was also compared to mass-trapping with Mosquisan + BioLure at 50/ha and spinosad bait sprays and all three treatments provided adequate control (Navarro-Llopis, V. et al. S13P13).

Session 14: Virus and virus-like diseases (Paul Fourie)

Bassanezi R.B. et al. There is no systemic movement of citrus leprosis virus so infections are localised. First found in Paraguay in 1930s. Spray once or twice per year. Many of the mites do not carry the disease (64%). Infected mites are closer to diseased trees. Of positive mites, 95% were within 7 m of symptomatic trees. Spot spraying is therefore feasible.

Svetlana Folimonova. Presented an overview talk on "Recent developments on *Citrus tristeza virus* research". In her own research she demonstrated super-infection exclusion of similar CTV strains as a mechanism of cross-protections (i.e. pre-infection with a certain CTV strain could not cross-protect against infection of a different strain). This offered a rather simple cross-protection recipe: ID severe strains, find mild isolate of similar strain (or make it) and cross-protect. However, in natural populations different strains can occur as mixed infections in one tree, which complicates matters. She referred to the recent paper from Gerhard Pietersen's group in South Africa (Scott et al.) that the cross-protecting GFMS12 source is composed of a mixture of different strains, and does not work in all situations. Folimonova also demonstrated through gene deletion and silencing that the P33 gene is needed in cross-protection. Ongoing research is looking for the decline determinants in CTV, which would allow possible engineering of mild

strains. To date, Folimonova and Bill Dawson's groups could not find naturally occurring cross-protecting strains in Florida.

Poster highlights

Ruiz-Ruiz et al. showed a rapid procedure to evaluate the protecting ability of *Citrus tristeza virus* mild isolates (MS) against severe stem pitting isolates (SP). They developed a quantitative real-time RT-PCR method using TaqMan locked nucleic acid (LNA) probes that enabled quantification of SP and MS variants in natural CTV populations.

Vidalakis et al. determined the molecular diversity of *Citrus tristeza virus* isolates collected over the past 50 years and maintained *in planta* collections in California. T30 genotypes were abundant in California but a few T36-, VT- and B165-like strains were also present.

Nunes and co-workers presented the nucleotide sequence of three genes of *Citrus tristeza virus* from selected isolates in the Brazil pre-immunisation program.

In Spain, many nurseries are still open-ground nurseries, presented the possibility of CTV infection from early rootstock seedling stages. Gorris et al. showed that the most susceptible rootstock species was *C. macrophylla* followed by *C. volkameriana*. Cleopatra mandarin showed an intermediate susceptibility, Carrizo citrange and sour orange very low susceptibility and Citrumelo non-susceptible. Aphid species visiting rootstock seedlings were monitored by the sticky shoot method. *Aphis gossypii* was the predominant vector species visiting nursery plants followed by *A. spiraecola*, and Spring was the most active vectoring stage, with up to 1700 *A. gossypii* individuals per *Macrophylla* plant. They proposed that individual testing of nursery plants by tissue print-ELISA could guarantee the CTV-free status in certified citrus plants, but suggested a system to test 10% of plants in a row (external row) in order to estimate the CTV prevalence.

Several detection techniques were presented. Cambra and co-workers presented simultaneous detection of *Citrus exocortis viroid* and *Hop stunt viroid* in citrus plants by direct tissue-print duplex real-time RT-PCR using two newly designed primer pairs and TaqMan probes; they patented this method. A direct tissue-print sample preparation method was tested and used to analyze samples from different citrus species and in different seasons. Results confirm that the real-time RT-PCR approach is as reliable as biological indexing and more sensitive than conventional sPAGE and molecular hybridization, enabling reliable detection of both citrus viroids at any season with the same accuracy. A kit for the detection of both citrus pathogens has been validated and its use could facilitate the sanitary controls required in certification programmes.

Pina et al. presented the citrus nursery tree certification programme in Spain. This programme includes four blocks of trees: 1) The protected foundation block, which is maintained at IVIA and includes healthy plants recovered by shoot-tip grafting *in vitro* (STG) from local or foreign varieties. Plants are grown in containers inside insect-proof screenhouses. 2) Foundation blocks propagated with budwood from the protected foundation block and also grown inside screen- or greenhouses. They belong to individual nurseries or nursery groups. 3) Budwood increase blocks including plants propagated directly from foundation trees to increase the number of buds for propagation of certified trees. They are also maintained inside screen- or greenhouses at each nursery. 4) Certified nursery trees are propagated with budwood from the increase blocks. Certified trees are produced in the open field, in screenhouses or in greenhouses. Plants of each block are periodically indexed by different methodologies according to the regulations. The program is operating with this outline since 1979, when the first healthy plants recovered by STG were released to the nurseries, which started selling plants from this origin to growers in 1982. Since then, 103 varieties have been propagated with a total of 150 million certified plants, with sweet oranges representing 51% of the certified plants, Clementines 27%, other mandarins 13%, lemons 6% and grapefruits 1%. Presently Carrizo citrange is used as rootstock for 59% of the nursery plants and *Citrus macrophylla* for 19%. A total of 40 nurseries are presently operating and they are grouped in 7 foundation blocks. Under this program practically all the Spanish citrus industry has been renewed with healthy plants. Today, traditional graft transmissible pathogens do not pose any problem for our citrus industry. Details, as well as the various diagnostic tests were presented on the poster.

In preparedness for possible CTV incursion in Tunisia, Najar et al. evaluated various rootstocks as alternatives for the exclusively used sour orange rootstock, which is highly susceptible to CTV. They evaluated Maltese demi-sanguine orange trees grafted on 8 rootstocks against CTV as well as exocortis and cachexia viroids. HSVd (CVIIB) caused a significant reduction of canopy volume and fruit yield of trees grafted on *C. macrophylla*. In addition, fruit quality was deteriorated. With citrumelo and *C. volkameriana*, canopy volume was decreased, while with sour orange and Cleopatra mandarin, tree volume was similar to non-inoculated trees. Similar yields were recorded on *C. volkameriana*, sour orange and Cleopatra mandarin

for the CVIIb inoculated trees and the noninoculated control. Yield of the Maltese plants on citrumelo and Carrizo citrange infected with this viroid, was slightly lower without affecting the quality. Concerning Exocortis, data showed that with *Poncirus trifoliata* and Rangpur lime, CEVd infection affected tree height by 25% and 20%, respectively, compared to non-inoculated controls and reduced yield. For Maltese grafted on Carrizo citrange and citrumelo infected with CEVd, yield was slightly decreased in comparison to control trees, but fruit quality was conserved.

Andreas Voloudakis introduced a COST project “Plant virus control employing RNA-based vaccines: A novel non-transgenic strategy” aiming to exploit RNA silencing for citrus virus disease control. RNA silencing is a natural, endogenous mechanism in plants leading to viral mRNA degradation via a sequence-specific process.

Session 15: Fungal diseases (Tian Schutte, Paul Fourie, MC Pretorius, Gideon van Zyl)

Dewdney, M.M. et al. When citrus black spot was discovered in 2010 in juice blocks of ‘Valencia’ sweet orange in southwest Florida, the area where the disease has been found expanded from 14 km² to 57 km² within two years. Upon discovery, quarantine measures were immediately applied to reduce further spread including covering fruit loads, safe debris disposal, fruit surface decontamination and fruit movement restrictions. Fungicide programmes consisting of copper and strobilurins were sprayed in affected groves.

Agostini, J.P. et al. Ascospore release was correlated with environmental variables. Ascospore release was monitored weekly with a Burkard spore trap and climatic data was obtained from a weather station. A predictive model for ascospore release was developed with daily maximum and minimum temperature, rain and days with more than 10 hours of leaf wetness were used for the model. Leaf wetness and the number of days with temperatures between 20 and 29° C had a correlation coefficient with the spore release of 82.1%.

Schutte, G.C. et al. Retention of 3 copper fungicides on citrus leaves and fruit were monitored over a period of 56 days by means of copper residue analyses and a spray deposition assessment using fluorometry, photomacrography and digital image analyses. Persistence of copper residues was similar and decreased at the same tempo during both seasons. The loss of copper residues was attributed to weathering, fruit growth and cumulative rainfall. All the copper formulations tested at registered rates at 35-day spray intervals were effective in controlling *Guignardia citricarpa*.

Huang, F. et al. *Phyllosticta* strains from mandarins, pomelos, oranges and lemons across China were selected for phylogenetic analysis. ITS1 & 2 analyses showed these *Phyllosticta* isolates clustered in four distinct clades corresponding to three known, and one undescribed species, named as *Phyllosticta citrichinaensis*. *P. citriasiana*, associated with tan spot of pomeloes, was isolated only from pomeloes, but not from lemons, mandarins and oranges. *P. citricarpa* was isolated from lemons, mandarins and oranges, but never from pomelos.

Goulin, E.H. et al. attempted gene silencing as a new method to study CBS symptom induction in detached fruits and pathogenicity. *P. citricarpa* was successfully transformed, by random T-DNA insertion of *Agrobacterium tumefaciens*.

Bassimba, D.D.M. et al. *Alternaria* brown spot conidia were monitored weekly by a spore trap and climate was recorded using an automated meteorological station in Spain. Presence of inoculum on affected leaves, shoots, leaf litter, and weeds was determined and infection periods were monitored weekly by exposing trap plants using Fortune and Nova. Only 5% of the isolates were pathogenic. Although affected leaves and shoots were the main source of inoculum, the survival of the fungus in the leaf litter was high. Pathogenic isolates were detected at low levels in weeds.

Van Zyl, J.G. et al. A deposition assessment protocol using fluorometry, photomacrography and digital image analysis was developed to study improvement of spray application which proved to be very accurate in determining deposition parameters on spray targets. A strong linear relation was found between treatment concentration, leaf area covered by fluorescent pigment particles and Cu residue analysis on Nova leaves. ABS control was modelled on %FPC and benchmarks for 50% and 75% control were calculated.

Pretorius M.C. et al. Causal factors involved in root disease related decline have not yet been fully elucidated and when tree decline is noticed, it is too late for the implementation of preventative management strategies. Continued multivariate analyses will allow identification of the progressive changes in soil environmental interactions that lead to tree decline.

Goes, A. et al. Control of postbloom fruit drop (PFD), caused by *Colletotrichum acutatum* and *C. gloeosporioides* consisted of four applications of fungicides at the appropriated flowering stages which were begun at pinhead stage. Spray programmes consisting of tebuconazole, trifloxystrobin and carbendazim were effective.

Hyan J.W. et al. Pathogenicity and genetic tests among 15 isolates of *Elsinoe australis* from Korea, USA, Argentina and Brazil were investigated on seven differential hosts. None of the isolates induced any symptoms on leaves of the seven differential hosts. Sequence analysis of Eaut-1, 2, 3, 4, EaNat-1 and 2 genes divided the isolates in three subgroups, natsudaikai pathotype group, south American isolates group and the USA group.

Ippolito, A. et al. An overview of Mal secco disease (MSD) in Italy caused by *Phoma tracheiphila* was given. Citrus cultivars such as lemons, cedar, lime, bergamot, chinotto, sour orange, rough lemon, Volkamer lemon, are mostly affected. The search for new and resistant rootstocks remains the only control method. Rootstocks evaluated include alemow, sour orange (S. Marina and undetermined selections), ichang lemon, yuzu orange, natsudaikai sour orange, siamelo, Yuma citrange, citrumelos (Sacaton, Swingle 4475, and Swingle FF9), and the hybrids Cleopatra x *Poncirus*, and *Poncirus* Christian x Cleopatra. Importantly, Ippolito also mentioned that the pathogen can be present in seed when seed is harvested from infected fruit. However, as only teguments are infected, and not the embryo, seedlings are not infected.

Session 16: Entomology and pest control (Sean Moore and Tim Grout)

Grafton-Cardwell, E.E. Citricola scale is the most serious pest. The red mite treatment threshold is 8 adult females per leaf. Important to avoid dust, water stress and broad spectrum pesticides. *Euseius tularensis* is used for biocontrol and also effective against thrips. Wet weather lowers thrips pupal numbers in the soil. Spinetoram is the most effective thripicide at the moment. Katydid (*Scudderia furcata*) is a serious pest because it often takes one bite and moves to another fruit. Diflubenzuron and Kryocide are the soft options but need to be used before petal fall to get the numbers down. Very low rates of broad spectrum sprays on the outside of the tree are effective and not disruptive. *Aphytis melinus* releases are made at 250 000/ha. Treatment threshold is 1000 male scale per card trap, but don't use pheromone traps as much as in the past because pyriproxyfen is more effective against males than females so can be misleading. Pyriproxyfen used every second year which is cheaper than *Aphytis*. Good trial results with red scale mating disruption. Citricola scale - using imidacloprid and acetamiprid more. Coragen registered for CLM but only used on young trees. Imidacloprid is affecting *Euseius*, *Rodolia* and *Aphytis* negatively. Asian citrus psyllid: when first found use pyrethroid and imidacloprid. Coragen expected to work against ACP. Several other treatments used in the San Joaquin Valley will work against ACP so will be less disruptive than in the south where programmes are softer. Earwigs are starting to become a problem.

Urbaneja, A. et al. <http://gipcitricos.ivia.es> Wanting to develop smart phone app. Pests that need chemical control are red scale, Medfly, *T. urticae* and aphids (*A. spiraecola* and *A. gossypii*). Releasing *A. melinus*. Vacas et al. 2011 Pest Management Science paper on MD for red scale. Compatible with biocontrol and increases developmental time in the 3rd instar. Apart from parasitoids, soil predators such as carabids (23% +ve) and *Pardosa* spider (5%) prey on Medfly. For *T. urticae* (a problem on Clementines) they use *N. californicus* and *P. persimilis* on young plants. Grass ground cover *Festuca arundinacea* is used as a banker plant. Horticultural oils are still being used in Spain.

Mazih, A. More than 80% of citrus in Morocco is on sour orange. This is very susceptible to CTV and *Toxoptera citricida* is present. Phytophthora is the most important disease. Have had *Eutetranychus orientalis* since 2008. *Prays citri* is a pest. They can still use OPs for red scale but some make releases of *A. melinus* at 100k to 200k per annum. Ants disrupt *Rodolia* - using sticky ant bands. Wash off Cottony cushion scale from trunk with high pressure water. *Lepidosaphes beckii* and *Parlatoria pergandii* are sometimes problematic. Medfly is the most serious pest. Bait only on 1 row of 3 or 4. Chemosterilisation with Adress (lufenuron) is being evaluated. There is still a heavy dependence on chemical control in general. Mealybug pests are *P. citri* and a *Pseudococcus* species.

Pyle, K.R and Jamieson, L.E. Citrus at 35-39°S. Windbreaks are essential. Satsumas, navels and lemons are most important. Kelly thrips and citrus flower moth and Aust. citrus whitefly are the most important pests. Kelly thrips treatments are acephate, avermectin and spinosad. Thiamethoxam and Thiacloprid have been excluded due to red mite repercussions. Surround was used but repercussions occurred and some residues could not be removed in the packhouse. A spray at 5000 L/ha for waxy scale gave better control than 3000 L/ha, with no obvious advantage from oil. Red mite controlled with milbemectin or abamectin or 0.5% oil. Copper is deleterious to the steel blue ladybird. Scab *Elsinoe fawcetti* is a big problem on lemons; they use

flusilazole + strobilurin mixtures, copper, mancozeb or folpet. *Botrytis* is important on lemons and some mandarins; fenhexamid is used, but timing of application remains a problem. Du-Wett adjuvant allows for lower spray volumes (500 L/ha compared to usual 2000 L/ha). Wall chart of Citrus IPM programme. Australian citrus whitefly, *Orchamoplatus citri*, has been introduced. Only one full generation per year. Pymetrozine kills adults. Importing *Serangium* and three parasitoids from Australia. *Prays nephemolina* is flower moth in NZ. Pristine is a new fungicide used for anthracnose control.

Stansly, P.A. Currently no IPM in Florida with ACP (appeared in 1998; HLB detected in 2005). 70% of US citrus production in Florida but industry has shrunk from 345 000 ha to 230 000 ha. Tap sampling for ACP is used widely. Strike a branch 3 times for one tap. 10 taps in 10 different spots per block. Biocontrol is 90% effective, but this is not effective enough. Pyrethroid with 1 d PHI is used in Jan. For young trees they are rotating neonicotinoids and Cyantraniliprole (Cyazapyr). Trying reflective metalised mulch under young trees which is better than white: it confuses ACP and results in lower numbers on trees. Insecticide plus nutrition is giving the best production on infected trees. Growers are optimistic about replanting and the future.

Supriyanto, A.S. and Nurhadi N. In Indonesia, between 1980 and 1990 most citrus was infected with HLB and production was <10 ton/ha. Now nurseries cannot meet demands from growers. HLB known there as Citrus Vein Phloem Degeneration disease. Found in 1964. By the time trees are 7 years old, 100% infected. Also use botanical pesticides. Have *T. radiata* and *D. aligharensis*. *T. erecta* plant and guava repellent. *H. citriformis* EPF.

Campos-Herrera R. et al. Advanced Citriculture Production System involves open hydroponics. Looked at landscape barrier like shade cloth on the ground as a physical barrier but EPNs are important for *Diaprepes* control and the landscape barrier increased *Phytophthora* and was detrimental to EPN biocontrol of *Diaprepes*.

Rogers, M.E. et al. In Florida, young trees were being protected with thiomethoxam, imidacloprid and clothianidin. Bonani et al. 2010 did work on feeding by ACP: 34% of time probing, 22% phloem ingestion and 35% time wandering around on untreated plant. When imidacloprid in plant have initial taste, then jump off. Imidacloprid is therefore preventing uptake. J. Econ. Entomol. 105-5: 1492. At least 6 weeks feeding disruption from soil systemics. With sprays much shorter disruption of feeding (e.g. fenpropathrin 2-3 weeks) but reduce populations. Compared only soil systemics every 6 weeks with sprays alone in field and a combination. PCRs were conducted on trees every 3 months. Found resistance to systemics alone. Soil systemics had 11.3% infection, control 3.8% and foliar 2.5%, combination 0%. Trees with systemics had more flush than controls, that was why more infection. Must do pesticide rotation to prevent resistance. Can only use for first 2 years, then the trees are too big for dosage allowed. Acetamiprid (Mospilan) is no longer used because of hormoligosis.

Miranda, M.P. et al. Shade cloth with UV blocking absorbs 99%, non UV blocking blocks only absorbs 20%. UV blocking shadehouses had much fewer psyllids throughout the area. With non UV blocking shade cloth the highest psyllid numbers were found nearest the edges.

Setamou, M. and Patt, J.M. Used penetrometer to measure hardness of flush. With lemon, mature leaves are still soft but oranges and grapefruit are not. Young flush has higher spectral reflectance and that is what psyllids prefer. SPAD values are correlated with chlorophyll. Adults feed on any flush stage. Immatures less abundant as flush hardens and eggs not laid on hard tissue. Choice tests with stage 2 and stage 4 leaves. Adults 60% on young leaves but much higher percentage for eggs being laid on young leaves. Psyllids use visual cues followed by softness of substrate. Aim control around flush. May be able to use NAA to reduce a flush but this may result in more later.

Van Ekert, E. et al. May help in the future to be able to block JH synthesis using RNA interference. *Diaphorina citri* no longer in Psyllidae but Liviidae.

Dao, H.T. et al. Red scale is still considered the most important citrus pest in Australia. Use winter solstice 21 June as start date for degree days. *Aphytis* ectoparasitoids more important than endos *Encarsia* spp., *Comperiella*. *Rhyzobius* leaves holes in scale covering when it feeds and does not remove the whole scale. *Microcera larvarum*, *M. coccophila*, *Tetracium coccicolum* and *T. novae-zealandica* – fungi that attack red scale. Ants move fungal spores around. Steel-blue ladybird *Halmus chalybeus* was the most effective natural enemy of CRS. Early season, 10% infested fruit as threshold but late season 20%.

Tena, A. et al. *Aphytis* synovogenic i.e. eggs develop after eclosion. Need sugars to live longer. Honeydew is poor sugar source. Only 1-3 eggs laid compared with 6 eggs when fed with sucrose. Sugars may increase longevity and egg production. Release of parasitoids with sugars result in higher numbers of eggs than just

releases alone - almost double the number. Spray of sugars (2M) best. Allow parasitoids to host feed and provide honey as well as sugar that is sprayed. Perhaps sugar alone may have had an impact on natural parasitoids. Sugar improves searching too.

Gomez-Marco, F. et al. *Festuca arundinacea* has shown a benefit for the control of *T. urticae*; they are now investigating whether it helps for aphids. Grass promotes early arrival of cereal aphids and predators can increase on these before aphids arrive in citrus. Results were that with bare soil they had an exponential increase in aphids in citrus but with *Festuca* the increase was slower. A 4 or 5 day headstart was gained by predators due to *Festuca* cover. EIL of 25% infested shoots in a frame never reached with *Festuca*.

Kararacoglu M. et al. In Turkey, Movento is registered for the control of mealybug on citrus. They are releasing *C. montrouzieri* and *L. dactylopii*, but on a small scale.

Navarro-Campos C. et al. Kelly thrips now in Morocco too and first found in Spain in 2005. Other host plants have more extended flowering period than citrus. Higher damage in an area if more late maturing varieties such as Ortanique, Lane late. Found 15 spp from 8 fams of soil predatory mites. *Gaeolaelaps (Hypoaspis) aculeifer* negatively correlated with thrips numbers. Very few phytoseiids in flowers. In NZ where weed control not good tend to have less Kelly thrips in citrus. No chemicals are registered in Israel against thrips.

Poster highlights

Releases of *Anagyrus* for control of citrus mealybug showed that 3 or 4 release points per ha would be adequate (Franco J.C. et al. S16P19). In Sicily they are still learning how to distinguish thrips scars from wind damage and have found that most of it is due to wind (Siscaro G. et al. S16P22) and research on predatory mites in the soil that may prey on thrips shows a dominance of Laelapidae, Macrochelidae and Phytoseiidae (Biondi, A. et al. S16P23). Due to the importance of *Tetranychus urticae* on citrus in Spain there were a few posters involving this pest including one by Pina, T. et al. (S16P24) which showed that *Festuca* pollen was of little benefit to *Euseius stipulatus* and another by Agut, B. et al. (S16P27) which showed that Sour orange rootstock resulted in much less infestation by *T. urticae* than Carrizo or Cleopatra mandarin due to the oxylipin pathway. There were a couple of posters on ants including one on how the management of the orchard floor influenced ant distribution by Martinez-Ferrer M.T. et al. (S16P29). The distribution of *Pheidole pallidula* nests was not affected, probably because all the nests were at the base of the tree where weeds were excluded. Research on *Prays citri* in Sicily by Conti, F. and Fiscaro, R. (S16P34) shows that B.t. and flufenoxuron give effective control and that an action threshold of around 120 moths per pheromone trap per week could be evaluated.

Session 17: Varieties (Hannes Bester)

'Sweet Sicily' and 'Early Sicily' triploids from Italy are two promising mandarin hybrids that were released in 2011, due to their promising characteristics. 'Sweet Sicily' is a cross between 'Comune' Clementine and 'Tarocco' sweet orange and 'Early Sicily' is a cross between 'Oroval' Clementine and 'Tarocco' sweet orange. The fruit of both these hybrids are very early ripening (mid-November) with good fruit size and excellent taste.

Performance of different late maturing navel selections around the world. Various Australian selections like 'Barnfield', 'Powell', 'Autumn Gold' and 'Chislett' were commercialised around the world during the early 1990's, especially in California, South Africa, Spain, Australia and Chile. In general, sub-optimal yields, too large fruit size and granulation resulted in general profitability not desirable. Lately, a number of late-maturing selections have been found in South Africa, like the 'Cambria', 'Witkrans', 'Glen Ora' and 'Karninka'. The characteristics include better return crops, smoother rinds and later hanging ability as compared to the Australian selections.

Yield and fruit quality of 'navelina' and 'Fukumoto', two early-maturing navel selections, in Spain. Significant differences were showed in fruit quality characteristics between these two selections. 'Fukumoto' induced more attractive rind colour, while 'navelina' had more rounded fruit. Total yield and internal quality showed no significant differences.

Late maturing commercial mandarin cultivars in Spain. The chemical and physical characteristics of 'Fina', 'Clemenules' and 'Hernandina' Clementines, 'Ortanique', 'nadorcott', 'Mor' and Ellendale tangor cultivars and 'Fortune', 'Kara', 'nova' and 'Yosemite' hybrid cultivars were analysed. 'Ellendale' and 'Kara' had the biggest fruit size and 'Hernandina' the smallest. 'Ellendale' also showed the highest juice percentage, but also had the highest number of seeds, while 'nova' had the lowest with no seeds. 'nova' and 'Fina' Clementines showed the highest TSS.

Lemon selections for the deserts of the US. 'Allen Eureka', 'Variegated Pink-Fleshed Eureka', 'Corona Foothills', 'Limoneira 8A Lisbon', 'Walker Lisbon', 'Femminello Santa Teresa', 'Interdonato', 'Limonero Fino 49', 'Limonero Fino 95', 'Messina', 'Seedless' lemon and 'Yen Ben' were evaluated for tree and fruit characteristics under desert conditions. 'Corona Foothills', 'Limonero Fino 49', 'Walker Lisbon' and 'Femminello Santa Teresa' have the greatest yields with 'Messina' and 'Variegated Pink-Fleshed Eureka' the least yield. 'Messina' had the largest fruit size, with 'Variegated Pink-Fleshed Eureka' and 'Yen Ben' the smallest.

Session 18: Rootstocks (Hannes Bester)

'Forner-Alcaide 5' ('FA 5') citrus rootstock released in Spain. 'FA 5' is a hybrid between 'Cleopatra' mandarin and *Poncirus trifoliata*, and is commercially used in Spain since 2005. 'FA 5' is resistant to CTV and citrus nematode and shows good tolerance to salinity, similar to 'Cleopatra' mandarin. It is also tolerant to *Phytophthora spp* and wet conditions. This rootstock consistently shows higher productivity than 'Carrizo' citrange, with good fruit size and internal quality.

Preliminary studies on high density plantings in Egypt. Yields of 'Clemenules', 'Hernandina' and 'nova' mandarins budded on sour orange rootstock at a planting spacing of 2x5 m was compared to yields at a planting spacing of 5x5 m from 3 to 7 years after planting (YAP). Trees planted at 2x5 m were significantly taller than those planted at 5x5 m, but the canopy volume of trees planted at 5x5 m was larger. The yield per acre from trees planted at 2x5 m was consistently higher than those planted at 5x5 m, but with a decreasing percentage year on year up to year 7 after planting.

Session 22: Citrus economics and trade

Meliá Martí presented "Spanish citrus cooperatives: keys to success and challenges for the future" indicating the unique situation in Spain with many small growers. 60% of units are classified as micro-units, while 5% of the units are classified as large. In total, micro units earn a mere 14% of income, while the 5% large units earn 50% of the income. To address the short-comings of the micro units (for example, lack of negotiating power) Spain has 3900 cooperatives, involving mostly small-medium units and 42% of output. 23% of these 3900 generate 75% of sales. They still face challenges, such as the Euro-crisis, common agricultural policy, scattered growers and demand concentration with three biggest retailers have 45% buying power.

Scuderi presented "Evolution of development models for Italian organic citrus growing: economic and environmental aspects". Italy has >20,000 ha organic citrus production and organic growers receive 500-750 Euro per ha government support. They receive a premium on organic produce of 5-30%, while orange and lemon production cost was 8.7% and 3% more expensive, respectively. However, yields of organically produced fruit declined.

Vaughan Hattingsh presented "The importance of research and technical services in the recent growth of the Southern African citrus export industry", giving an excellent overview of the South African research and technical service model.

PRE, MID AND POSTCONGRESS TOURS

Pre-congress unofficial tour on postharvest plant pathology (13-16 November 2013)

Arno Erasmus and Ncumisa Njombolwana

Tecnidex

Tecnidex, a Spanish postharvest technology company servicing the Spanish citrus industry, sponsored a three day tour. The aim was to introduce the South African postharvest citrus plant pathologists to the Spanish citrus postharvest industry. Five packhouses were visited during this period; Ripoll and Carcaixent, Copal, Canso, Frutisol and Beltran. A discussion of interesting and innovative concepts learnt from the Spanish citrus packhouses follows below.

Small crates. All packhouses used smaller crates for harvest and fruit transport, this will definitely decrease fruit injury and pressure on fruit (Figs. 7.1.1 and 7.1.2). The packhouses have automatic tippers (Fig. 7.1.3) and the impact on the fruit is much less severe than it would be with larger crates. These crates are easy to manage and handle. The bottoms are well perforated (Fig. 7.1.4) for optimum drenching (Fig. 7.1.5).



Figure 7.1.1. Small crates stacked



Figure 7.1.2. Small crates prior to tipping



Figure 7.1.3. Small crates being tipped



Figure 7.1.4. The bottom of a small crate



Figure 7.1.5. Small crates being drenched

Inline drenching and presorting of colour for degreening. Packhouses that do not employ a pre-degreening drench outside the packhouse, drench fruit inline (Fig. 7.1.6). Fruit are dry tipped (Fig. 7.1.3) and sorted for

injuries and infections. Fruit are then washed before it enters a flooder type system (Fig. 7.1.6) where it is treated with fungicides. After the flooder it is colour sorted and re-binned (Fig. 7.1.7) before it is transferred to specific degreening room as per colour count. This will result in excellent degreening and a smaller loss in terms of fruit quality and decay. The inline drencher has an automatic top-up and dosing system, which will result in better and more effective application (Fig. 7.1.8).



Figure 7.1.6. An inline drencher (flooder)



Figure 7.1.7. Fruit colour-sorted and re-binned prior to degreening



Figure 7.1.8. The automatic top-up and dosing system of an inline drencher



Figure 7.1.9. The automatic top-up and dosing system of an outside drencher

Automatic top-up and dosing systems. Most of the fungicide applicators have automatic top-up and dosing systems (Fig. 7.1.8 and 7.1.9). The fungicides are made up in high concentration stock solutions. The system is then dosed from these stock solutions. This could improve the accuracy and affectivity of an application if manage well.

Water recycling. Tecnidex developed a system to recycle used water to either release it in the sewage system or re-use for wash water (Fig. 7.1.10). All fungicides and contaminants can be removed. As water management becomes a more pressing issue, systems like this will become more and more important to investigate.



Figure 7.1.10. The Tecnidex water recycling plant at a citrus packhouse

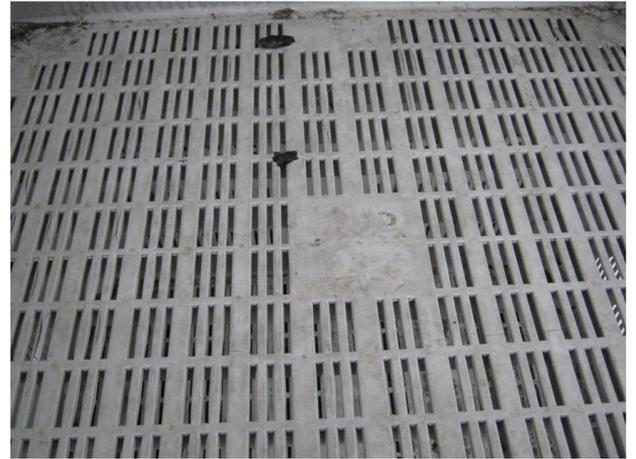


Figure 7.1.11. Bottom of a large Spanish harvest bin

Design of large harvest bins. The Spanish design of the large harvest bins differ from the South African counterpart in terms of the amount of openings in the bottom of the bin. The Spanish bins have a grid-like bottom (Fig. 7.1.11), which will definitely improve the flow of water through a stack of bins when drenched. The Tecnidex head office was also visited in Valencia (Fig. 7.1.12). There products and fungicide application was discussed and the in-house experimental packline was demonstrated (Fig 7.1.13).



Figure 7.1.12. Ncumisa and Arno at the Tecnidex head office in Valencia, Spain.



Figure 7.1.13. The Tecnidex experimental packline

IVIA – Luis Palou

The municipal research centre of Valencia (IVIA) was visited. Dr. Luis Palou hosted the visit (Fig. 7.1.14). A detailed tour of facilities and the experimental farm was undertaken. The availability of the newest technology and equipment was impressive. There is a full experimental packline (Fig. 7.1.15). The facility also holds 6 highly sophisticated incubators where temperature, humidity and gaseous exchange can be manipulated and measured.



Figure 7.1.14. Ncumisa and Luis Palou at IVIA



Figure 7.1.15. The experimental packline at IVIA



Figure 7.1.16. Highly sophisticated incubators for research use at IVIA

Official pre-congress tour (15 – 17 November)

This tour included three 1-day visits to the southern, middle and northern citrus areas around Valencia. The first day we visited the largest citrus cooperative in Spain – Copal Cooperative, one of the largest citrus plantings in the Valencia Community – El Realengo orchard and the beautiful mountain area of Tavernes de Validigna where citrus is planted downhill in small terraces. The orchard of El Realengo is in the area where tristeza epidemics first became problematic. Problems were solved when they started to use pathogen-free certified trees. One of the highlights was to meet Mr El Bachir Nadori who discovered the Nadorcot (Afourer) mandarin. On the way back we visited the Albufera Nature Park – once a saltwater lagoon but due to dilution and increasing sand banks it was converted into a fresh water lagoon where rice is produced. The second day we visited the Zumos Valencianos del Mediterraneo S.A. processing plant where high quality juices are produced and the orchard Les Trencaes. This orchard of about 50 ha is covered in nets to protect the fruit against wind and hail damage. On the third day we visited the Viveros Alcanar Nursery. This is the largest producer of nursery citrus trees in Spain. All trees are made from material from the IVIA protected collection of the Citrus Germplasm Bank. We were allowed to enter the greenhouses and screenhouses but were not allowed to touch any plant/seedling! Due to bad weather we could not visit the traditional plantings of two soft citrus varieties.

Mid-congress Bayer CropScience tour

Sean Moore participated in this field tour. Bayer sought to showcase their “farm to fork” approach to a contingent of mostly farmers from around the world. Bayer acknowledges that many growers consider their products to be expensive. Bayer tries to remedy this by helping growers to use and manage their products more effectively, by regular on-farm consultancy, thus reducing their overall cost of production. We visited four citrus farms in Valencia where Bayer is implementing their programme (out of a total of 21). What was striking on all farms was the poor orchard sanitation. As Spain does not have cryptic phytosanitary pests, unlike South Africa, orchard sanitation is probably not considered as important. Additionally, the labour to conduct regular orchard sanitation is probably unaffordable in Spain. Consequently, it was not difficult to find

fruit fly adults in orchards. Calibration of spray machinery was demonstrated, resulting in an average application of around 3500 L per ha for a full cover spray. My impression was that this was inadequate. At one of the farms, closest to Valencia city (Museros) thrips damage on fruit was noted. However, we were informed that Kelly thrips did not yet occur in this region. On another farm we noted shells of the predatory snail, *Rumina decollata*. Brown snails and white dune snails were also observed in trees. On one farm, *Festuca* ground cover was noted in the interrows, as a means to encourage biocontrol of mites and aphids. A Movento trial site was visited, where comparison with a standard programme (chlorpyrifos, pyriproxyfen and oil) was shown. Far less red scale was observed in the Movento-treated block. Bayer also has a mass-trapping option for Medfly control called Decis trap. The traps are sold with attractant and toxicant inside and are made from thin, cheap plastic. They are used at 50 per hectare in citrus and cost around €3 each.



Disposable Decis trap used at 50/ha for Medfly control

Mid-congress “fungal diseases” tour (21 November 2012)

All the invited speakers and other prominent citrus plant pathologists were taken on a mid-congress tour. We visited the Bayer Crop Science plant in Valencia and then went to visit the Spanish citrus historical museum in Burriana, Castellon and also went to look for *Phytophthora citrophthora* branch canker in the same region, but could not find any in the orchard we inspected. We then visited a 'nova' mandarin orchard at Onda, Castellon to look at *Alternaria* brown spot. What was noted is how poorly their commercial spray applications were as *Alternaria* brown spot was seen everywhere, even on the outside fruit. Thereafter we visited another farm also close by (which we also visited during the pre-congress tour) where we could show the unique symptoms of the disease on 'Hernandina' Clementines to the tour group. This tour also provided for an opportunity to meet all the co-workers on the *Guignardia citricarpa* population genetics project.



Alternaria brown spot infection on a 'nova' Mandarin fruit and flush in an orchard at Onda, Castellon



Phytophthora trunk and branch canker on 'Hernandina' Clementine at Onda, Castellon

Mid-congress tour with Citrosol

A postharvest technology company, Citrosol, hosted a mid-congress tour, especially arranged for CRI personnel. The group consisted of Dr. Hennie le Roux, Hannes Bester, Dr. Paul Cronje, Ncumisa Njombolwana and Arno Erasmus. The tour started at the head office of Citrosol in Potries, Valencia. Here the group was hosted by Dr. Benito Orihuel, CEO of Citrosol (Fig. 7.1.17). The laboratory facilities and wax manufacturing plant was visited. In the second part of the tour two packhouses were visited. Here similar technology was showcased as with the Tecnidex tour, which included automatic dosing systems (Fig. 18), inline drenchers and wax applicators.



Figure 7.1.17. Dr. Benito Orihuel and Ncumisa at the Citrosol head office at Potries in Valencia, Spain



Figure 7.1.18. The Citrosol automatic doser for a drench applicator

Mid-congress official tour

The official tour stopped at four locations in the Valencia region. One stop was the Masía del Doctor experimental field station where trials with *Festuca* ground cover were being conducted and a variety block was visited. The farm also had persimmons that are becoming an increasingly popular replacement for citrus. The Rincón de Gausa farm owned by Fontestad in the Sagunto province was visited and a walk around the orchards was taken. Drip irrigation is used and the drippers are underground to prevent squirrel damage. Young trees also have to be protected from rabbits. All trees on the farm (160 ha) are annually pruned by hand and some were being shaped with strings pulling branches in certain directions. The Decis fruit fly trap was being used on this farm but perhaps just for demonstration purposes. Red scale, aphids and red mite are controlled by sprays. The Instituto Valenciano de Investigaciones Agrarias (IVIA) in Moncada was visited. This is the Spanish equivalent to the ARC and holds the IVIA Germplasm Bank (IGB). This bank was initiated in 1975 with the main objective of maintaining the highest variability of *Citrus* and related genera of the Aurantioideae subfamily. Presently the bank comprises 620 accessions (330 of them selected in Spain and 290 from foreign countries). It includes 425 genotypes belonging to 51 species of *Citrus*, 53 genotypes of 44 species of 20 Citrus relatives, and 142 intra- and inter-specific hybrid genotypes. There was an impressive variety block with a wide range of *Citrus* species which we walked through, and many screenhouses. Not far from IVIA an old farm Finca Campo Anibal was visited where we had a traditional paella for lunch.

Post-congress unofficial IPM tour

Vaughan Hattingh, Tim Grout and Sean Moore participated in this tailor-made three-day tour, organised by Tim Grout. On Saturday, we visited IVIA, hosted by Alberto Urbaneja and accompanied by Phil Stansly (Fig. 7.1.19). We were given a tour of the facilities, including observing cultures of *Nesidiocoris tenuis* (a mirid bug) and *Amblyseius swirskii*. These are generalist predators, which can solve a number of the pest problems experienced on tomatoes, including *Tuta absoluta*. However, if there is no prey present, then *N. tenuis* can damage the tomato plant. In order to prevent this, a selective insecticide (e.g. indoxacarb) is sprayed to temporarily suppress *N. tenuis*, which will recover on its own after a period. They also had cultures of the red palm weevil *Rhynchophorus ferrugineus* that is killing *Phoenix* spp. of palms in Spain. This is a large red weevil that was imported with palm trees from Egypt in the 1990s. We were also taken to visit orchards where woolly whitefly is a problem. The presence and effectiveness of the parasitoid, *Cales noacki*, was conspicuous.



Figure 7.1.19. Vaughan Hattingh, Sean Moore, Alberto Urbaneja, Phil Stansly and Tim Grout at IVIA where mirids are being used to control *Tuta absoluta*.

On Sunday we were hosted by Ferran Garcia-Marí, head of the Applied Entomology unit at the Mediterranean Agroforestry Institute of the Polytechnic University of Valencia. We were also accompanied by two of his students. We visited a young orchard in bloom that was infested with *Pezothrips kellyanus*. This pest is particularly problematic where it can survive on other flowering hosts adjacent to the citrus. We visited a few orchards where we saw a fairly heavy infestation of *Delotococcus aberiae* (previously *elizabethae*). The Spaniards claimed that this species was recently introduced from South Africa. We refuted this, as we never see this sort of infestation and accompanying deformation (Fig. 7.1.20) on citrus in South Africa. We also observed female Mediterranean fruit fly on fruit in an orchard and a couple of predatory fly species, *Platypalus* and *Coenosia* (Fig. 7.1.21). Ferran told us that he is often contacted to identify pest insects found in imported agricultural produce, including citrus from South Africa. He confirmed having positively identified FCM larvae on several occasions. He reported that *Ecdytolopha aurantiana* from Argentina is another major cryptic lepidopteran phytosanitary pest.



Figure 7.1.20. *Delottococcus aberiae* and the deformation it causes in Spain (yellow animals are tydeid mites not mealybug crawlers)



Figure 7.1.21. Ovipositing fruit fly (top), predatory fly (centre) and decaying fruit.

On Monday we visited the Spanish Mediterranean fruit fly SIT facilities (Tragsa) – rearing and release. We were hosted by Rafael Argilés. The rearing facility is 80 km outside of Valencia, in the village of Caudete de las Fuentes. The genetic strain of fly produced is the Vienna 7, which is ts1 (temperature sensitive) and wp (white pupae). The larval diet is 60% water and uses sugar beet pellets (as a bulking agent), sugar (12%), Brewer’s yeast (40%) and preservatives (sodium benzoate, HCl and Nipagin) and has a pH of 3. Larval development takes 6 days, followed by 5 days in the “jumping” room. 50 000 larvae are produced per tray. 20% of eggs laid remain in the mother culture. The remaining 80% is bulked up through 5 generations before being sterilised. Each generation experiences a 10-fold growth on the previous. After the 5th generation, eggs are incubated in water at 34°C, which kills the females. Irradiation is conducted under apoxia i.e. in a vacuum bag, as this reduces damage to the flies. The irradiation source is cobalt, which is extremely slow. Therefore irradiation is conducted at a nearby unrelated company, which has an electron-

beam irradiator (Beta irradiation), which is much faster. The normal irradiation dose is 50 KGy. The release facility is at IVIA in Valencia. 10 million flies are released per day – twice a week in all areas. Releases are conducted over 160 000 ha – mainly citrus. Flies are stored at 4°C and released at 12°C (Fig. 7.1.22). Flies are released by fixed-wing aircraft at a height of 300 m. A report on a computerised variable release rate technology, authored by Briasco *et al.* was obtained and forwarded to Xsit.



Figure 7.1.22. Refrigerated, irradiated Medfly adults ready for release

TITLES OF ORAL PRESENTATIONS AND POSTERS FUNDED BY CRI

Assessment of retention and persistence of copper fungicides on orange fruit and leaves using fluorometry and copper residue analyses. Schutte G.C., Kotze C., Van Zyl J.G., and Fourie P.H.

Association and interaction of edaphic factors with root disease related citrus decline. Pretorius M.C., Labuschagne N., Kotze C., and McLeod A.

Can imidacloprid cause lepidopteran pest repercussions? by Sean Moore, Rachel van der Walt, Wayne Kirkman and Derek Du Preez.

Citrus water use in South Africa. Vahrmeijer J.T., Annandale J.G., Gush M.B., and Taylor N.J.

Could ethylene influence peteca spot incidence of lemon fruit? Cronjé P.J.R.

Curative and protective control of *Penicillium digitatum* following imazalil application in aqueous dip and wax coating. Njombolwana N.S., Erasmus A., and Fourie P.H.

Influence of light on carotenoid accumulation in Star Ruby grapefruit. Lado Lindner J., Lado Lindner J., Cronje P.J.R., Rodrigo M.J., and Zacarías L.

Modelling of *Guignardia pseudothecium* maturation and ascospore dispersal in citrus orchards. Fourie P.H., Schutte G.C., Serfontein S., and Swart S.H.

Population Genetics of *Guignardia citricarpa* in South Africa. Carstens E., Linde C.C., Slabber R., Langenhoven S., Schutte G.C., Fourie P.H., and McLeod A.

Practical impact of imazalil resistance on control of postharvest citrus green and blue mould. Erasmus A., Rikhotso V., Lesar K.H., Lennox C.L., and Fourie P.H.

Silicon uptake in citrus and the validation of an analytical method. Vahrmeijer J.T., Asanzi N.M., and Taylor N.J.

Spray deposition benchmarks for control of *Alternaria* brown spot and evaluation of adjuvants to improve fungicide spray deposition in citrus orchards. Van Zyl J.G., Schutte G.C., and Fourie P.H.

The importance of research and technical services in the recent growth of the Southern African citrus export industry. Hattingh V.

The status of citrus IPM in South Africa. Grout T.G.

7.2 S.D. MOORE

7.2.1 REPORT ON VISIT TO ITALY AND FRANCE – 12-17 NOVEMBER, 2012

Introduction

This visit preceded a visit to Spain, to participate in the International Citrus Congress, and took place from 12-17 November 2012 (in Spain from 17-27 November). I was invited to participate in a Du Pont seminar in Bolzano Italy, entitled “Lepidoptera on Top Fruit in Europe, Middle East and Africa - Current status and new threats”. This coincided with the Interpoma Fair in Bolzano. En route from Bolzano to Valencia, a day visit was made to Sylvan Bio in Loches, France.

Itinerary

Date/s	Destination	Institution/venue	Activity	Mode of travel
12-13 November	Verona	-	Travel via Johannesburg & Paris	Air
13-15 November	Bolzano	Du Pont Symposium & Interpoma	Participate in symposium & visit Interpoma	Road
15 November	Paris	-	-	Air
16 November	Loches	Sylvan Bio	Visit EPF production facility & discuss production of CRI isolates	Train
16 November	Paris	-	-	Train
17 November	Valencia	-	-	Air
SPAIN TRIP REPORT INCLUDED IN A JOINT REPORT BY ALL CRI PERSONELL WHO PARTICIPATED IN THE ICC MEETING				
26-27 November	Port Elizabeth	-	Travel via Paris & Johannesburg	Air

Purpose of trip

1. To attend and participate in a Du Pont organised symposium in Bolzano, Italy, on invitation by and at the expense of Du Pont.
2. To visit Sylvan Bio in Loches, France, to observe and learn about their production of entomopathogenic fungi (EPF) and to arrange details for their production of CRI/Rhodes University's EPF isolates.

Trip details

BOLZANO, ITALY: DU PONT SEMINAR

Du Pont seminar: Lepidoptera on Top Fruit in Europe, Middle East and Africa - Current status and new threats

The following presentations were made, with some notes recorded. Note the presentation which I made in session 3.

Session 1: Introduction

Changes in Lepidoptera pest expansion, related problems and solution strategies – a global overview: John Andaloro, DuPont

Amongst the diamides, Du Pont and Syngenta have chlorantranilipole (anthranilic diamide), however, Syngenta combines theirs with either thiamethoxam, lambda cyhalothrin or abamectin. Bayer and Nihon Noyaku have flubendiamide (phthalic diamide). By 2020, Du Pont and Bayer will each have 5 diamide products, Nihon Noyaku will have 4 and Syngenta will have 8. Resistance already occurs in localised areas! No tolerance yet seen on tree fruit pests. World-wide recommendation is that a diamide should not be used against successive generations (including codling moth). I pointed out to Dr Andaloro that the SA registration for FCM directly recommended the opposite. He was unaware of this.

How Rynaxypyr® has changed the Lepidoptera control strategy in Top Fruit growing: Andrea Bassi, DuPont

Trials conducted 2-4 years after the introduction of Rynaxypyr for control of codling moth (CM) on apples. Two applications of Rynaxypyr, 21 days apart, reduced CM damage on apples by 76-86%. Five applications, 10 days apart, reduced CM damage by 86-92%. Five applications of the standard (unnamed) reduced CM damage by only 43-48%. Also effective against *Panonychus ulmi*, *Tetranychus urticae* and woolly apple aphid. Rynaxypyr shown to be softer than carbaryl, spinetoram, thiacloprid and other neonicotinoids against *Aphelinus mali*.

Requirements of the Top Fruit industry regarding fruit production and fruit quality: Luca Granata, Melinda consortium, Italy

On average, 500 million tons of fruit are harvested per annum worldwide. This can be broken down as follows: apples – 13%, other deciduous – 12%, grapes – 13%, oranges – 13%, other citrus – 10%, bananas – 16%, other tropical – 22%, berries – 1%. 42% of the world's apples are produced in China; 17% in the rest of Asia; 16% in the EU; 7% elsewhere in Europe; and only 3% in Africa. The biggest producer in Europe is Poland (24%), followed by Italy (22%) and France (16%). The main phytosanitary issue experienced in apples in northern Italy is scab. Insects and mites cause negligible problems. Germany used to be the main export destination for Italian fruit. However, German retailers are very demanding and do not pay well. Italy is therefore now sending far more exports to Africa. I later reviewed South African apple exports and noted a similar trend i.e. reduction in European exports and an increase in African exports.

Session 2: Biology and Control Strategies of Important Lepidoptera

Cydia molesta on pome fruits in the United States: Larry Hull, Pennsylvania State Univ., USA

OFM problems started in the USA in 1998. In the last few years it has caused greater losses than codling moth. Probable resistance to OPs and carbamates and new selective insecticides (e.g. tebufenozide and methoxyfenozide) are less effective. Interestingly – differentiate between CM and OFM based on absence/presence of anal comb, in the same way we do with FCM and carob moth. Host plant has a significant effect on biological parameters e.g. pupal weight, longevity, oviposition period, fecundity. Have developed an OFM egg-hatch model, which helps with spray timing. Are very active flyers, potentially dispersing far. Females fly 2-4 times further than males! Unmated females fly twice as far. Mated males fly further than unmated males. Rynaxypyr – similar initial efficacy to Acetamiprid and Spinetoram (all better than Flubendiamide). However, Rynaxypyr far more persistent – noted at 21 and 29 days after application. Acetamiprid and Spinetoram expressed significant residual activity against CM egg hatch for up to 7 days; only fresh residue of Rynaxypyr (day 1) provided some ovicidal activity; Rynaxypyr and Spinetoram provided direct mortality of CM and OFM males and females with all three routes of exposure; Rynaxypyr and Spinetoram reduced the fecundity and fertility of CM and OFM females exposed to the product (sublethal effects); OFM adults are more sensitive to direct and sublethal effects of Rynaxypyr and Spinetoram than CM.

Key leaf rollers on pome fruits in Poland: Remigiusz Olszak, Institute of Applied Pomology, Poland

18 species of Tortricids in Polish fruit orchards. Only 3-5 species are economically important: *Adoxophyes orana*, *Pandemis heperana*, *Spilonota ocellana*, *Hedya nubiferana*, *Archips rosanus*. The main insecticides recommended: Spin Tor (spinosad), Steward (indoxacarb), Runner (methoxyfenozide) and Coragen (Rynaxypyr).

Effects of the host plant on the survival of larvae of the codling moth *Cydia pomonella*: Herman Helsen, Wageningen UR / Praktijkonderzoek Plant & Omgeving, Netherlands

Two generations per year in Holland. Big difference in survival of CM on apple and pear, and this changes over time. Survival on apples much higher in June, but by late August, survival in pears was higher. This is

related to the relative hardness of the fruit (pear initially much harder), but no formation of stone cell tissue in pear after end July.

Anarsia lineatella and Cydia molesta on stone fruits in Italy: Fabio Molinari, Italy

C. molesta females produce a sex pheromone, which is well studied. Larvae developing in shoots grow faster than those developing in fruit, but are smaller. Natural mortality can be broken down as follows: 5% eggs do not hatch; 50% mortality during wandering around looking for a penetration site; 43-88% during larval development, depending on fruit type; up to 70% looking for a pupation site; 40% during overwintering. Natural enemies are not a significant mortality factor, unlike *A. lineatella* (up to 45% parasitism).

Session 3: New Pest Control Experiences

Psylla spp and Aculus sp: the threat of disease transmission and the concern for fruit quality: Gino Angeli, Plant Protection Department, Trento, Italy

The apple disease, *Candidatus phytoplasma mali*, is vectored by *Cacopsylla picta* and *C. melanoneura*. Multiplies 30-fold in the salivary glands.

Drosophila suzukii is a new threat to Italian fruit production. First invasion observed in autumn 2009. Wide host range but main hosts are berries, grapes and apricots. Also attacks several wild host species. Phosmet, dimethoate and Decis were effective for at least 12 days.

Drosophila spp and other fruit flies and their control: Adriana Escudero-Colomar, IRTA, Spain

For Medfly control, it is usually necessary to use a combination of more than one control measure. Three component lure (FERAG) used for mass trapping of both male and female Medfly (more effective than BioLure). Have not yet determined a threshold for when to spray.

D. suzukii appeared in these European countries in the year listed – Italy and Spain 2008; France 2009; Slovenia 2010; Switzerland, Germany, Austria 2011; Belgium 2012. Medfly attractants ineffective for *D. suzukii*. Use cider vinegar (2.5%) + water, replacing weekly.

On the lookout for appearance of *Bactrocera invadens*.

Drosophila suzukii – evolution of the situation in France: Pascal Borioli, Du Pont, Italy

Most effective of 7 products tested was Cyazypyr.

Zeuzera pyrina, share of control experiences in Israel: Shlomo Glidai, Gadot, Israel

Attacks apple, pear, quince, olive, pomegranate, pecan, loquat. Occurs in Med, Europe, Asia, America, Africa. Coragen more effective than Calypso and teflubenzuron.

False codling moth on citrus and other crops in the South Africa: Sean Moore, Programme Manager: IPM. Citrus Research International, South Africa

Emphasis was placed on the extensive research efforts being made, the multitude of effective control options available, and the poor dispersal capacity of FCM, thus negligible risk for any citrus importing country.

Session 4: Implications of the New EU Laws on Top Fruit Production

EU directive on Sustainable Use of crop protection products, Integrated Pest Management and the zonal regulatory frame work - implications on product registration and biological data generation:

Andreas Huber/ Stefan Peterka, DuPont.

Update on tools to control lepidopteran pests in Integrated Pest Management systems in top fruit (including semiochemicals, pheromones, and new chemicals): Fabio Molinari, Italy

There has been a dramatic reduction in the use of chemical pesticides in Italy. Integrated production is now a prerequisite for the large-scale retail trade. The next IPM General Configuration in Europe is scheduled for January 2014. IPM implementation will become mandatory, without grant aid. EU grant aid will only be available for voluntary advanced IPM implementation. A table of retailers and extra-regulatory residue requirements was presented.

SESSION 5: ROUND TABLE: Sustainable Use Directive, IPM and its implementation in top fruit (monitoring, resistance management, crop protection products, etc.): Francesc Miret (Spain), Tiziano Galassi (Italy), Tim Belien (Belgium). Tbc (if it makes sense) an IPM specialist from Poland, Germany, France and / or the Top Fruit industry. Facilitator: Verena Rappaport

Interested parties can contact me for more information on the presentations or copies of presentations.

Interpoma

Visited Interpoma Fair – similar to Fruit Logistica, but smaller and specifically for the apple industry.

LOCHES, FRANCE: SYLVAN BIO

I was met and hosted by Olivier Potin, Project Manager Process Development. He gave me a guided tour through the Sylvan Bio production facility in Loches. He was very open with showing me the equipment and processes and in sharing information with me, bar a few proprietary steps. We also spent some time discussing CRI's requirements for EPF production. Olivier said that Sylvan Bio would conduct a series of tests in order to determine the appropriate production protocols for the three EPF isolates which we had contractually agreed we would send to them. Olivier said that the test process should take approximately 6-10 weeks. Thereafter we can discuss production requirements in detail and associated costs. Production of the required amount of spores may take as short as six weeks.

Detailed notes were made of the production process, however, these are not included in this report in order to avoid dissemination of any information which Sylvan Bio would prefer to keep confidential. However, it can be mentioned that the attention to hygiene and sterility was impressive. The product which will be supplied to CRI can be:

1. Extracted fungal spores, or
2. The substrate – without spores extracted – can be finely ground for spraying, or
3. The substrate after extraction of spores (the vast majority) – for soil application at approximately 10 kg/ha.

There are approximately 10^{10} to 10^{11} spores per gram. Therefore if 10^{13} spores are required per ha, we will need 100 g to 1 kg of spores per ha.

Value and summary of visit

Neither the visit to Italy nor France were the primary purpose of the trip to Europe (the primary purpose was to participate in the International Citrus Congress – see CRI combined report), however both legs of the trip were very valuable. Although the focus at the Du Pont seminar was on apples, it was very valuable for me to learn about their pests, their management, the products and techniques used to control them. Much was learned about Rynaxypyr (Coragen), newly registered for FCM on citrus in South Africa and other diamides. Additionally, we can take note of new invasive pests in Europe, such as *Drosophila suzukii* and be forewarned of their invasive ability. It was also valuable to communicate the extensive research being done on FCM in South Africa, the effective control measures being applied against the pest and its poor invasiveness.

The visit to Sylvan Bio was equally valuable. I have confidence that we have entered into a partnership with a reputable and competent company to mass produce our locally isolated entomopathogenic fungi (EPFs) for large scale field trials. I also gained a first-hand idea of production requirements for EPFs.

Follow-up actions to be taken

1. Revitalise, plate and courier selected EPF isolates to Sylvan Bio.
2. Communicate production criteria to students at Rhodes University working on EPFs.

Acknowledgements

Citrus Research International is thanked for authorising and funding the trip. Du Pont is thanked for inviting me to Italy and for sponsoring this leg of the trip.

7.3 ARUNA MANRAKHAN

7.3.1 Report on IAEA Expert Mission, Madagascar 3-7 September 2012

Project: To design an action plan to detect and suppress *Bactrocera invadens* in Madagascar (IAEA PROJECT MAG5021)

Objectives of mission

The specific objective of the assignment was to design an action plan to detect and suppress *B. invadens* in Madagascar.

The duties for the assignment were to:

1. Revise the actual status of *B. invadens* in Madagascar.
2. Develop an action plan that involves the surveillance and suppression responses to *B. invadens*.

Bactrocera invadens is an invasive fruit fly pest of Asian origin which was first detected in Africa (in Kenya) in 2003. This invasive pest was subsequently found present in several other countries on the African continent (East, West, some Southern countries) and in two Indian Ocean islands (Comores and Madagascar). *Bactrocera invadens* was found to cause considerable damage to many commercial fruit crops such as mango, citrus and banana in countries where it occurs and is also a pest of phytosanitary concern.

In Madagascar, the pest was found for the first time in December 2010 in Antananarivo (Central) and Tamatave (East). The pest poses a threat to fruit production in the island. Commercial fruit in Madagascar are mainly produced for local trade and consumption except for litchi which is also produced for export mainly to the European Union.

Status of *B. invadens* in Madagascar

In a poster presented by Raelijaona et al. (2012) during the second symposium of the Tephritid workers of Africa, Europe and the Middle East in Crete, Greece, between 3rd and 6th July 2012, it was stated that "*B. invadens* is now considered established in Madagascar. It already shows a wide distribution throughout the country". Currently in Madagascar, monitoring of *B. invadens* is being carried out in 6 locations (Central: Antsirabe, Antananarivo; East: Farafangana, Tamatave; North-West: Mahajanga; North: Antahala). Out of these locations, *B. invadens* was not found present to date in 1 of the locations- in Antahala (in the north). However in Antahala very few traps (2 methyl eugenol and 2 Biolure 3 component baited traps) have been placed since January 2012 and the trapping network in that region is not extensive enough to confirm pest absence. As such, no revision of the status was deemed necessary at this stage. However to date, there has been no official notification of the presence of *B. invadens* in Madagascar on the International Plant Protection and this has to be carried out.

Weekly trapping data from Antananarivo (Central) since December 2011 indicate that peaks of *B. invadens* population (up to 30 flies per trap per day) occur between January and February. Fortnightly trapping data from Mahajanga (North-West) since October 2011 indicate that peaks of *B. invadens* population (up to 20 flies per trap per day) occur between October and November. The high numbers of *B. invadens* caught during some parts of the year indicate that control actions are necessary in those areas.

The presence of *B. invadens* in Madagascar is possibly recent. Between 2005 and 2007, following a report of the pest in Comores, traps baited with methyl eugenol, a powerful attractant, to which *B. invadens* males responds to, were placed in Mahajanga- in the north west of Madagascar- and no *B. invadens* was recorded there during that time (C. Raelijaona, pers comm.).

In Madagascar, there are three other fruit fly pests of main economic importance: *Ceratitis malgassa* (widely distributed across the country), *Ceratitis cosyra* (in the north-west, north and central parts of Madagascar), and *Dacus demmerezi*. *Ceratitis malgassa* is endemic and is a pest of quarantine importance affecting citrus, deciduous fruit and guava. *Ceratitis cosyra* is a pest of mango in the west and north-west parts of the island. *Dacus demmerezi* is also endemic to the island and is a pest of cucurbit crops. *Ceratitis capitata*, a notorious fruit fly pest in many parts of the world, is also present in the island but it seems to have a limited distribution on the island, only occurring in the east of the country. *B. invadens* was found to occur in sympatry with existing pest species in areas where it was found and thus management actions that target the exotic pest should also in part at least target the three other existing pest species.

Develop an action plan that involves the surveillance and suppression responses to *B. invadens*

Surveillance

Adult trapping. Monitoring through adult trapping must continue in the existing sites and must be expanded in the northern, eastern and central parts of Madagascar. In the northern part of Madagascar, the expansion of monitoring will mainly aim to determine pest presence/absence. Two regions should be selected in the north for placement of additional traps: Sava and Diana. In each region, trapping sites will be selected based on availability of personnel for trap placement and servicing. In Sava, traps should continue to be maintained in Antahala and should be additionally placed in Sambave and Andapa. In Diana, traps should be placed at Diego Suarez and Nosy Be. In the eastern part of Madagascar, the aim of an increased monitoring will be to determine extent of spread of the pest and population levels. The eastern region of Madagascar produces litchi for export. Information on pest distribution and population level might be required by export markets despite that litchi is currently not listed as a host for *B. invadens*. For the delimiting survey in the east, traps should be placed along a transect from Tamatave to Fort Dauphin at locations where regional plant protection services are available for trap servicing. The following locations should be included: Tamatave, Vatomaniry, Mahanoro, Mananjary, Manakara, Farafagana, Vangaindrano and Fort Dauphin. There is currently no information with regards to *B. invadens* presence and distribution in the central parts of Madagascar which are below and above the capital Antananarivo, in the south of Antsirabe (Ambositra) and in Ambatondrazaka respectively. The south of Antsirabe is an important deciduous and mango production area. Ambatondrazaka is an important mango and citrus production area. Fruit fly monitoring has previously been carried out in Ambatondrazaka and *C. malgassa* was found to be present there. In all new and existing monitoring sites, there must be at least 2 methyl eugenol baited traps. These traps must be checked on a weekly to fortnightly basis throughout the year for at least 2 years in order to obtain information on the presence of the pest and if present, on the annual and seasonal abundance of the pest. Information on abundance of the pest will be important for timing of appropriate control efforts.

Host sampling. The aim of host sampling will be to establish a complete list of host fruit- both commercial and wild- for *B. invadens* in Madagascar. This information is important for determining control actions in various fruit types. At all new and existing sites where traps have been placed, a list of host present near the traps should be made and should be sampled in different regions at the time of availability of ripe fruit.

Suppression actions

The proposed package for suppression of *B. invadens* is a combination of bait application technique, male annihilation technique, orchard sanitation and biological control using the solitary hymenopteran ovo-pupal endoparasitoid -*Fopius arisanus*. This package should be tested in pilot suppression programmes over 2 years with the biological control component phased in only in the second year. Inclusion of the biological control component will take time as training of the DPV team and the necessary facilities will be required for setting up *B. invadens* and *F. arisanus* cultures. The DPV team could receive cultures of *F. arisanus* from Reunion Island and training on rearing techniques from CIRAD, Reunion.

For suppression, bait application technique should be applied only at the time of fruit ripening for a period of about 8 weeks. Bait application technique could be either in the form of sprays or stations. During baiting, all host plants in selected plantations should be covered. Baits which could be used for sprays include (i) GF-120 at the recommended rate and application methods on the label (1 L of GF-120: 4 L water), (ii) mixture of protein hydrolysate (e.g NuLure) and malathion EC, with protein hydrolysate used at the rate of 400 ml per 100 L of water and malathion EC used at the rate of 175 ml per 100 L of water. Bait sprays should be applied weekly on a limited area of the canopy of each tree. For GF-120, 10 ml of the mixture could be applied per tree on every tree and for the protein hydrolysate and malathion mixture, approximately 100 ml could be applied per tree. These sprays should be applied with knapsack sprayers. Various bait stations have been developed over the years in different parts of the world and could also be used. The M3 bait stations have been used for fruit fly control in South Africa and found to be effective on *B. invadens* in Kenya. M3 bait stations can be deployed at the rate of 300-400 stations per ha. It would be important to test various types of bait applications which could suit different growers both in terms of logistics available for bait application and affordability of these baits. Some insecticides such as malathion and spinosad are already registered for fruit fly control in Madagascar. Malathion (Callimal 50 EC, Calliope SA) is registered for control of fruit flies on fruit trees at the rate of 1.5-2.5 L/ha. Spinosad (Laser 480 SC, Agricom) is registered for control of *Bactrocera* sp. on cucurbits in Madagascar at the rate of 100 ml/ha.

The Male Annihilation Technique (MAT) component in the package should be maintained throughout the year to keep population levels low at all times. MAT will involve the distribution of square (5 cm x 5 cm) 1.3 cm thick fibre-board/soft board blocks soaked in a mixture of methyl eugenol and malathion EC (500g/L) or malathion UL (1130 g/L) at a ratio 3:1, methyl eugenol to malathion respectively. MAT blocks should be deployed at the rate of 400 blocks per km² and should be replaced every 12 weeks.

Cultural control methods should be incorporated in the program to further increase efficacy of control. During fruit availability, dropped fruit and fruit left over after harvest should be removed from the orchard. Fruit removed from the orchard should be properly disposed of by placement either in black plastic bags in the sun (although this might be not adequate to contain other lepidopteran fruit pests) or under an augmentorium which is a tent like structure to keep fruit. The tent like structure consists of a netting material with mesh size adequate for keeping fruit flies bred from infested fruit inside the tent and at the same time large enough to allow the exit of smaller fruit fly parasitoids.

The aim of incorporating biological control in the suppression programme in the second year is to target wild hosts which could be breeding grounds for *B. invadens* outside of commercial fruit plantations.

Pilot *B. invadens* suppression programmes should be conducted in three locations in Madagascar: Antananarivo (Central), Mahajanga (North West) and Tamatave (East). At each location, 3 sites should be selected. In each site, the types of bait application could vary. The sites selected could be commercial fruit plantations and prior agreement for conduct of suppression programmes should be sought with the owners of the plantations. The programmes would serve as an evaluation of the control methods and as on field demonstration for training of extension officers and growers. The training would ensure sustainability of *B. invadens* control in fruit production areas in Madagascar. Suppression programmes would include control methods and fruit fly monitoring through adult trapping and fruit infestation survey. Adult trapping and fruit infestation surveys should commence at least 1 month before start of suppression programmes and should continue throughout the year in order to determine treatment effects. Pilot suppression programmes could be implemented in the selected locations by October 2012 to coincide with the mango and litchi fruiting season in particular in Mahajanga and Tamatave.

In this mission, an action plan for surveillance and suppression of *B. invadens* in Madagascar was put in place. The plan was tailor made to fit the current capacity of the DPV team and the facilities available in Madagascar.

7.4 T.G. GROUT

7.4.1 INTERNATIONAL CONFERENCE ON PESTICIDAL PLANTS, NAIROBI, KENYA 21-23 JANUARY 2013

SUMMARY

Due to the need to find products that can be used against soft bodied insects such as thrips, woolly whitefly and leafhoppers, shortly before harvest, I attended the first international conference on pesticidal plants held at ICIPE in Nairobi. Several international speakers were there and good contacts were made with researchers in Canada, United Kingdom, India and Italy. Fairly detailed notes were taken during three full days of presentations. The quality of the research ranged from trial-and-error herbalist experiments to genetic/molecular/biochemical investigations. Several papers were presented on Intellectual Property issues and it is clear that after the court cases involving *Hoodia gordonii* and CSIR/Unilever/Pfizer, few companies are interested in dealing with South Africa on any indigenous plant products. Some plants such as the weed *Tephrosia vogelli*, which contains several rotenoids, featured in many talks but with most countries in the world not having an MRL for rotenone it is unlikely that this will be acceptable late in the season. Often plants that are being used medicinally with water as a solvent can be insecticidal when a more effective solvent is used or just when a surfactant is added to the water in the extraction process. Different chemotypes sometimes exist where plants that look identical contain different chemicals, so a single source of a plant should not be used in trials. Research should include commercial botanical products as contact insecticides and perhaps locally-manufactured products that could later be commercialised. Email addresses of the speakers and an abstract book are available.

INTERNASIONALE KONFERENSIE OOR PLAAGDODENDE PLANTE, NAIROBI, KENIA 21-23 JANUARIE 2013

OPSOMMING

As gevolg van die behoefte om produkte te vind wat teen "soft bodied insects" soos trioziids, blaaspootjies, wollerige witvlieg en blaarspringers, kort voor oes, gebruik kan word, het ek die eerste internasionale konferensie oor plaagdodende plante wat by ICIPE in Nairobi gehou is, bygewoon. Daar was verskeie internasionale sprekers en goeie kontakte is met navorsers in Kanada, die Verenigde Koninkryk, Indië en Italië gemaak. Redelik volledige notas is gedurende drie vol dae van aanbiedings geneem. Die gehalte van

die navorsing het gewissel van “trial-and-error” kruiedokter eksperimente tot geneties/molekulêre/biochemiese ondersoeke. Verskeie referate is oor Intellektuele Eiendomskwessies aangebied en dit is duidelik dat ná die hofsake van *Hoodia gordonii* en WNNR/Unilever/Pfizer, net 'n paar maatskappye belangstel om met Suid-Afrika oor enige inheemse plantprodukte te onderhandel. Sommige plante soos die onkruid *Tephrosia vogelli*, wat verskeie rotenoïedes bevat, was deel van baie praatjies, maar met die meeste lande in die wêreld wat nie 'n MRL vir rotenone het nie, is dit onwaarskynlik dat hierdie láát in die seisoen, aanvaarbaar sal wees. Dikwels is plante wat medisinaal gebruik word, met water as 'n oplosmiddel, insekdodend wanneer 'n meer effektiewe oplosmiddel gebruik word, of slegs wanneer 'n plante wat identies lyk, verskillende chemikalieë bevat, so 'n enkele bron van 'n plant moet nie in proewe gebruik word nie. Navorsing moet kommersiële botaniese produkte insluit soos kontak-insekdoders en moontlik plaaslik-vervaardigde produkte wat later gekommersialiseer kan word. E-pos adresse van die sprekers en 'n abstrakboek is beskikbaar.

Notes on presentations

Josphat Matasyoh – local organiser/MC.

Joshua Ogendo – Introduction and thanks

Phil Stevenson – international organiser and principle investigator.

African Dryland Alliance for Pesticidal Plant Technologies (ADAPPT) EU funding with Univ of Greenwich and African partners through African-Caribbean Programme.

Focus currently on health of environment, plants, humans and animals at ICIPE with emphasis on role of insects in each.

Murray Isman – Harnessing pesticidal plant technologies: An African success story in the making?

USA created “reduced risk” insecticide category which includes microbials (and derivatives such as spinosyns), IGRs, botanicals and some newer chemistries e.g. neonicotinoids, fipronil and indoxacarb. Most plant secondary compounds have evolved as defence mechanisms. Perhaps 100 000 or more of these, and hundreds have been shown in the lab to be active against insects. Bioactivity can be behavioural (repel or prevent oviposition) or physiological. Most existing botanical insecticides are neurotoxins or muscle poisons. Conventional chemical screening focused on acute toxicity but insect-plant chemical interactions are more subtle. Long-established botanicals include pyrethrum, rotenone and nicotine. Recently introduced botanicals include neem and various essential oils (*Eugenia*, *Cymbopogon*, *Thymus*, *Cinnamomum*, *Citrus*). Pyrethrum is from *Tanacetum cinerariaefolium*, now half of world production in Tasmania. Drawback is being very labile in sunlight. Neem has 2 major limonoids and mainly has IGR effects and antifeedant properties. Very low mammalian toxicity but quite photolabile so some formulations have a sunscreen. Essential oils include monoterpenes and phenols. Common essential oils from rosemary, cloves, cinnamon, lemon grass, mint and thyme. EPA List 25B Exempted active ingredients (1996) includes several of these and don't need registration. They may therefore not be very effective but easy and cheap to use. Have rapid mode of action due to membrane disruptors, octopamine antagonists and at lower dosages can be repellent. Especially effective against soft bodied insects, sucking insects and mites. Don't break down in sunlight but are volatile so do not last long e.g. thyme oil which has rapid action on caterpillars. EcoSmart Technologies (Isman's company) sells products like this. Some for animal use for fleas and ticks. Brandt sells Ecotec with rosemary oil and peppermint oil for use in agriculture. Invincio makes products for use in homes. 1% rosemary oil is a good repellent to *T. urticae*. Ecotec compared with spinetoram. Ecotec worked better against aphids than thrips but the combination with spinetoram at lower dosages of each worked better against WFT than higher rates of spinetoram alone. California produces more than 50% of organic fruit and vegetables in USA. Botanical usage in 2003 was 37 439 lbs a.i. and in 2009 3X more. But botanicals still only 0,06% of all pesticides used in USA. Only 8.4% of biopesticides in USA are botanicals. Availability and sustainability of plants to produce products is often a problem. Also needing to prove the safety of the products is expensive for small companies. Requiem is a recent success story from *Chenopodium ambrosioides*. Bayer CropScience bought developer Agraquest in July 2012. Ecoflora in South America. In China use *Melia azedarach* (syringe) extracts that not very effective on their own but combined with nicotine work well. Two paths of development: industrialised countries and developing countries. Latter countries often with little pesticide regulation and lower efficacy still considered valuable. Custard apple (*Annona* spp.) seeds have very effective insecticides. In Brazil and Thailand unregulated use of plant extracts is permitted. *Tephrosia vogelli*, *Tagetes minuta*, *Eucalyptus* spp. produce effective insecticides. Need less research on finding new botanicals than on the preparation and use of known plants. Many patents on plant materials that have been used for years will be difficult to defend. Thymol used in beehives to control *Varroa* mite. It will be valuable to find out about negative effects of these products on pollinators. High rate of volatilisation allows timing of sprays when little pollinator activity. Improved technology for detecting pesticides also applies to botanicals. Very common that medicinal plants also make good insecticides so low LD50s in rats

will not be well accepted in agriculture. However, nicotine and rotenone have very low LD50s but are used at very low dosages in the field. Probably large scope to investigate toxins in fungi but taxonomy is complicated. CAB abstracts shows an explosive growth in botanical insecticides but most do not have chemical characterisation of extracts or do not have controls, so much is of little use.

Baldwyn Torto – Exploiting plant volatile semiochemicals in the control of pests and disease vectors.

Research at ICIPE concerning food security. Those suffering from chronic hunger were 239 M in sub-Saharan Africa out of 925 M worldwide in 2010. Mosquitoes need energy from plants to find hosts so plant volatiles can play a role. Mosquitoes like visiting *Ricinus communis* and *Tagetes pilosa*. Plant volatiles sometimes more attractive than human odour but CO₂ increases attraction. Collaborating with Rothamsted: John Pickett working with Zeyaur Khan and the “push and pull” approach. Napier grass used as a pull plant as more attractive than maize but larvae cannot develop properly on it. The legume *Desmodium* is used as a push plant between the rows of maize. However, *Desmodium* is not popular as it is not a food legume and is only used for fodder. In the USA, non-host birch trees inhibit pheromone response in spruce bark beetle. Work with the chemicals as repellents. EPNs attracted to volatiles produced by citrus roots when damaged by *Diaprepes*. *Maruca* sp. is a major pest of cowpea. Extract of cowpea is required for mating of moths. Could we find the genes to silence the production of these key chemicals? Can combine pheromones with plant volatiles for increased effect. Perhaps could be used for mass-trapping. Antennal responses will not show whether it is attraction or repellence. Push-pull is difficult to use in tree crops because will need other trees to be at the same height as the host.

S1-7 **Akanmu et al.** Botanicals inhibited *Fusarium* pathogens of millet seedlings.

Paper by Lakshman and Dilip 2006 on use of botanicals for *Fusarium*. Subsistence farmers want to use botanicals that can be obtained in their environment. Used mango and *Jatropha curcas* extracts at high concentrations. *J. curcas* gave some promising results at 45% against *F. verticilloides*. Also works as fertilizer at this dosage so improved growth could be due to both effects.

S1-10 **Mudita and Jongomanzi.** Thorn apple – *Datura stramonium* against aphids on rape.

Rape used as relish in food but production constraints due to aphids. Can't afford to buy synthetic chemicals. Heiken 1999 showed that *Datura* could be used as a natural insecticide. Plant leaves were dried and ground. The powder was mixed with one litre of water, stirred and left overnight. Dosages 2-8 g per litre. Got a dosage response. Rape leaf yield increased up to 6 g but 8 g decreased due to scorching of leaves (too much soap). Multiple treatments required. 4 g/L had almost double the yield of 2 g/L rate. 6 g was even better without scorching. Results confirmed by other workers in Namibia in 2008. Impact of sprays on human health were not determined.

S1-11 **Mchingula and Bandason.** Evaluating efficacy of fresh leaf extract *Tephrosia vogelii* with and without soap in the control of aphids, forage thrips and jassids in cowpeas.

Fresh extracts from *Tephrosia vogelii* (common weed) were more effective when dishwashing soap was included in the mixture. Need to work on dosage of soap as sometimes burn. Effect of soap alone also reduced insects. Used 125 ml. *Tephrosia* contains rotenoids whose solubility may increase with soap.

S1-12 **Kosgei et al.** Use of Pymarc in management of bean fly (*Ophiomyia* spp.) on common beans (*Phaseolus vulgaris* L.).

Pymarc (Pyrethrum daisy cake remaining after pyrethrum extraction) was effective in reducing bean fly and improved leaves. Sprinkled as a powder on the new leaves.

S1-15 **Mwatawala et al.** Incorporating a botanical based bait in an integrated pest management programme of fruit flies attacking mangoes in Tanzania.

Control based on monitoring, sanitation and spot application of bait. Local bait containing molasses, brewers waste yeast and crude extracts of *Derris elliptica* as a toxicant tested as a substitute for GF120. Sanitation alone was as effective as GF120 and *Derris* etc for all 3 fruit fly species.

S1-17 **Demeke.** Efficacy of some botanicals on stem borers on sorghum in Ethiopia under field conditions.

As powder in leaf whorl. Reduced damage with several products, almost as good as endosulfan.

S1-18 **Phil Stevenson.** Phytochemical variation: a limiting factor in pesticidal plant applications for small holder farmers in Africa.

Tephrosia vogelii is a legume so can fix nitrogen. Most popular for insect control. Bruchid lays eggs on cowpeas and they get infested. Rotenoids from *Tephrosia vogelii*: Deguelin most abundant and Tephrosin. Another species *T. candida* was found to be a synonym of *T. vogelii* but Tv is pesticidal while Tc was not. Tc did not contain rotenoids. The rotenoids are not very water-soluble and water extraction is not

efficient. If add some soap (5% Tween) it improves extraction and spreading when sprayed. *Tephrosia* pesticide could be commercialized.

S1-19 **S. Okweche et al.** Comparative efficacy of pesticidal plant products and carbofuran in the management of maize stem borers in Nigeria.

Tephrosia vogelii widely used in Africa as is neem. *Gmelina arborea* also used in trial. As effective as carbofuran.

S1-20 **Pofu et al.** A ground leaching technology for application of bio-nematicides from indigenous plants in small holder farming systems of South Africa.

Use fruits of *Cucumis* spp. (spiky cucumbers). Also using rootstock for watermelon to provide resistance. Ground leaching of powdered product on soil around plant. Requires irrigation. Dry *C. myriocarpus* fruit for 72 h at 52°C, then grind up. Material is active for 56 d. Reduced nematodes by 92% in root and 86% in the soil. Almost as good as aldicarb. Did not affect pH of soil but improved EC.

S1-23 **Nampeera et al.** Potential of crude leaf extracts of *Cupressus lusitanica*, *Nicotiana tabacum*, *Azadirachta indica* and *Lantana camara* for control of sweet potato weevil.

Bores inside the stems and damages the tubers. Used most susceptible variety. 2 kg plant leaves pounded with 250 ml water. One kg used per plot and poured at base of plant.

Mexican cedar and tobacco most effective. Neem and Lantana not very effective.

Extra talk: **Bagarama.** Soil organic amendments reduce root-knot nematode infestations in tomato in Tanzania.

Goat manure and bat manure controlled nematodes but not cow manure. Tobacco leaves had similar controlling effect.

S2-P **Steve Belmain.** Optimisation of pesticidal plants for post-harvest systems.

On-farm storage in rural Africa is generally not insect proof. If could store grain for a while it could be sold for a better price. Grain borers mainly rely on aggregation pheromones to find a host that is already infested. The first beetle almost finds the host by chance. Important to replicate lab work in the field. Safety: tirucallicine in *Euphorbia tirucalli*. Ascaridine, nicotine. Everything can be poisonous at the wrong dosage. Paracelsus 1536 said "Poison is in everything and no thing is without poison. The dosage makes it either a poison or a remedy." Sampling bias may lead to wrong conclusions, e.g. wrong time of year, wrong species/chemotype, wrong concentration. *Securidaca longepedunculata* African violet tree has many traditional uses, winged samara like *Tipuana*. Methanol extract gives good control of *Sitrophilus* on maize. But root bark is what is used so it is not sustainable. Has a distinct menthol smell due to methyl salicylate that provides bioactivity. Other bioactive chemicals are saponins which are soluble in water so can be extracted that way. It requires less material but is more labour intensive so is not popular. Usage is therefore not always correlated with best efficacy. In some parts of Africa the same plant does not have saponins and is not toxic. Need to know if polar or non-polar solvents are required. When and where to collect material. Get the taxonomy right. How toxic is acceptable? Think of new application methods, e.g. push-pull technique. Indigenous knowledge is not held equally but needs to be protected to ensure that it is not lost. Need new registration process for low dosages of chemicals that have been used for generations. *Tephrosia* grows rapidly and is easy to grow but others are very slow. Soaps can also bind to active ingredients and may cancel the effect so again you need to know the chemistry.

S2-1 **Sarah Arnold et al.** Intraspecific variation in the response of *Callosobruchus maculatus* to methyl salicylate, a botanically-derived repellent.

Securidaca longepedunculata root powder and methanol extract repel *Sitrophilus zeamais*. Methyl salicylate is responsible for this behaviour. Occurs in many plants. Tried against *C. maculatus* which is dimorphic with one morph being more active than another. Used EthoVision motion-tracking software. Air goes through charcoal filter before going through odour source. At 1 mg/ml females definitely repelled but not males. Active beetles are not repelled as much by methyl salicylate as inactive forms.

S2-2 **Serame et al.** Chemical composition and insecticidal effect of essential oils of some aromatic plants of Burkina Faso.

Lippia multiflora one of plants used for essential oils. Also used as a tea and in traditional medicine. *Cymbopogon schoenanthus*, *Cymbopogon giganteus*, *Laggera aurita* has some healing properties. *Laggera oloptera* has pinene and limonene main components. *Anona senegalensis*, *Ocimum basilicum* for repelling mosquitoes and very repellent to weevils.

S2-3 **Kitonde et al.** Phytochemical and utilization of *Vernonia glabra* in the management of food spoilage and poisoning in Kenya.

Toxins from *Aspergillus niger* can cause kidney failure and liver cancer. *V. glabra* evaluated for its effect against *A. niger*, *S. aureus* and *E. coli*. Leaves and flowers showed most microbicidal activity based on inhibition zones. Could be used to treat food to prevent people from getting sick.

S2-7 Asogwa et al. Evaluation of ethanolic plant extracts for protection of *Cola nitida* against Kola weevils in storage.

Used *Cedrela odorata*, *Khaya*, *Chromolena odorata* and other plant extracts at 4 concentrations. Soaked nuts in extracts. All extracts generally suppressed oviposition and emergence from nuts.

Extra talk **Akhatar et al.** Toxicity and feeding deterrent effects of a pea extract and protein-rich pea flour.

Pea flour was found to be toxic to various stored-product beetle pests. High concentrations would have to be used and it leaves a visible residue and odour. A peptide and soyasaponin had a synergistic effect in extracts from the flower and were much more effective than either alone. Methanol, isopropyl alcohol or ethanol extracts could be used. Lab screening on *Trichoplusia ni* using 0.25% to 2% on leaf disks. Use scanning digitometer to quantify how much of the leaf disks had been eaten. Not seen to be all that active in deterring feeding but extract is much more toxic as contact insecticide than crude powder. Spider mites seem to be more susceptible than *T. ni* as are stored product beetles.

Keynote - **Ahmed Hassanali**. Bioprospecting for phytochemicals or control of vectors of animal and human diseases: building on ethno-practices.

Mosquito repellents for *Anopheles gambiae*: *Corymbia citriodora* burning of leaves gives 51.3 % repellency but thermal fumigation (heating on clay plate) increased to 74%. *Ocimum suave* gave 53% repellency with thermal fumigation. Potted plants like *Lantana camara* caused 33% repellency just in room. Could be taken outside during the day and brought inside in the evening. *Coryza newii* has distinct odour and essential oil gives knockdown of mosquitoes. Monoterpenes perillaldehyde and perillyl alcohol plus geraniol. Being investigated now for fumigation properties. About 8 essential oils have repellency that is greater than DEET but most too volatile. Put in oil base at about 2-5% essential oil and heat from below with candle or paraffin lantern. *Ocimum kilimandcharicum* being developed in postharvest protection of maize and legumes, mosquito repellent and flowers are very attractive to bees. It has about 8% camphor. Now being grown in fields in Kakamega area of Kenya and in Tanzania. Have equipment to produce own oil and products being sold locally. Looking at terpenoids with low volatility for postharvest use and personal repellents, e.g. p-Menthane diol from *Corymbia* is effective for personal protection against mosquitoes. Mentane diol at 8% gave 100% protection after 8 h equivalent to 20% DEET. Product called Mozigone. Found how to convert citronella from grasses to Mentane diol as available in large quantities. Two other sesquiterpenoids with low volatility also being investigated. Tick *Rhipicephalus appendiculatus* (brown ear tick) is vector of East Coast Fever and kills calves. *Tagetes minuta* and *Tithonia diversifolia* used to treat whole animal but only lasts 24 h. Anal odour repels ticks towards head and ear odour attracts ticks. Evaluated essential oils of above plants at 10% in Vaseline in ears and got significant repellency for 2 weeks. Using push-pull techniques on each cow with possible trap with acaricide. Using similar approach for tsetse with odours from hosts and non-hosts.

S2-24 Mwangi et al. Activity of extractives from *Albizia anthelmintica* and *Teclea trichocarpa* as biorational alternatives to control the maize weevil (*Sitophilus zeamais*)

Albizia used as grain protectant. *Teclea* bark antifeedant for *Spodoptera exempta* (African army worm). Extracts from powdered leaves using hexane, chloroform, ethyl acetate and methanol. Brine shrimp used as an indicator of toxicity. Triterpenoids from hexane extract and alkaloids with other solvents. *Teclea* extracts similar activity to Actellic super standard.

S2-25 Ogemah. The feeding deterrence effect of neem oil on the larger grain borer *Prostephanus truncatus* (Horn).

Neem oil used at 0.5 to 4%. Measured live weight of beetles after being offered treated maize. First instar more susceptible, 2nd instar only susceptible to 2 and 4%, 3rd instar not susceptible. Feeding deterrence on grains was better than on flour. High rate is effective.

S2-26 Bett. Insecticidal activity of two Kenyan plants against *Tribolium castaneum* and *Acanthoscelides obtectus*.

Lab studies with *Eucalyptus saligna*, *Cupressus lusitanica* against above beetles. Evaluated essential oils as fumigants with up to 20 uL/L air. Better results against *A. obtectus*. Similar results to those others have found with other Lamiaceae spp.

S2-27 Ng'eno. Phytochemical studies of some indigenous plants as grain protectants against *Sitophilus zeamais*.

Investigated *Lippida kituiensis*, *Plectranthus sylvestris* and *Chenopodium chenopoides*. *Plectranthus* leaves contains globulol, caryophyllene. Acetone extracts were placed on half a filter paper which when dry was placed in a petri dish with half of an untreated disk. Then observed where the beetles spent their time. Also mortality tested with grain treated with extracts and effect determined after various periods. *Lippida* was as effective as pyrethrum when used at 1%. Others caused little mortality, including *Ricinus communis*. Camphor, limonene and eugenol considered to contribute most to mortality.

S2-29 **Deng et al.** Toxic and repellent properties of *Cupressus lusitanica* and *Eucalyptus saligna* leaf essential oils against *Callosobruchus chinensis* and *Sitophilus zeamais*.

Fumigation and repellent effects evaluated. *C. chinensis* more susceptible to fumigant at high dosages than other beetle after 7 d evaluation. Only *Eucalyptus* was effective in topical applications. Repellency in petri dishes not convincing for *C. chinensis*. A bit better for *Sitophilus*.

S2-30 **Kenneth Wilson et al.** Can the biofuel crop *Jatropha curcas* be used as a locally

Market for biofuel has collapsed in Zambia so search for alternative uses. Realism versus control in designing experiments is a challenge to get good data. Need to try to conduct research at all levels (lab, field and farm). Green living movement has been doing farm work. Treatments included 2% *Jatropha* powder and 5% *Jatropha* solution as a dip. Dried the leaves over several weeks then crushed into a fine powder. This was mixed with the maize. For solution, soaked with water overnight then dipped maize in mixture, then left to dry in the shade until dry. Then put in bags and stacked with replicates together. Samples taken at monthly intervals after treatment. Powder was acting as a slight attractant. Soaking the seed in water for a minute increased the number of beetles. Wet *Jatropha* treatment was even worse for large number of insects and damage to seeds. Wet seeds were lighter. Perhaps *Jatropha* soln could be used as an attractant in push-pull. Perhaps water is not suitable for extracting the insecticidal components. Not much oil in the dried leaves so not responsible for attraction of treatments.

S3-16 **Murungi.** Chemical composition of essential oil of *Solanum sarrachoides* and its bioactivity against *Tetranychus evansii*.

Solanum sarrachoides known as hairy African nightshade and can be eaten but not preferred due to pubescent leaves. The latter may confer resistance to *T. evansii*. Major components in leaves are camphor, monoterpenes and fatty acids. Made serial dilutions in 5% DMSO in water. Leaf oil effective at 100 ug/ml due to monoterpenes. Fatty acids don't contribute much to mortality.

S3-P **Eustace Kiarii.** Status of organic agriculture and use of pesticidal plants by organic farmers in Kenya. CEO of Kenya Organic Agriculture Network (Sponsor).

Have a system where consumers can order organic fruit and veg in a basket that is delivered directly to their home. *Lantana camara* leaves are being used against weevils as a postharvest treatment.

S3-1 **Saskia Vermeylen.** The poisonous and the sensuous in Chibobo's community lab: Lessons learned from a farmers'-led biopesticidal experiment in Zambia.

Jatropha introduced on a large scale in Zambia about 10 years ago. Asked farmers to keep diaries about farm management. Meetings with extension worker to discuss strategies with growers. Have been using various botanical extracts or powders in their agriculture. Challenge will now be to share new knowledge with others.

S3-3 **Matasyah.** Natural botanical larvicides for the control of the malaria vector *Anopheles gambiae*.

Climate change may result in malaria appearing in parts of Europe. Product Carplarvex is a larvicide. Float a mesh-topped container with larvae and treated water on a water source for trial purposes. Alkaloids from *Zanthoxylum lemairei* as larvicides and *Z. leprierii*. Fungus *Podospora* sp. in *Laggera alata* (Asteraceae) has anthraquinone derivatives that toxic to *Anopheles*. However, some say it is a relative of aflatoxins and cannot be put in water sources.

S3-4 **Munyua and Wagara.** Potential and uncertainties in utilization of pesticidal plants in pest management among small-scale farmers in Nakuru, Kenya.

Some growers use stinging nettle with Omo for blight on tomatoes. *Tagetes minuta* used for spider mites, *Capsicum* spp. for various insects, etc. even though not found to be very effective. Info was not obtained from a reliable source. Trial and error approach to using pesticidal plants. Lack of info on dosages and possible side effects.

S3-5 **Chikwanda et al.** The effectiveness of *Sphenostylis erecta* in controlling blue tick (*Boophilus decoloratus*) infestation.

Sphenostylis erecta is a legume used as food, fish poison and dye. Has a bulbous root that is used as the acaricide. For lab tests put bulb extract in olive oil on filter paper in petri dishes and exposed ticks for

different times. Over 72 h, 10% conc caused 55% mortality and higher mortalities were obtained with higher dosage. 100% conc equivalent to Amitraz and caused 100% mortality after 3 d. Other published work on *Lippia javanica* for use in the same way.

S3-7 Jambo and Magreta. Effects of land ownership on land investments: a case of Malawi.

90% of Malawian population are small-holder farmers. Agriculture is declining due to climate and erosion. Land ownership security has no effect on control approaches used. Need to get farmers to implement known knowledge.

S3-8 Mdangi. Farmers' knowledge and perceptions on the use of pesticidal plants for rodent control in Maize farming systems in Tanzania.

Commercial rodenticides have non-target effects. After drought, rats are biggest threat to grain farmers. Farmers were willing to use pesticidal plants but only 10% are using them.

Keynote Monique Simmonds. Plant compounds in pest control: challenges and opportunities.

Since 1985 have been working on 20 000 diff. species of plants and 15 000 insect-plant interactions. Around 2000 many of the companies interested merged so fewer companies were involved. Around this time more work on authentication started too. Work on chemosystematics and authentication has been more valuable than random screening which has produced very little. Work has been influenced by the new phylogenies found with molecular work. About 28 300 spp. of plants have pesticidal activity. Only 460 of these with solid evidence. Certain families more likely to have them than others. The "older" plants have more pesticidal activity e.g. Ginko. Legumes have a lot. Info on the pesticidal plants is in public domain. Types of IP involved: Patents, Know how (trade secret), Copyright, Database rights, Design rights and Trademarks. Copyrights and Database rights vary from country to country. Information should be retained in the country where it originated so that other usage is secondary. Convention on Biological Diversity has had a large impact on terms used. Access to genetic resources – Article 15. Had various guidelines since then e.g. Bonn Guidelines on how to share info. Delhi Declaration 21 Jan 2005 very important for neem. Have to disclose country of origin of plant, not where the plant is actually growing. Aichi Nagoya Japan Summit on biodiversity 18-29 Oct 2010 on benefit sharing and launch of economics of ecosystems and biodiversity www.cbd.int/doc/press/2010/. Example of getting a product to market: Dan Janzen in 1980 sent some seeds from Costa Rica to Kew because were not being eaten by insects. Found DMDP was responsible. Asked another group BTG if they would investigate it and declined. Then included in an abstract so went into public domain. Then found to have nematocidal properties so patented. Made collaborative agreements with Costa Rica and permitted them to use it as a nematocide. Developed into a revenue sharing agreement. Then found DMDP in blue bells and Hyacinths. Could use discarded material from flower industry. Licence agreements then assigned to INBio and used for control of nematodes in bananas and coffee. Formulation is important as it is water-based. 50% of royalties going back to Costa Rica. Industry is now starting to protect biodiversity. Every year plants are being misidentified. In China 596 plant species officially used in medication, water soluble and taken by mouth; 267 of these with pesticidal activity. Traditional Chinese Medicine (TCM) in Chinese Pharmacopoeia sometimes with different names. Names can be wrong in publications e.g. 215 records in Genbank but 75% incorrect for one particular species. www.gp-tcm.org. Need to know what is a quality extract and what compounds are responsible. In medicines plants are often combined together to help penetration of membranes or uptake. Less likely to be a single chemical that most effective. Lessons learnt: solid evidence that have a lead. Check plant and review literatures (look for 3 asterisks). Talk with someone with knowledge about IP regulations. Make collaborations early. Involve industry to contribute to formulation. Involve growers of plants and formulators. Quirks: Darwin 1876 reported ivy rootlet secretes yellowish matter while climbing a surface. Identified as nanoparticles by Zhang in 2008. Difficult to work on South African plants because information is not accessible. If make a new chemical from a plant chemical the plant chemical needs to be acknowledged but can patent the new chemical. Would have to get benefit back to original country.

S4-P Opendor Koul. Botanical insecticides: An Asian perspective.

We concentrate on structure, complexity and toxicity. Koul 2003 in Recent Trends In Chemistry shows lists of plant family names with pesticidal properties. Writing book on Naturally occurring insecticidal toxins. 650 known compounds that are purely insecticidal 1160 compounds considered to be antifeedants. Natural chemicals that are currently marketed are 89. Toosendanin from *Melia toosendan* (synonym for *M. azedarach*) from bark extract. Has Chinese patent. *Sophor flavescens* has sesquiterpene polyester and an alkaloid plus plant volatile oil sold as BBM and patented. Chinese registration procedure has some exemptions for full analysis of chemicals. Registered products with cold pressed neem oil in USA for bed bugs. In India only Azadirachtin and Pyrethrum registered botanical pesticides. No Chinese products registered there. Have found other limonoids besides azadirachtin in neem. *Entandrophragma candolei* also have limonoids but no dominant toxin. Sugar esters have been studied in India and glycol diesters which effective against various pests such as whiteflies, mealybugs. Have also looked at effect of essential

oils in preventing oviposition behaviour. Cyclotides from Violaceae, Rubiaceae and Cucurbitaceae are also insecticidal. Ecwin producing botanical pesticides in South Korea. Specific constraints in Asia: 1. Selection of ideal plants. Should be perennial, wide distribution, should have additional uses. 2. Should be systematic studies. Many different approaches to antifeedant studies. 3. Patents cannot protect traditional knowledge in the Asian-Pacific region. 4. Reasonable regulation processes are required to assist registration.

S4-1 Nyalala, Petersen and Grout. How growing conditions and developmental stage of *Gynandropsis gynandra* influence emission of potential miticidal leaf volatiles.

Reduces thrips damage in beans. when intercropped in roses it reduces spider mites. Emits bioactive acetonitrile (methyl cyanide) and bioactive volatiles against spider mites. Did some work in greenhouses in Denmark and Edgerton Univ. In both places under greenhouse conditions methyl cyanide was the main chemical produced but quantity can vary with temperature. Various isothiocyanates are also produced when the plant is quite old. Also some aldehydes like hexanal that had an effect on spider mites that were being produced by old plants.

S4-4 Zida et al. Seed treatment with a binary pesticide and aqueous extract of *Eclipta alba* (Asteraceae) for improving sorghum yield in Burkina Faso.

Aqueous extract from leaves and stems. Plant material dried then crushed to powder which mixed with water. Used at 6-25% to treat sorghum seed. There was a fungicidal effect but in the field there were no significant differences in yield.

S5-PL Chege. Intellectual property issues in the research and development of innovations.

Churchill: "the empires of the mind are the empires of the future". Everything we do is knowledge driven and based on intellectual creation. Patents are used to protect inventions of a technical nature. Phases of innovation: 1. Research phase, 2. Design and development phase, 3. Commercialisation phase. In 1 need to have material transfer agreements, collaboration agreements and institutional IP policies. In 2 could involve development of prototypes and private companies. Phase 3 requires market testing and collaboration with private companies. Patent will usually last 20 years. Don't undervalue or underestimate your work at the initial stages.

S5-1 Sola et al. Botanical pesticides production, trade and regulatory mechanisms in sub-Saharan Africa; making a case for plant pesticide products.

Neem and *Tagetes minuta* have been the most common sources in the past. *Tephrosia* and *Securidaca* are widely used but no commercial products. Kenya only supplying 20% of pyrethrum these days. Also grown in Tanzania and Burundi. For pyrethrum production, leaves are picked, dried and powdered. Then processed overseas. Often a limitation by the growing of plants for the pesticide extraction.

S5-2 Severine. Governance structures for bridging the access and utilization gap of bio-pesticides produced by different farmers in Tanzania.

75% of pesticide products imported into Tanzania are reported to be fake. Government structures do not address commercialisation of biopesticide pesticides.

S5-5 Magreta et al. Economic evaluation of optimized pest management options on bean cropping systems in southern Africa.

Using *Vernonia amygdalina* and *Tephrosia vogelli* treatments. Tv at 2 and 5% was giving best marginal rate of return. Strong smell when pounding is not appreciated and blockages when spraying require fine filtering or stocking.

S5-6 Omolo et al. Bioprospecting for phytochemical control agents of the stored product pest, *Prostephanus truncatus* from some western Kenya flora.

Larger grain borer (nickname Osama Bin Laden). Labiatae, Verbenaceae, Euphorbiaceae and Compositae. Extracted essential oils by steam distillation. *Conyza newii*, *Plectranthus marruboides* and *Tetradenia reparia* were good fumigants.

S5-7 Innocent. Translating science into community life: an experience at the institute of traditional medicine.

Annona squamosa and *Annona senegalensis* bark and stem extracts are effective against mosquito larvae.

S6-P Muthoka. Conservation and propagation of pesticidal plants for sustainable use.

www.centerforplantconservation.org has some info about some of the plants being used.

S6-1 Mulanda et al. Prospects for a rapid *in vitro* regeneration system for propagation of the pesticidal tree *Melia volkensii*.

If could clone plants would have a more consistent pesticidal product. Callus induction from zygotic embryos. Whole process took about 105 d to viable plant.

S6-? **Njogu et al.** Use of detached leaf bioassay to evaluate host plant chick pea susceptibility to *Helicoverpa armigera*.

Exposed different chick pea genotypes to neonate larva and larger larvae collected from the field. No varieties were completely resistant but some resulted in lower weight larvae. Four genotypes should be avoided because of high susceptibility.

8 VOORLIGTING 2012-2013

Deur Hennie le Roux, Hannes Bester, Keith Lesar, Dawid Groenewald, Andrew Mbedzi en Melton Mulaudzi (CRI)

Die 2012 Seisoen

Die skatting vir 2012 vir sitrus-uitvoere uit suider-Afrika was aanvanklik 102.9 miljoen kartonne, is later afwaarts aangepas tot 96.3 miljoen, en uiteindelik het die totale volume verpak die 100 miljoen kerf verbygesteek. Finale pomelo-volumes gepak was 12.8 mil, suurlemoene 10.6 mil, nawels 23.6 mil, Valencias 46.2 mil en sagtesitrus 7.6 mil om 'n totaal van 100.8 miljoen kartonne te gee.

Die gehalte van die vrugte was aansienlik beter as die 2011 seisoen, veral ten opsigte van bederf, wat ongeveer die helfte van die vorige seisoen was. Die mees algemene probleme gedurende 2012 was 'pitting' op Valencias, laat manderyne en satsumas en dan sagte, pofferige vrugte in die geval van nawels. Vruggrootte was oor die algemeen effens kleiner.

Die algemene siening onder produsente is dat die 2012 seisoen beduidend beter in verskeie opsigte as die vorige seisoen was.. Verdienste terug op die plaas was ook beter as gevolg van die gunstiger wisselkoers, goeie kwaliteit en beter verkoopswaardes.

Aan die negatiewe kant is die Sitruswartvlek (CBS) onderskeppings in die EU 'n bron tot kommer, veral met die oog op die 2013 seisoen. Indien die EU gaan volstaan met hul onrealistiese vereistes tov onderskeppings van CBS in 2013, voorspel dit niks goeds vir die suidelike halfgrond se sitrusbedryf nie.

Die 2013 Seisoen

Die totale oesskatting vir 2013 is bykans 4.0 miljoen kartonne hoër as in 2012, waarvan die grootste toename in pomelos is. Die totale skatting vir 2013 is 106.8 mil kartonne met Valencias 47.7 mil, nawels 24.8 mil, pomelo's 14.9 mil, suurlemoene 11.1 mil en sagtesitrus 8.2 mil kartonne.

Die grootste uitdaging vir 2013 gaan waarskynlik die beheer van swartvlek en Valskodlingmot (FCM) na die EU wees. Op hierdie vroeë stadium is die FCM lokvalvangste redelik laag in meeste areas, wat toegeskryf kan word aan beter bestuurspraktyke wat gevolg word. Dit is veral opsigtelik in areas waar SIT toegepas word en streng boordsanitasie plaasvind. CBS beheerprogramme word nougeset gevolg, maar CBS word wel op buiteseisoen vrugte waargeneem veral in suurlemoene in die Sondagsriviervallei. Die EU dreig om die hele EU te sluit vir Suid Afrikaanse sitrus sou daar meer as vyf onderskeppings vir swartvlek wees. Daar was in 2012 sewe-en-dertig voorvalle van swartvlekonderskeppings. Dit is dus duidelik dat die EU die mark vir Suid Afrikaanse sitrus wil toemaak ongeag die feit dat die siekte nie deur vrugte versprei nie en ook nie in 'n Meditireënsse klimaat kan vestig nie. Hierdie is nie alleen 'n bedreiging vir Suid-Afrika nie, maar vir die hele suidelike halfgrond se sitrus. Sowat 35-40% vandie Suid-Afrikaanse sitrus gaan na die EU. As hierdie vrugte na alternatiewe markte gestuur word, sal hierdie markte onder druk kom agv oorvoorsiening omdat dit bloot nie hierdie groot volumes kan absorbeer sonder 'n neerdrukkende effek op prys nie.

Bactrocera invadens het ook verander van 'n potensiële bedreiging in verskeie van die sitrusproduserende streke in die Limpopo Provinsie, Mpumalanga, Noord-Wes en Swaziland na 'n wesentlike bedreiging. Hierdie vrugtevlug kom wydverspreid in die genoemde provinsies voor. Die tellings wat gevang word, is egter steeds laag. Die wyse waarop die verskillende gebiede se produsente reageer, verskil dramaties. In een situasie sal daar gevind word dat die produsente saamstaan en onmiddellik metyl eugenol blokkies (MAT) uithang, area-wye lugbespuitings doen en seker maak dat hulle proteïenlokaas spat-aksies op datum is. In hierdie gevalle het die Bi tellings afgekom na nul. In ander areas het produsente na twee maande nog nie die vereiste aantal MAT blokkies uitgehang nie en was die effektiwiteit van die lugbespuitings ook nie na wense nie. In hierdie gevalle het die tellings nie noemenswaardig afgekom nie. In talle gevalle sal die Bi beheer moet word soos enige vrugtevlug. Agv huistuine en verwaarloosde boorde kan die Bi in sekere gebiede nie meer uitgewis word nie. Mangoes en maroelas blyk die gasheerplant by uitstek te wees. Dit is net 'n kwessie van tyd voordat dit ook die Kaapse sitrusproduserende gebiede sal bereik.

Die vruggrootte is soortgelyk aan verlede seisoen en mag selfs effens kleiner wees waar groot oeste geset is danksy goeie reëns in September. Die uitvoerders is bekommerd oor die invloed wat klein vrugte op die prys kan uitoefen. Hierdie is 'n onderwerp wat weereens by die CRI Sitrus Produksie Werksinkels aangespreek sal word.

CRI Na-oes Tegnieese Forum (CRI-PTF)

Daar is onverpoosd gefokus op die gehalte van pakmateriaal en die ontwikkeling van meer koste-effektiewe verpakking, asook die hantering deur die logistieke ketting. Heelwat aandag is ook gegee aan die

volhoubaarheid tov verskaffing van papier vir die vervaardiging van kartonne en die evaluasie van nuwe papier.

In samewerking met Houers is 'n proef gedoen om T64 Supervent kartonne by Groep 91 te evalueer. Die proef het in Japan aangekom en die "i-Button" inligting oor temperature is terug ontvang. Die voorlopige resultate het getoon dat daar nie betekenisvolle verskille in die verkoelingstempo's is nie. Omdat daar net vyf T64 kartonne per laag op die palette gepak word, is die lugvloei deur die paletvragte beter as met A15C kartonne wat 10 per laag gepak word. 'n Volledige verslag hieroor is opgestel.

Beskikbaarheid van goeie kwaliteit papier vir uitvoerkartonne is 'n bron tot kommer aangesien die bedryf totaal van Sappi afhanklik is vir die vervaardiging van "Virgin Kraft Linerboard". Sappi het finaal besluit dat alle "Virgin Linerboard" voortaan slegs by hulle Ngodwana meule in Mpumalanga vervaardig sal word. Dit sluit Stackraft in. Die ooreenkoms was dat die bedryf genoeg tyd gegun sal word om die nodige laboratorium-toetse en veldproewe te doen totdat CRI-PTF tevrede is dat Ngodwana se Stackraft op dieselfde standaard as dié van Tugela is. Ongelukkig het hulle nie by die ooreenkoms gehou nie en Houers is in kennis gestel dat Stackraft vanaf Julie 2012 slegs by Ngodwana vervaardig sal word. Houers is meegedeel dat die 71 ton wat hulle nog van Tugela af moes kry, nou vanaf Ngodwana gelewer word. Einde 2012, in samewerking met Houers, is toetse met Sappi Tugela Linerboard (TLB) en met die eerste eksperimentele kartonne gedoen. Meer omvattende proewe met A15C kartonne, wat met 200g/m² TLB vervaardig is, is gedurende die eerste week in Maart weer by Houers in Letsitele gedoen. Tydens die normale spoed waarteen kartonne kommersieël vervaardig word, het die TLB ongelukkig gekraak. Sappi gaan sekere verstellings aan die vesel samestelling maak en dan sal die proef herhaal word. Tesame met die TLB is die eerste proewe met Sappi se nuut ontwikkelde Spray Starch Stackraft (SSK) ook geloop, wat 'n verbeterde produk op die Ngodwana Stackraft (SK) is en die doel is om te verseker dat die Ngodwana SK op dieselfde standaard as die Tugela SK is. Die nuwe Ngodwana SSK het goeie resultate gelewer en grootskaalse proewe sal gedoen word.

Daar word gerugte versprei dat Sappi op een of ander stadium met die vervaardiging van "Kraft Linerboard" (KLB) en Fluting gaan staak en slegs "Chemical Cellulose Pulp" gaan vervaardig. Tydens die laaste gesprek met Sappi (8 April 2013) het hulle CRI-PTF vir die soveelste keer die versekering gegee dat hulle nooit sal ophou met die vervaardiging van KLB en Fluting nie. Op versoek van Houers se direksie beplan Dawid Groenewald en Wimpie Mostert (Houers) om so spoedig moontlik met die CEO van Sappi te vergader. Sappi sal versoek word om hulle verbintenis op skrif te plaas. Die CRI-CTF bly ook op 'n voortdurende basis in kontak met instansies wat KLB en Fluting invoer.

Wanneer sitrus in wit kartonne uitgevoer word, word Sappi Printpride of Mpack Paper se Baywhite papier gebruik. Om ekonomiese redes het Sappi die vervaardiging van Printpride gestaak en die vervaardiging van wit papier na Tugela verskuif. Intussen het Capepsan besluit om 100% oor te skakel na wit kartonne, gevolglik is daar nou 'n groter vraag na wit papier. In Maart 2013 kondig Sappi amptelik aan dat hulle geheel en al uit die wit linerboard mark onttrek, aangesien dit nie meer lonend vir hulle is om wit linerboard te vervaardig nie. Wat die voorsiening van wit linerboard betref, is die bedryf nou, wat plaaslike verskaffers betref, 100% in Mpack Paper se hande. Die CRI-PTF is betrek en tydens samesprekings met Mpack Paper is die versekering gegee dat hulle in die vraag sal kan voorsien op voorwaarde dat die kartonvervaardigers hulle bestellings vroegtydig plaas. Verder is die vervaardiging van wit linerboard nie omgewings- en "carbon footprint" vriendelik nie en die CRI-PTF is versoek om uitvoerders te oorreed om eerder by bruin kartonne te bly. Die feit dat Sappi nie meer wit linerboard vervaardig nie, mag lei tot buitensporige verhogings en dit is kommerwekkend.

Twee hoëvlak samesprekings met Mpack Paper het plaasgevind oor die ontwikkeling van 'n nuwe verbeterde "fluting" vir sitrus kartonne. Die "typical values" en die eerste lab resultate lyk baie belowend. Dit vergelyk op hierdie stadium uitstekend met Sappi se Superflute. Hierdie ontwikkelingswerk gaan voort. Laboratoriumtoetse word tans gedoen om te bepaal hoe Nampak se Rosslyn "Fluting" se waardes vergelyk met goedgekeurde "Semi-chemical Hi-yield" en "Superflute", om as "high performance fluting" vir die maak van kartonne gebruik te kan word. Voorlopige resultate lyk belowend en verdere toetse sal volg.

Gegewe die kommer oor die beskikbaarheid van plaaslik vervaardigde "Virgin Linerboard" word daar op 'n voortdurende basis gekyk na die moontlikheid om hierdie produk in te voer. Dit lyk baie belowend en in samewerking met sekere kartonvervaardigers is daar alreeds proewe aan die gang. Op hierdie stadium dui alles daarop dat kartonne met die ingevoerde papier op hierdie stadium effens goedkoper kan wees. Die wisselende sterkte van die Rand speel hier egter 'n belangrike rol en dit sal nie noodwendig in die toekoms die geval wees nie. Hierdie ondersoek geniet dringend aandag. As gevolg van die gekompliseerdheid van die situasie word hierdie werk met groot omsigtigheid en verantwoordelikheid gedoen.

Die aangeleentheid rakende karton kodes is na die Verpakkingswerkgroep verwys om te standardiseer. Die lys met aanbevole kodes is uitgestuur vir kommentaar. Die standardisering van karton kodes is terugverwys na die CRI-PTF (verpakkingswerkgroep), waarna dit gefinaliseer en die finale lys met die aanbevole gestandaardiseerde kodes is gedurende Februarie onder alle belanghebbende instansies en rolspelers versprei. Nuwe kartonne sal in die toekoms by die Verpakkingswerkgroep geregistreer word. Die aangeleentheid rondom karton-gewigte is in opdrag van die CMF opgevolg, eers deur die ETP en daarna deur 'n kleiner groep van belanghebbendes en kundiges. Karton-kodes is gesamentlik met karton-gewig aangespreek om te standaardiseer en te vereenvoudig.

Limpopo produsente is deur 'n firma in Namibia genader oor ingevoerde kartonne vanaf Dubai, wat 35% goedkoper is vir A15C kartonne as wat plaaslik verskaf word. Produsente het die verpakkingswerkgroep versoek om dit te ondersoek.

Die moontlikheid om sitrus in plastiese kratte uit te voer, is ondersoek. Plastiek is egter nie so koste-effektief soos papier riffelbord nie.

Die Citrus Logistics Forum (CLF), in samewerking met Seatrade en 360° se Quality: Vessel and Terminal Audits, se gereelde terugvoering en verslae met ondersteunende foto's oor verpakkings-verwante probleme oor die algemene toestand van die vrugte, palette, kartonne en ander pakmateriaal is met die betrokke pakhuis gekommunikeer om regstellende stappe te neem. Hierdie verslae was van onskatbare waarde en het CRI-PTF instaat gestel om probleme direk aan te spreek. Die reaksie van produsente is baie positief en groot dank word uitgespreek vir hierdie terugvoering. Geen klagtes is oor kartonne ontvang nie. Palette en hoekstukke bly egter 'n baie groot probleem. Juis a.g.v. probleme met palette en hoekstukke is daar gedurende Augustus 'n tweede omsendskrywe aan alle pakhuis uitgestuur waarin Pakhuisbestuurders dringend versoek is om nie minderwaardige "goedkoop" palette te gebruik nie. In Junie is die eerste soortgelyke omsendskrywe uitgestuur, maar ongelukkig was die reaksie nie goed nie.

Swak kwaliteit palette was ongetwyfeld die grootste enkele probleem en alles moontlik sal gedoen word om hierdie probleem op te los. Die CRI-PTF is onder baie druk om die paletvervaardigers te akkrediteer. Die volledige akkreditasie proses (instuur van palette deur vervaardigers en pakhuis asook volledige toetse deur 'n SANAS geakkrediteerde laboratorium) is ongelukkig 'n moeilike en duur proses en 'n gebrek aan befondsing maak dit tans onmoontlik. Verskeie opsies word ondersoek, waarvan die bou van 'n apparaat wat deur pakhuis self gebruik kan word, een is.

Om vinnig te toets of palette se houtkomponente se afmetings en die spasiëring tussen die bo-dek planke aan die spesifikasie voldoen, is 'n templaot ontwerp en by Schoeman Boerdery getoets. Dit werk baie goed en pakhuis kan intussen hierdie spesiale templaot gebruik om die afmetings en spasiëring van die bo-dek planke en die blokke na te gaan. Een van die groot paletvervaardigers het voorgestel dat die spasiëring van die bo-dek planke op uitvoerpalette verander moet word. D.m.v. 'n rekenaar ondersteunde stelsel is 'n nabootsings gedoen en hierdie werk het getoon dat dit nie 'n verbetering vir vertikale lugvloei sal meebring nie.

Navraag om meer inligting oor Euro Palette is vanuit die Marble Hall streek ontvang en deurgegee, en produsente is ook gemaan om versigtig te wees. Euro Palette is nie geskik vir gebruik vanaf SA pakhuis nie. Freshgold SA het navraag gedoen oor plastiese palette. Hierdie palette is aansienlik duurder as die standaard houtpalette. Daar is ander nadele ook, oa beskadigde plastiese palette wat nie in pakhuis of voorverkoelingstore herstel kan word nie. Verskaffers is genooi vir samesprekings, maar tot op datum is nog geen reaksie ontvang nie.

FPT is deur die loop van die jaar besoek om opnames te maak van die toestand van kartonne en palette. Dit was verblywend om te sien dat verpakking/palette baie beter lyk as voorheen. Geïsoleerde gevalle met swak kwaliteit hoekstukke en hoekstukke wat te lank is, moet verder aandag geniet. Die ISPM 15 merk op palette is dikwels 'n probleem en pakhuisbestuurders is ook weer versoek om seker te maak dat hulle palette baie goed vasgemaak en gestabiliseer is voordat vragmotors hulle pakhuis verlaat.

Tesco se instruksie dat die SA Sitrusbedryf met die aanvang van die 2013 seisoen weer die sogenaamde "Interstackable" karton sal terugbring, het daartoe gelei dat CRI-PTF oorval is met versoeke om motiverings op te stel wat gebruik kon word om Tesco te ooreed om van hierdie belaglike idee afstand te doen. Die bedryf is in 1997 daarin geboelie, wat gelei het tot stakings en ander ernstige probleme. Dit het die bedryf destyds baie geld gekos en die karton is na drie maande uitgegooi. Voorlopige koste-berekenings toon dat net die "stereo" en kostes in die omgewing van R2,28 miljoen sal beloop. Addisionele kartonvou-masjiene sal aangekoop moet word teen 'n minimum koste van R650 000 per masjien. Tesco het bes gegee die instruksie later teruggetrek.

Op versoek van die ETP is 'n proef by Schoeman Boerdery gedoen met korter hoekstukke. Die proef is gedoen met agt korter stukkie in plaas van vier vollengte hoekstukke per palet. 'n Volledige verslag is opgestel. Dit is deel van voortdurende werk wat gedoen word om te kyk waar kostes bespaar kan word. Dit was nie suksesvol nie. Die gebruik en opsit van agt korter hoekstukke was tydrowend, meer arbeid-intensief en het baie onstabiele paletvragte tot gevolg gehad.

Op versoek van die ETP is laboratoriumtoetse gedoen om te bepaal of vol drukwerk op 600x400mm oop vertoon-kartonne (Sainsbury ens.) 'n nadelige uitwerking op die stapelsterkte van die kartonne het. Die werk is in samewerking met Houers en die Sappi Technology Centre gedoen. Die verskil in stapelsterkte tussen normale drukwerk (Outspan, Dole ens.) en vol drukwerk was nie betekenisvol nie. Kartonne met geen drukwerk op nie se stapelsterkte was wel betekenisvol hoër. 'n Volledige verslag is opgestel.

Ook op versoek van die ETP is 'n proef met "bubble pack interleaves" gedoen met die doel om alternatiewe lae wat toegedraai word, te vervang. Die "interleaves" het die vertikale uitbuiging (bulge) onaanvaarbaar hoog gemaak. Vrugte nes nie mooi inmekaar nie en die verpakking op die verskillende lae het soos 'n "jumble pack" vertoon. Die "interleaves" se koste per karton sal tussen R0,45 en R0,50 hoër wees teenoor toedraai van vrugte. 'n Volledige verslag is opgestel met die slotsom dat die verwydering en vernietiging van die "bubble pack interleaves" as 'n groot probleem gesien word. Die toedraai papier verhoed verder die verspreiding van bederf en veral spore as dit voorkom, wat nie met die "interleaves" die geval sal wees nie.

'n Verskaffer van pakmateriaal, en onder andere ook goedkoop kartonne wat van herwinde papier gemaak word, het sitrusprodusente genader. Hulle bied 600x400mm oop vertoon-kartonne aan teen 'n prys wat gemiddeld R2,50 per karton laer is. Hulle het geen toerusting om kartonne self te vervaardig nie en is in gesprek met van die geakkrediteerde kartonvervaardigers om hulle te ooreed om saam met hulle in besigheid te gaan. Volgens hulle is die "baie sterk/goedkoper" karton nou gepatenteer. Hulle is die eienaars/houers van die patent. Die geakkrediteerde kartonvervaardigers moet die kartonne vervaardig, die karton vou-masjiene verskaf, alle risiko's aanvaar en dan boonop 'n 10% tantiem betaal. Houers se direksie het versoek dat CRI saam met Houers 'n volledige ondersoek doen. Prototipe kartonne, in verskillende bordkombinasies, is op 'n rekenaar ondersteunde apparaat gesny en die eerste laboratorium toetse is gedoen. Voorlopige resultate toon dat die aantreklike prys uitsluitlik toegeskryf kan word aan die feit dat die instansie wat die kartonne promofeer 'n ligter bordkombinasie aanbeveel. Laboratorium toetse toon dat die "nuwe ontwerp" wel aan die stapelsterkte vereistes voldoen, maar a.g.v. verskeie ander praktiese probleme het die CRI-PTF ernstige bedenkinge oor die toekoms van hierdie karton. 'n Kleinskaalse proef was beplan maar a.g.v. ernstige praktiese probleme in die pakhuis het die proef nog nie plaasgevind nie. Dis 'n tydrowende proses om die kartonne in die pakhuis te vou en te gom en agv die feit dat die "flute direction" in die teenoorgestelde rigting loop word probleme voorsien wanneer die kartonne aan hoë humiditeit en sikliese toestande onderwerp gaan word.

Die opnames vir die behoefte aan "Compliance Audits" (CA) in die hawens en innamepunte is ontleed en a.g.v. kommer oor "nog ekstra" kostes vir die bedryf, wat alreeds gebuk gaan onder verskeie ander kostes (waarvan PPECB se kostes nogal sterk na vore gekom het) en ook 'n gebrek aan belangstelling, is daar besluit om nie daarmee voort te gaan nie. 'n Omsendskrywe met die nodige verduideliking waarom daar nie met CA voortgegaan word nie, is opgestel en aan alle produsente/pakhuis uitgestuur.

Die Akkreditasie-proses wat vir die kartonvervaardigers geïmplementeer is, gaan deurlopend voort. Heelwat tyd is by die Sappi Technology Centre spandeer aan die akkreditasie van kartonvervaardigers. Dit is 'n sensitiewe aangeleentheid, wat met groot omsigtigheid hanteer word. Sappi gaan gedurende 2013 weer die toetse op hulle koste vir die sitrusbedryf doen. Opregte dank en waardering aan Sappi en die personeel van die STC in Pretoria. Die skedule vir die instuur van kartonne deur die vervaardigers, CRI en pakhuis is opgestel en versprei.

Houers se Algemene Jaarvergadering op 29 November 2012 het saamgeval met die herdenking van hulle 30ste bestaansjaar. CRI-PTF is uitgenooi om die vergadering by te woon en 'n toespraak te maak. Daar is 'n skitterende verhouding tussen Houers, hulle lede (wat baie groot rolspelers in die sitrusbedryf is) en CRI, gevolglik ondersteun Houers baie van die navorsingswerk wat deur die Verpakkingsforum gedoen word as samewerkers in proewe. Hulle versoek nog nouer samewerking met CRI-PTF.

Op versoek van SRCC het CRI-PTF betrokke geraak by die toets van toedraaipapier wat oorsee gebruik word. Alle inligting dui daarop dat toedraaipapier nie meer plaaslik vervaardig gaan word nie. Gesprekke word tans met vervaardigers van ander tipes papier gevoer en hulle ondersoek nou die moontlikheid om 'n 18g/m² papier vir dié doel te vervaardig. Navrae i.v.m.pakmateriaal vereistes en spesifikasies word deurlopend hanteer.

Die Zimbabwe-produisente is deur CRI-PTF van kostes van pakmateriaal voorsien, sodat hulle kan aansoek doen vir finansiering vir die aankoop van pakmateriaal deur SA Banke.

Die 'Packaging Material Specifications and Palletisation Protocols for the 2013 Citrus Export Season' is opgestel en gedurende die eerste week in Maart aan alle betrokke instansies uitgestuur.

Na-oes voorligting

'n Baie suksesvolle reeks landswye sitrus pakhuis besoeke/konsultasies is gedurende 2012 afgehandel. Pakhuise is op 'n een-op-een basis besoek en die terugvoerig was baie positief omdat belangrike informasie en aanbevelings met die pakhuis bespreek kon word. Pakhuisbestuurs is meer bereidwillig om hulle idees en vertroulike informasie ivm terugvoering oor bederf, residu-resultate, ens, te bespreek wanneer dit op 'n een tot een basis is en nie tydens 'n werkswinkel nie.

Die bedryf was weer, soos in 2011, met baie skilprobleme op satsumas, Clementines en nawels oorval, weereens as gevolg van wisselvalige omgewingstoestande. Bome is onder stremming geplaas agv uitermatige en wisselende hoë temperature. In sekere streke waar die probleme ondervind is, het die dag temperature gewissel tussen 30 en 45°C oor 'n tydperk van 19 aaneenlopende dae tydens pluk. Bome was onder stremming en dit het swak vrugskil-gehalte veroorsaak. Die vrugskil-gehalte van hierdie kultivars is verder deur fito-brand tydens voorontgroening-storting ("drench"), en te lank in ontgroening (> 72 ure), benadeel.

Heelwat bederf is op uitskot vrugte waargeneem as gevolg van vrugte wat te lank in ontgroening gestaan het, en ook van die aantal groen/blouskimmel vrugte, na ontgroening, wat nogsteeds in die vrugwas-stelsels gedompel word en nie vooruit gesorteer word nie. Hierdie is nog steeds 'n groot praktiese probleem in baie pakhuis. Die "miljoene" swampore in die was-stelsels, veral die dompelbaddens, kan duidelik gesien word. Dit bly steeds 'n resep vir bestandheid en 'n toekomstige ramp.

Nog twee probleme wat tot die baie bederf op die uitskot vrugte bygedra het, was die aantal klein vrugte (satsumas, Clementines, pomelos, nawels ens.), en die hoë volumes vrugte in vergelyking met die lae volumes in 2011. Sekere pakhuis het uitpak verhoudings van 50-50% gewys op groot en klein vrugte. 'n Aantal pakhuis het met hulle verpakkings-programme agter geraak as gevolg van die hoë volumes vrugte wat na ontgroening in kratte opgehoop gestaan het, en ook daarna reëval in die Wes-Kaap. Dit was 'n resep vir bederf.

Kraakskil op nawels, Clementines en ander kultivars was ook 'n geweldige probleem wat hierdie seisoen waargeneem is. Twee pakhuis het onderskeidelik 12 en 15% uitskot van hulle hele nawel-oes gehad agv kraakskil.

Palettisering in sommige pakhuis was steeds 'n probleem en moes aangespreek word. Sommige palette staan regop en lyk asof daar gepoog is om dit reg te doen, terwyl ander soos die "toring van Pisa" lyk. Dit lyk asof van die kartonne, op een van die palette, enige oomblik in die middel van die kartonne gaan bars of induik. Party palette se hoekstukke loop van bo af tot heel onder die palet tot op die vloer. Ander palette se hoekstukke loop tot die middel van die palet se voetstuk, en ander hoekstukke eindig by die onderste karton en raak aan die begin van die voetstuk van die palet. Te veel palette met gebuigde hoekstukke is ook waargeneem.

Die herinstelling van die Voorseisoen Pakhuiswerksinkels, nou bekend as die CRI Na-oes Werksinkels, en die aanstelling van 'n na-oes voorligter is besig om vrugte af te werp, aangesien die bederfsituasie aansienlik beter is as verlede seisoen. Produisente is baie in hul skik met die individuele aandag wat aan pakhuis gegee word om bederf te help bestry. Die meerderheid pakhuis poog om hulle kritiese beheer punte goed te bestuur en die sanitasie komponent het in baie van die pakhuis verbeter. Die 2012 seisoen was, oor die algemeen, nie 'n "bederf probleem jaar" nie. Nogtans is heelwat bederf op uitskot vrugte waargeneem as gevolg van vrugte wat te lank in ontgroening gestaan het, veral die sagte sitrus wat gedruk word om vroeër in die markte te kom.

Die groot meerderheid na-oes probleme was agv. groenskimmel. Daar is 'n mate van suurvrot op sekere kultivars gesien, en 'n toename in die latentepatogene was ook 'n probleem. Terugvoering vanaf die uitvoerders vanuit die markte, het ook latentepatogene uitgewys as 'n probleem. Dit het ingesluit antraknose, Diplodia- en Phomopsis stingelentvrot. Duidelik is die beserings op vrugte 'n groot probleem en dit waarskynlik agv dooiehout in bome. Te min aandag word aan die verwydering van dooiehout in boorde bestee. Sekere pakhuis het openlik genoem dat van die produisente nie veel tyd aan hierdie praktyk spandeer nie. Dit kan groot probleme vir die bedryf in die geheel skep, indien 'n volgende hoë-bederf jaar weer voorkom.

Die grootste na-oes probleme tydens die 2012 seisoen was die skilprobleme, meestal op die satsumas, in die WesKaap, en tot 'n mindere mate in die ander gebiede. Die ander sagtesitrus kultivars, en sekere Valencias, het ook gehalteprobleme getoon. Sekere boord en na-oespraktyke, soos die vroeër pluk van vrugte met sensitiewe skille vir vroeër toegang tot die markte, wat te lank in ontroening gestaan het, het bygedrae tot swakker skille en 'n korter raklewe.

Die gebruik van ongeregistreerdemiddels in die sitruspakhuse is steeds kommerwekkend. Dit sluit hoofsaaklik die "saniteermiddels" en benatters, wat deur die chemiese verskaffers versprei word in, sonder om eers vir CRI te raadpleeg of die middels goedgekeur is, of nie.

Die 2013 sitrus seisoen het op 'n baie positiewe noot afgeskop, in vergelyking met 2012 waar daar teen dié tyd, terugvoering vanaf verskeie gebiede, oor fitotoksisiteit (brand) op die Satsumas ontvang is. Die eerste Satsuma besending hierdie seisoen, vanaf die Burgersfort gebied, waar daar in 2012 verskeie gevalle van skilbrand voorgekom het, is goedgekeur as "good to excellent". Die eerste suurlemoene vanaf Letsitele, en die eerste suurlemoene en pomelos vanaf Tshipise en Hoedspruit se algehele gehalte is ook baie goed. Die eerste Satsumas vanaf Citrusdal, waar daar ook baie gevalle van skilbrand tydens 2012 waargeneem is, en die eerste Satsuma besending vanaf Clanwilliam se algehele gehalte is gerapporteer as "baie goed". Satsumas en suurlemoene vanaf die OosKaap (Kirkwood en Patensie) is ook as "baie goed" aangedui. 'n Mate van brand is wel op die Satsumas vanaf Katrivier gesien, maar dié is binne die spesifikasies. Die Satsumas vanaf Paarl en Robertson is ook goeie gehalte. In Mpumalanga is daar verskeie Satsuma boorde waarvan die interne kwaliteit te swak was om die vrugte uit te voer.

Die meerderheid gevalle van brand op die Satsumas tydens die 2011 en 2012 seisoene is na "drench" alleen en/of saam met ontgroening van die vrugte waargeneem. Van die begin af het almal die verhoogde konsentrasie van guazatine, vanaf 500 tot 1000 dpm, die skuld gegee. Proewe in 2012 het gewys dat die toediening van 250, 500 en 1000 dpm guazatine vrugte onder sekere omstandighede kon brand. Dit is alreeds 'n bekommernis om te hoor, vanaf seker bronne, dat 'n paar pakhuse die guazatine konsentrasie in die "drench" gaan verminder of guazatine heeltemal uit die mengsel gaan onttrek. Dit na al die reënval in die Noordelike gebiede en nou onlangs in die Suide, en die moontlike risiko van suurvrot en groen/blouskimmel infeksies.

Die "Compendium of Postharvest Citrus Diseases" is voor die begin van die "Pakhuiswerkswinkel" vergaderings voltooi. Die verkope van die boek tydens die vergaderings het goedgegaan. Die "final draft" van die "Citrus Packhouse Checklist for Auditing" is vir kommentaar gestuur. Die CRI Na-oes Produksie Riglyne is in die proses om opgedateer te word.

7de CRI Sitrusnavorsingssimposium

Die uitstaande gebeurtenis binne Voorligting die afgelope jaar was verseker die 7de Sitrusnavorsingssimposium wat vanaf 19 – 22 Augustus by die Champagne Sports Resort in die Drakensberge aangebied is. Die enigste barometer om te bepaal of dit suksesvol was, is die terugvoer van die teikengroep of gehoor wat dit bygewoon het. Die bywoning was weereens uitstekend met net minder as 500 simposiumgangers en, gemeet aan die terugvoer, kan die simposium as 'n reuse sukses beskou word.

Die simposium is ge-open deur dr Hoppie Nel waarna Dr Tim Gottwald van die USDA in Florida wat as die hoof gasspreker opgetree het 'n lesing aangebied het getiteld: "Biodefence for arboreal pathogens: From incursion prediction to epidemic mitigation, monitoring and management strategies". Tydens hierdie praatjie het hy onder andere die situasie tov Asiatiese vergroening (Huanglongbing) in Florida geskets asook die potensiële gevaar wat dit vir Suid Afrika kan inhou.

Die res van die gassprekers het ingesluit Prof Vaughan Hattingh (Market Access, Biosecurity & the South African Citrus Industry), Prof Martin Hill (Good Science is the key to understanding applied systems) en dr Klaus Eckstein van Bayer Crop Science, die hoofborg (Challenges of developing a modern crop protection remedy), dr G. Stammier (Evaluation of Qol resistance risk of fungal species associated with CBS) en dr Charlene Jewell van California (Novel imazalil application systems in the United States.) Justin Chadwick het die Wêreld Sitrus Uitvoertendense bespreek terwyl Paul Hardman 'n lesing gegee het oor Citrus Consumption and Human Health.

Daar was sowat 55 lesings en 30 plakkate wat deur die CRI groep van navorsers aangebied is om terugvoer te gee oor die vordering wat die afgelope twee jaar gemaak is met navorsing. Uitstaande punte was die lae waarskynlikheid dat daar swartvlekbestandheid teen die strobiliriene kan ontwikkel, die beheer van *Bactrocera invadens* en die na-oes risiko bestuur van VKM met koue-behandeling waarvoor Wayne Kirkman, Sean Moore en Vaughan Hattingh die prys ontvang het vir die beste aanbieding. Die prys vir die beste

plakkaat het gegaan aan P. Sithole en JF Dames vir “Investigating the role of mycorrhizal associated bacteria”.

Die CRI Sitrusnavorsingsimposiums word uitstekend ondersteun deur die onderskeie bedrywe wat betrokke is by die sitrusbedryf. Die volgende instansies het as borge opgetree in 2012: Diamant: Bayer Crop Science, Platinum: SAPPI, Ruby: Arysta Lifescience, Emerald: Yield en River Bioscience, Goud: BASF, Nulandis, Villa Crop Protection, Du Pont, Makteshim Agan, Budget, Laeveld Agrochem, Insect Science, Netafim en Maf Roda. Silwer: XPS, Compac, Agri-Soil, CRI Grondvesblok, Orro Agri, Farmsecure, Philagro, Du Roi Kwekery, Improcrop, PlusNet/Geotex, AV Designers, DOW AgroScience, SASCCON, Corporate Guarentee, Southtrade en die CRI Diagnostiese Sentrum. Brons: ICA, Nampak, Dana, PPECB, Magalies, Jansen PMP, Engen, Sun Valley, Houers Orange River Cellars, Nexus en Esselen Kwekery.

Die enigste ernstige beswaar teen die simposium was die feit dat al die lesings op een na in Engels was. Daar is 'n versoek van die produsente dat meer van die lesings in Afrikaans moet wees aangesien daar meer produsente is wat in die toekoms die simposium sou wou bywoon. Daar sal dus in die toekoms gekyk moet word na vertalers wat tydens die lesings kan optree. Gehoorstukke kan dan verskaf word aan die sowat 50 persone wat nie Afrikaans magtig is nie om na 'n vertaling van die Afrikaanse lesings te luister.

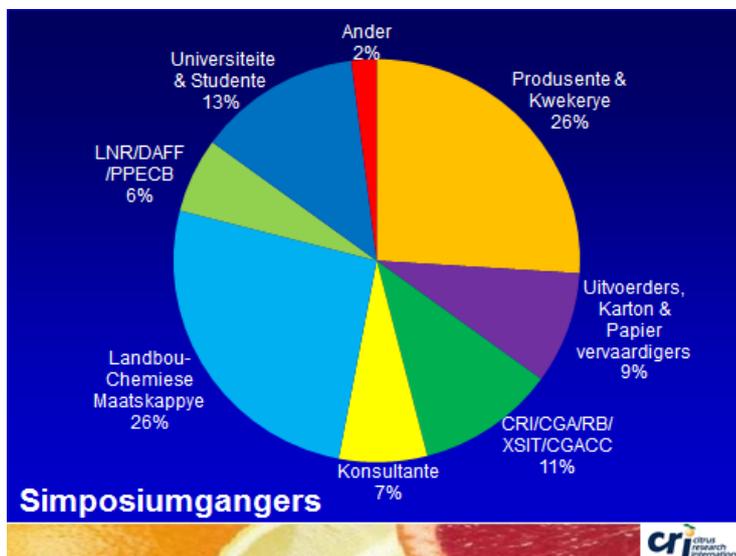


Fig 8.1. Bywoningsamestelling van simposiumgangers.

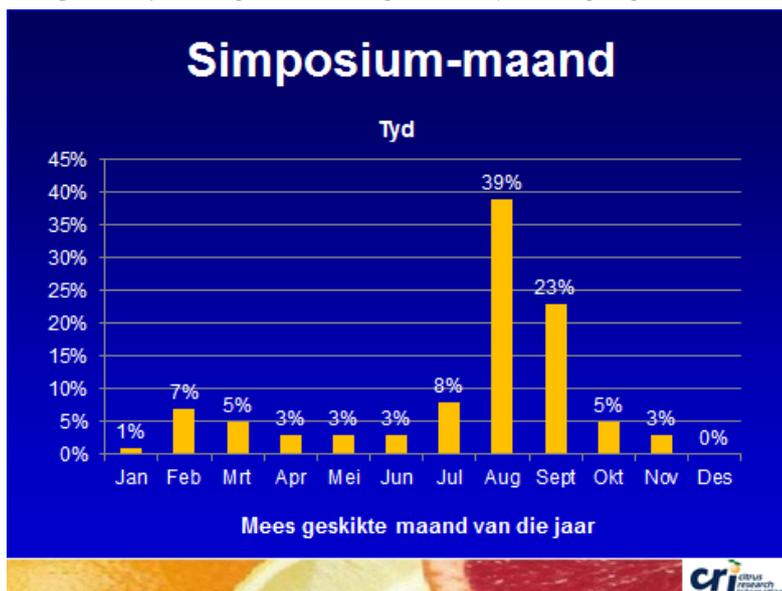


Fig 8.2. Mees geskikte maand om die simposium te hou.

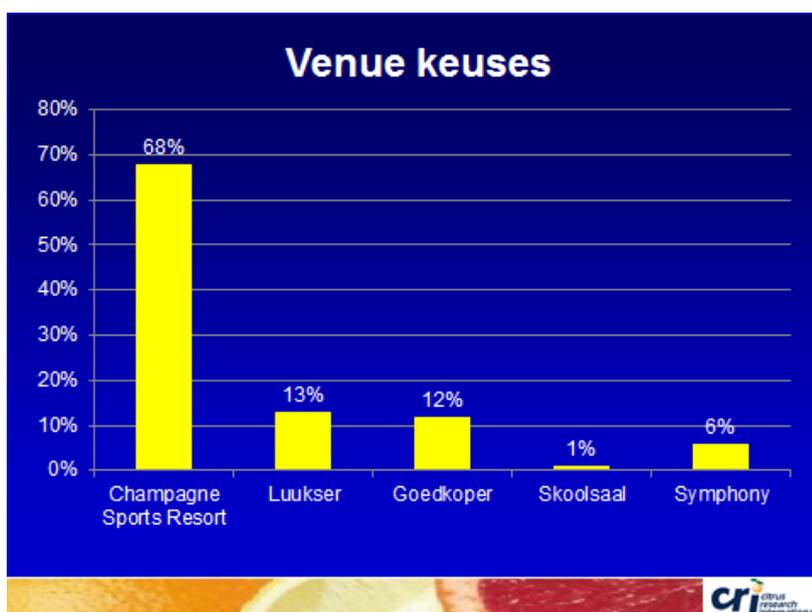


Fig 8.3. Venue vir die volgende symposium.

PROGRAMME / PROGRAM		
VENUE / PLEK: CHAMPAGNE SPORTS RESORT, DRAKENSBERG		
SUNDAY – 19 AUGUST 2012		
YIELD GOLF DAY 10:00 – 17:30 REGISTRATION 14:00 – 18:00 ARYSTA LIFESCIENCE - WELCOME & DINNER 19:00 – 21:00		
MONDAY – 20 AUGUST 2012		
07:30		
08:30	REGISTRATION	
	SESSION 1: INTRODUCTORY SESSION	
	<i>Welcome: Hoppie Nel</i>	
	<i>Global Citrus Trends</i>	
08:30	Welcome to the 7th CRI Citrus Research Symposium	Page
08:40	Hoppie Nel	
08:40	Biodefense for arboreal pathogens: From incursion prediction to epidemic mitigation, monitoring, and management strategies	
09:15	Tim R. Gottwald	
09:15	Market Access, Biosecurity and the South African citrus industry	
09:35	Vaughan Hattingh	
09:35	Good science is the key to understanding applied systems	
09:55	Martin Hill	
09:55	Challenges of developing a modern crop protection remedy	
10:15	Klaus Eckstein	
10:15	TEA/COFFEE + POSTER VIEWING	
	SESSION 2: INTEGRATED PEST MANAGEMENT	
	<i>Chairperson: Sean Moore</i>	
11:00	Fruit Flies: Are we winning the war?	
11:20	Aruna Manrakhan	

11:20 11:35	Learning to control <i>Bactrocera invadens</i> in other parts of Africa Tim Grout, Peter Stephen	
11:35 11:50	Post-harvest false codling moth risk mitigation through cold treatment Wayne Kirkman, Sean Moore, Vaughan Hattingh	
11:50 12:05	Can imidacloprid cause false codling moth repercussions? Sean Moore, Rachel van der Walt, Wayne Kirkman and Derek du Preez	
12:05 12:20	The expansion of the SIT programme for false codling moth suppression, to the Sundays River Valley, E. Cape province Rob Stotter, Eugene Nepgen, Sampie Groenewald	
12:20 12:35	Entomopathogenic fungi for control of soilborne life stages of false codling moth, <i>Thaumatotibia leucotreta</i> (Meyrick) (Lepidoptera: Tortricidae) Candice Coombes, J.F. Dames, M.P. Hill, S.D. Moore	
12:35 13:45	LUNCH	
13:00	CGA Board meeting	
	SESSION 3: DISEASE MANAGEMENT (Fruit + Foliar and Soilborne Diseases) Chairperson : Tian Schutte	
13:45 14:00	Evaluation of QoI resistance risk of fungal species associated with Citrus Black Spot G. Stammler, G.C. Schutte, S. Miessner, J.B. Speakman, P.W. Crous	Page
14:00 14:15	Spray programmes for the control of fruit and foliar diseases in South Africa G.C. Schutte, C. Kotzé	
14:15 14:30	Modelling of <i>Guignardia pseudothecium</i> maturation and ascospore dispersal in citrus orchards Paul Fourie, G.C. Schutte, S. Serfontein, S.H. Swart	
14:30 14:45	Spray deposition benchmarks for control of Alternaria brown spot and evaluation of adjuvants to improve fungicide spray deposition in citrus orchards Gideon van Zyl, G.C. Schutte, P. Fourie	
14:45 15:00	Holistic approach for the control of soilborne diseases on citrus M.C. Pretorius, C. Kotzé, A. McLeod, N. Labuschagne	
15:00 15:15	The effect of compost soil treatments with beneficial organisms on tree condition and general disease resistance W. van der Pypekamp, A. Fourie, J.J. Serfontein	
15:15 15:30	Are mycorrhizal fungi important in the citrus industry? J.F. Dames	
15:30 15:45	Control of <i>Botrytis cinerea</i> Pers. on lemons G.C. Schutte, C. Kotzé	
15:45 16:30	TEA / COFFEE & POSTER VIEWING	
	BREAK AWAY GROUP	
16:30 17:30	South African Subtropical and Citrus Consultants (SASCCON) Tom van der Meulen, et al.	
16:30 17:30	SESSION 4: POSTER VIEWING	
Poster 1	Growing citrus under shade cloth can result in higher pesticide residues at harvest Peter R Stephen and Tim G Grout	Page
2	The distribution of the Woolly whitefly, <i>Aleurothrixus floccosus</i> (Maskell) (Hemiptera: Aleyrodidae), and its parasitoid, <i>Encarsia</i> sp. (Hymenoptera: Aphelinidae), in the Eastern Cape Tanya Pretorius, M.P. Hill and S.D. Moore	
3	Screening of entomopathogenic fungi against citrus mealybug and citrus thrips V. Chartier Fitzgerald, J.F. Dames, M. Hill and S. Moore	
4	Studies on existing and new isolates of <i>Cryptophlebia leucotreta</i> granulovirus (CrleGV) on FCM populations from a range of geographic regions in South Africa John K. Opoku-Debrah, Martin Hill, Sean Moore and Caroline Knox	
5	Delegate *250 WG Johan Janse van Rensburg, Dow Agrosience	
6	Relative attractiveness of virgin female false codling moth from different regions to sterile FCM males Dan Niland, Martin Hill, Tanya Pretorius, Sean Moore and Wayne Kirkman	

7	Ovipositional preferences and host susceptibility of navel orange varieties by the false codling moth, <i>Thaumatotibia leucotreta</i> (Meyrick) (Lepidoptera: Tortricidae) C.N. Love , M.P. Hill and S.D. Moore	
8	Ant bait research moves towards commercialisation Tim G Grout and Kim C Stoltz	
9	Comparison of four methods for isolating RNA from agricultural lepidopteron pests J.A. Ridgeway , M.H. Villet and A. Timm	
10	Augmentation of <i>Anagyrus sp.</i> near <i>pseudococci</i> for control of mealybug on citrus Sean Moore, Wayne Kirkman , Rob Stotter, Moshe Cohen, Shimon Steinberg and Rami Friedman	
11	Controlling false codling moth and fruit fly with entomopathogenic nematodes Sean Moore , Ralf-Udo Ehlers, Aruna Manrakhan, Wayne Kirkman, John-Henry Daneel, Jean de Waal, Paul De Wet and Antoinette P. Malan	
12	An investigation into a means of mass-transporting facility-reared irradiated false codling moths Rob Stotter , Eugene Nepgen, Sampie Groenewald	
18:00	CGA, CRI & River Bioscience's AGM	
18:30	RIVER BIOSCIENCE - HAPPY HOUR	
	TUESDAY – 21 AUGUST 2012	
	BREAK AWAY GROUP	
08:00	South African Citrus Nurserymen's Association (SACNA) Annual General Meeting Peter Kingston et al.	
	SESSION 5: DISEASE MANAGEMENT (Post Harvest) Chairperson: Paul Fourie	
08:00 08:25	Novel imazalil application systems in the United States Charlene Jewell	Page
08:25 08:40	Practical impact of imazalil resistance on control of postharvest citrus green and blue mould Arno Erasmus , V. Rikhotso, C.L. Lennox, K. Lesar, P.H. Fourie	
08:40 08:55	Evaluation of curative and protective control of <i>Penicillium digitatum</i> following imazalil application in wax coating N.S. Njombolwana , A. Erasmus, P.H. Fourie	
08:55 09:10	Thiabendazole residue loading and control of green mould and chilling injury on citrus fruit M. Kellerman , N.S. Njombolwana, A. Erasmus, P.J.R. Cronjé, P.H. Fourie	
09:10 09:25	Development of a citrus hygiene management plan for reduction of <i>Penicillium</i> decay S.B. Coetzee , Lise Korsten	
09:25 09:40	A Heated Imazalil Flooder – New technology for South African packing houses W. du Plooy , A. Erasmus, C. Jewell, P. Fourie	
09:40 09:55	Optimal use of pyrimethanil, a new postharvest fungicide, for the control of green mould on citrus in South Africa E. Liebenberg , A. Erasmus, P.H. Fourie	
09:55 10:10	Use of potassium silicate and biocontrol agents to reduce postharvest disease and chilling injury in citrus fruit N. du Rand , M. Laing	
10:10 11:00	TEA / COFFEE & POSTER VIEWING	
	SESSION 6: HORTICULTURE (Post Harvest) Chairperson: Tim Grout	
11:00 11:15	Could ethylene metabolism in lemon fruit influence peteca spot incidence? P.J.R. Cronjé	
11:15 11:30	Postharvest application of thiabendazole reduces chilling injury of navel citrus fruit Jeanine Hordijk , U.L. Opara, P.J. R. Cronjé	
11:30 11:45	Postharvest rind disorders of 'hardorcott' mandarin are affected by rootstock in addition to postharvest treatments P.J.R. Cronjé	
11:45 12:00	Computer simulation and experimental studies on airflow patterns and fruit cooling rates inside different designs of citrus packaging Linus Opara , M. Delele & P.J.R. Cronjé	

12:00 12:15	Managing airflow inside reefer containers benefits citrus fruit temperatures and relative humidity of the storage air Malcolm Dodd	
12:15 13:15	SESSION 7: HORTICULTURE (Pre-Harvest) Chairperson: Paul Cronjé	
12:15 12:30	Novel usage of 2,4-D to increase citrus fruit quality O.P.J. Stander, K.I. Theron, P.J.R. Cronjé, J.S. Verreyne	
12:30 12:45	Phenology of Alternate Bearing 'hardorcott' Mandarin trees I.S. van der Merwe, K.I. Theron, P.J.R. Cronjé, J.S. Verreyne	
12:45 13:00	Effects of potassium humates and fulvate on the leaching of N, P and K A.J. Gatabazi, P.C. de Jager, J.T. Vahrmeijer	
13:00 13:15	Silicon uptake in citrus and the validation of an analytical method N.M. Asanzi, N.J. Taylor, J.T. Vahrmeijer	
13:15 14:00	LUNCH	
14:00	SESSION 8: CULTIVARS Chairperson: Hannes Bester	
14:00 14:15	Establishment of a molecular genotype reference database for mandarin cultivar verification A.A. Severn-Ellis, A. Sippel, N. Combrink, Z. Dlamini, B. Manicom	
14:15 14:30	Cultivar maturity charts for specific citrus production regions (northern areas) J.J. Joubert	Page
14:30 14:45	Cultivar maturity charts for specific citrus production regions (southern areas) R. Fenwick	
14:45 15:00	Future cultivar recommendations based on cultivar selection and adaptation in diverse environments Z. Bijzet, N. Combrink, A. Sippel, M. Booyse	
15:00 15:15	Flow cytometry – a novel method for identifying new citrus rootstock hybrids K. Hannweg, G. Visser, Z. Bijzet, N. Combrink, A. Sippel	
15:15 15:30	Induction of new favourable traits in citrus by using mutation breeding I.J. Froneman, J. Husselman, J. Maritz, C. Human, S. Willemse, A. Sippel	
15:30 15:45	Inheritance data is essential to a successful citrus breeding programme N. Combrink, Z. Bijzet, A. Sippel, M. Booyse	
15:45 16:30	TEA / COFFEE + POSTER VIEWING	
16:30	SESSION 9: VIROLOGY Chairperson: Glynnis Cook	
16:30 16:45	<i>Citrus Tristeza Virus</i> cross-protection of Star Ruby grapefruit: field trial results J.H.J. Breytenbach, S.P. van Vuuren, G. Cook	
16:45 17:00	CTV diversity in southern African grapefruit orchards and the improvement of cross-protection D.A. Read, S.P. van Vuuren, G. Pietersen	
17:00 17:15	Investigation into the seasonal population fluctuation of <i>Trioza erytraeae</i> and infection with the greening organism, <i>Candidatus Liberibacter africanus</i> G. Cook, V. Maqutu, S.P. van Vuuren	
17:15 17:30	Alternative hosts of <i>Candidatus Liberibacter africanus</i> amongst indigenous members of the Rutaceae in South Africa R. Viljoen, E.T. Steenkamp, G. Pietersen	
17:30 17:45	<i>Citrus Tristeza Virus</i> in South Africa: geno-type diversity and development of an unbiased Illumina sequencing pipeline O.D.J. Zablocki, G. Pietersen	
17:45 18:00	Characterization of two candidate pre-immunizing <i>Citrus Tristeza Virus</i> sources J.W. Lubbe, G. Pietersen	
19:00 for 19:30	SAPPI GALA DINNER	
	WEDNESDAY – 22 AUGUST 2012	
08:00 09:00	SESSION 10: POSTER OVERVIEW SESSION	
Poster 13	CropLife SA's key activities Tom Mabesa, CropLife/AVCASA	Page

14	Citrus Black spot free production areas in South Africa Elma Carstens , HF le Roux, L van Rooyen, J Coetzee, R Wentzel, GC Schutte, W Laubscher, Z Dawood, PH Fourie, A Mcleod and V Hattingh	
15	Resistance of citrus rootstocks against <i>Phytophthora</i> root rot M. Sakupwanya , N Labuschagne & Z Apostolides	
16	'n Holistiese benadering vir die vermindering van <i>Guignardia citricarpa</i> askospoor inokulum SH Swart, JJ Serfontein & A Fourie	
17	The Diagnostic Centre (CRI): A diagnostic service to the citrus industry Elaine Basson , Timothy Zulu and MC Pretorius	
18	Investigating the role of mycorrhizal associated bacteria P. Sitole and J.F. Dames	
19	Evaluation of Break-Thru S240 and Break-Thru Union at Different Spray Volumes in South African Citrus Orchards Johannes G. van Zyl , Dave Viljoen, and Paul H. Fourie	
20	Evaluating sweet orange clones for greening resistance Fanie van Vuuren , Glynnis Cook & Kobus Breytenbach	
21	Influence of potassium humates and fulvate on the culturable soil microbial community AJ Gatabazi , A Van der Merwe, PC de Jager, JT Vahrmeijer	
22	Differential susceptibility to peel pitting in fruit from citrus cultivars with different albedo thickness Paul. J.R. Cronjé , Fernando Alférez and Lorenzo Zacarías	
23	Citrus rootstock breeding at the ARC-ITSC and selections for further evaluation J.H. Husselman , Z. Bijzet, N.K. Combrink, J.G.J. Maritz, L.P. Zuma and A.D. Sippel	
24	Comparison of late Mandarin cultivars in the ARC-ITSC'S Citrus Cultivar Evaluation programme at the ADDO Research Station J.G.J. Maritz , N.K. Combrink, L.P Zuma, Z. Bijzet and A.D. Sippel	
25	Seed stimulation in two citrus mandarin cultivars through cross pollination R.B. Cronje , J.G.J., Maritz, N.K., Combrink, C.F., Human , I.J., Froneman & A.D. Sippel	
29	Improvements to the CRI Extension model Hennie le Roux , Hannes Bester, Andrew Mbedzi, Melton Mulaudzi, Dawid Groenewald & Keith Lesar	
30	CRI Organogram incorporating the CRI Group Henry Skinner	
SESSION 11: FUTURE PROSPECTS Chairperson: Hennie le Roux		
09:00	World Citrus Export Trends	
09:20	Justin Chadwick (CGA)	
09:20	Citrus Consumption and Human Health: Literature Review	
09:40	Paul Hardman (CGA)	
SESSION 12: HORTICULTURE (Pre-Harvest Con't.) Chairperson: Paul Cronjé		
09:40	Citrus water use: Quo Vadis?	
09:55	J.T. Vahrmeijer , J.G. Annandale, M.B. Gush, G.R. Backeberg, N.J. Taylor	
09:55	Understanding the dynamics of citrus water use	
10:10	N.J. Taylor , J.G. Annandale, M.B. Gush, J.T. Vahrmeijer	
10:10		
11:00	TEA / COFFEE + POSTER VIEWING	
SESSION 13: INTEGRATED PEST MANAGEMENT Chairperson: Aruna Manrakhan		
11:00	Movement of entomopathogenic nematodes in different soil types	
11:15	Antoinette Malan , Sean Moore	
11:15	Mass culturing <i>Steinernema yirgalemense</i> using <i>in vitro</i> liquid technology	
11:30	Tiarin Ferreira , Antoinette Malan	
11:30	Improved taxonomic understanding and the development of a LUCID key for tortricid moth pests in South Africa	
11:45	Monique Rentel , Pia Addison, H. Geertsema, J.W. Brown	
11:45	The control of <i>Planococcus citri</i> , the citrus mealybug, using entomopathogenic nematodes	
12:00	Sonnica van Niekerk , Antoinette Malan, Sean Moore	

12:00 12:15	Biochemical responses of false codling moth, <i>Thaumatotibia leucotreta</i> , to low temperature and controlled atmospheres Leigh Boardman, J.G. Sørensen, T. Grout, J.S. Terblanche	
12:15 12:30	Examining field ecology and diapauses in false codling moth, <i>Thaumatotibia leucotreta</i> (Lepidoptera: Tortricidae) Zoë de Jager, John Terblanche, Pia Addison	Page
12:30 12:45	Volatile emissions as a tool for detection of false codling moth infestation in Navel oranges Rachel van der Walt, Sean Moore, Melissa Gouws, Vaughan Oosthuizen, Ben Zeelie	
12:45 13:00	SESSION 14: CLOSING SESSION Hannes Bester	
13:00	Take-away lunches will be provided	

CRI Voorligtingswerkswinkels

As gevolg van die sukses van die vyf CRI Pakhuiswerkswinkels, nou bekend as die CRI Na-oes Werkswinkels, wat elke jaar gedurende Februarie in Limpopo, Mpumalanga, KwaZulu-Natal, die Oos-Kaap en die Wes-Kaap aangebied word, is besluit om in elke streek twee addisionele CRI Voorligtingswerkswinkels per jaar aan te bied waartydens voor-oes tegnologie oorgedra sal word. Hierdie drie werkswinkels wat elk in vyf van die belangrikste sitrusproduserende streke gehou sal word sal tot 'n groot mate die rol van die CRI sitrusstudiegroepe vervang. Die studiegroepe bly egter voortbestaan indien daar gebiede is wat die behoefte het dat CRI Voorligting vir hulle sprekers moet reël om spesifieke aangeleenthede aan te spreek en om jaarliks die verskillende areas se Navorsingsprioriteite te bepaal.

Die eerste rondte hiervan is gedurende September in Mpumalanga en Limpopo aangebied om terugvoer oor die 7de Sitrusnavorsingssimposium te gee, asook die lenteploeg-kompleks en sitruswartvlek te dek. Soortgelyke werkswinkels is gedurende Oktober in die Oos- en Wes-Kaap aangebied. Hoewel die bywoning nie bevredigend was in Mpumalanga nie, agv kort kennisgewing, botsende aktiwiteite en onkunde oor presies wat die werkswinkels behels, was die terugvoer uitstekend en was daar versoeke om dit by sekere studiegroepe weer aan te bied. Die versoeke is ook baie duidelik dat daar in die toekoms met hierdie werkswinkels voortgegaan moet word. Dis duidelik dat, soos in die geval met die Pakhuis-werkswinkels, hierdie Voorligtingswerkswinkels ook oor tyd opgebou sal moet word.

Die CRI Na-oes Werkswinkels is gedurende Januarie en Februarie in die vyf groot sitrusstreke aangebied. Die wye reeks onderwerpe wat gedek is, het goeie terugvoer ontlok en bywoning was, soos verlede jaar, uitstekend in al die areas met 670 persone wat dit bygewoon het. Citrosol/Wenkem was die hoofborg en, agv die sukses van die die werkswinkels, het hulle reeds versoek dat hulle volgende jaar weer die hoofborg wil wees. Borgskappe was ruim en die borge voel dat hulle goeie blootstelling gekry het.

Die CRI Produksie Werkswinkels word gedurende Mei en Junie aangebied. Die uitdaging is om hierdie werkswinkels net so suksesvol as die CRI Na-oes werkswinkels te maak en maksimum bywoning te probeer kry. Die werkswinkels word in die grootste produksiestreke gehou, nl Letsitele vir Limpopo, Nelspruit vir Mpumalanga, Sondagsriviervallei vir die Oos-Kaap en Citrusdal vir die Wes-Kaap. KZN & Swaziland verkies om hul werkswinkels in Durban te hou. In teenstelling met die CRI Na-oes werkswinkels wat gevestig is, is borgskappe by die CRI Produksie Werkswinkels nog 'n probleem.

Internasionale Sitrus Kongres (ICC) in Valencia (Spanje)

Verskeie van CRI se personeel het die ICC in Spanje bygewoon. CRI se navorsers het lof ontvang vir die hoogstaande gehalte van hul aanbiedings en plakkaat. Dit kan as 'n baie suksesvolle kongres beskou word waar goeie kontakte opgebou is en waar Suid-Afrika met reg trots kon voel oor die gehalte van die navorsing wat hier gedoen word.

Die voorkongres toer het verskeie interessante besoeke aan plase opgelewer, asook aan 'n indrukwekkende sap-aanleg, die grootste van sy soort in Europa. 'n Dag is ook saam met Citrosol spandeer waartydens hul fasiliteite besoek is, asook pak-aanlegte waar van hul produkte en toerusting gebruik word. Citrosol beplan om aggressief in Suid-Afrika uit te brei in vennootskap met Wenkem, en hulle het gesamentlik as die hoofborg vir die CRI Na-oes Werkswinkels opgetree.

Swartbemaatiging (BEE)

BEE binne die sitrusbedryf bly steeds 'n groot uitdaging. Ten spyte van die uiters goeie werk wat deur die twee Voorligters, Andrew Mbedzi en Melton Mulaudzi gedoen word, bly die uitdagings meer as die oorwinnings. Dit verg steeds Salomo se wysheid om saam met die onderskeie provinsiale departemente van landbou te werk. Die meeste sukses word behaal in die Limpopo Provinsie met 'n mate van sukses in die

KwaZulu-Natal provinsie. Beide die Mpumalanga en die Oos-Kaap Provinsies het tot op hierdie stadium nog nie samewerkingssooreenkomste met die CGA tov Voorligting gesluit nie. Hopelik sal die Mpumalanga Departement van Landbou die samewerkingssooreenkoms in Mei 2013 teken. Die werk wat deur die Voorligters tot op hede in hierdie gebiede gedoen is, is hoofsaaklik sonder enige insette van die provinsie gedoen.

CRI is ook versoek om behulpsaam te wees met die sogenaamde "Extension Recovery Plan" van die regering. Die bedoeling met hierdie plan is goed, maar dit sal geen positiewe resultate oplewer indien hulle dit wat hulle aanvanklik be-oog het gaan uitvoer nie. CRI het insette gelewer wat betref ons siening van die redes waarom transformasie binne die landboubedryf misluk. Dit is beslis nie weens 'n gebrek aan mannekrag nie, maar 'n gebrek aan kundigheid, werkstrots en middele om die werk te kan doen.

Die regering het ook verskeie van die bemagtigingslandgoedere met miljoene rande geherkapitaliseer, maar geen geld bewillig vir die onderskeie mentors nie. Die gevolge hiervan is dat die geld nie noodwendig op die mees sinvolle wyse gespandeer gaan word nie en herkapitalisasie waarskynlik oor 'n jaar of twee weer nodig gaan wees.

Opsomming van Voorligtings aktiwiteite: April – Junie 2012

Datum	Studiegroep/ Aktiwiteit	Onderwerpe/Aksies	Betrokkes/ Sprekers
2 Apr 2012	Pakhuisbesoeke: Southern Fruit Growers PSB Unifrutti	FCM-EU Systems Approach	Sean Moore Hannes Bester
	Vergadering met Ndala van die MEC se kantoor in Nelspruit	IOCV simposium	Gerhard Pietersen Glynnis Cook Ethne Cameron Hennie le Roux
3 Apr 2012	Vergadering met New Era Packaging Bestuur	Beskikbaarheid van Linerboard en Fluting	Dawid Groenewald
	Besoek die Empangiswene gemeenskap naby Glückstadt	Beoordeel die geskiktheid om sitrus te vestig	Hennie le Roux Andrew Mbedzi
4 Apr 2012	Vergadering met Josef Malan en Andrew Cooper Naranja Burgersfort	Imazalil en TBZ residu oorskrydings agv. swak waksaanwending	Keith Lesar
16 Apr 2012	Sondagsrivier boord-besoeke	Kultivar-besigtiging met Citrusdal produsente	Hannes Bester Jonny Roberts Andy Lee Richard Fenwick
16 Apr 2012	Vergadering met Houers en Sappi	Papier voorsiening en Stackraft proewe	Dawid Groenewald
	Hoedspruit Boerevereniging	Waternavorsings- kommissie verg	Hennie le Roux
	Letsitele	Ondersoek "Blossom – end –clearing" by Mahela	Hennie le Roux
17 Apr 2012	Patensie boordbesoeke	Kultivar-besigtiging met Citrusdal produsente	Hannes Bester Jonny Roberts Andy Lee Richard Fenwick
17 Apr 2012	Vergadering met Capespan, Tzaneen	Japanese T64 Supervent proef	Dawid Groenewald
17 Apr 2012	Vergadering met James Warrington, Barry Schiever and Andrew Muller by Karino Koop	Konsultasie oor chloor en swamdoder behandelings	Keith Lesar
18 Apr 2012	DAFF meeting in Pretoria (Transformation)	PPWG meeting	Hennie le Roux, Lukhanyo

			Andrew Mbedzi
19 Apr 2012	Vergadering met Nic v Wyk by Joubert en Seuns pakhuis	Konsultasie oor bestuur van chloor, swamdoder en waks aanwendings	Keith Lesar
19 Apr 2012	CFB	CFB evaluasie	Hannes Bester Thys Du Toit Michelle Johan Joubert Richard Fenwick
	JBT vergadering by CRI	Oorsese besoekers van JBT besoek CRI om te bepaal wat is die moontlikhede om die sapbedryf uit te brei	Hennie le Roux
23 Apr 2012	Vergadering met Chris Thompson van Laeveld Agrochem	Verkeerde na-oes aanbevelings	Keith Lesar
23 Apr 2012	Vergadering met Danie Strooh van Schoeman Boerdery	Identifikasie van moontlike Endokserose/Boor te kort op Schoeman suurlemoene	Keith Lesar Hennie le Roux
24 Apr 2012	Watervorsings-kommissie	Waterverbruiks-werkswinkel	Teunis Vahrmeijer Tim Grout Hennie le Roux Hannes Bester Dr Backeberg Prof Annandale Dr Nicky Taylor
3 Mei 2012	Vergadering met Schalk Visser van Wenkem	Citrosol sitrus wakse, Citrocide en Fortisol	Keith Lesar Arno Erasmus
3 Mei 2012	Beplanning van opleidings-DVD	Snoei	Hannes Bester Jacomien De Klerk
3-4 Mei 2012	Magalies	Ekonomie van sitrus verwerking	Hennie le Roux
7 Mei 2012	Katrivier/Riverside pakhuis: Katco, Riverside en Eden Agri	Besoeke/konsultasie	Keith Lesar
	SAPPI vergadering by CRI	Borgskap van die 7de Sitrusnavorsingsimposium	Hennie le Roux Henry Skinner Jean de Gasperi Louw van Wyk
8 Mei 2012	Sondagsrivier/Kirkwood pakhuis: Vergadering met SRCC pakhuis, Sun River Sitrus, Safe Kirkwood en Son Sitrus	Besoeke/konsultasie	Keith Lesar
8 Mei 2012	Patensie Pakhuis: PSB Southern Fruit Growers	Vrugtevlieg afkeurings	Hannes Bester
9 Mei 2012	Vergadering met Nampak Bestuur.	Akkrediasie Proses en lokale Nampak Fluting	Dawid Groenewald.
9 Mei 2012	Sondagsrivier/Kirkwood pakhuis: Sitrus Rand, Good Hope organiese pakhuis, Summerville, Cape Citrus, Atwell Citrus en Hankey	Besoeke/konsultasie	Keith Lesar
10 Mei 2012	Patensie Pakhuis: Ventershoek, Endulini, SFG (Southern Fruit Growers),	Besoeke/konsultasie	Keith Lesar

	Oorlogspoort en vergadering met Martina en Kobus Oordendal		
10 Mei 2012	Boordbesoek Niel Mellville	Swak prestasie van jong bome	Hannes Bester Thys Du Toit Paul Fourie
	Schoeman Boerdery Groblersdal	Ondersoek endokserose probleem	Paul Cronje Hennie le Roux
	Tian Kruger Boerdery	Ondersoek Phytophthora wortelvrot probleem	Hennie le Roux Dave Woods
11 Mei 2012	Besoek Schoeman Boerdery boorde en pakhuis	Vergesel UP ingenieurs student	Hennie le Roux Jaco Burger Frans Olivier
14 Mei 2012	Swellendam Studie-groep	Bemarkingsuitdagings Kultivars SVS Phytophthora River Bioscience	Hannes Bester Jonny Roberts Hannes Bester Hannes Bester Keith Dankwerts
	Casmar Kwekery Mooinooi	Ondersoek kwekerypraktyke	Thys du Toit Peter Kingston Hennie le Roux
15 Mei 2012	Boland Studiegroep	Bemarkingsuitdagings Kultivars SVS Phytophthora River Bioscience	Hannes Bester Jonny Roberts Hannes Bester Hannes Bester Keith Dankwerts
	Citrusdal Studiegroep	Bemarkingsuitdagings Kultivars SVS Phytophthora River Bioscience	Hannes Bester Jonny Roberts Hannes Bester Hennie le Roux Jacques Fouché
16 Mei 2012	Benede-Oranjerivier Studiegroep	Bemarkingsuitdagings Kultivars SVS Phytophthora River Bioscience	Hannes Bester Jonny Roberts Hannes Bester Hannes Bester Jacques Fouché Keith Dankwerts
16 Mei 2012	Strategiese sessie met Visy personeel van Australia	Invoer van Visy papier	Dawid Groenewald
	Stephan Kamburov Pretoria	Rakende die afhandeling van sy sitrus boek	Hennie le Roux
17 Mei 2012	Vaalharts Studiegroep	Bemarkingsuitdagings Kultivars SVS Phytophthora River Bioscience	Hannes Bester Jonny Roberts Hannes Bester Hannes Bester Jacques Fouché Keith Dankwerts
	Virologie vergadering Nelspruit	Bespreek algemene problem in industrie bv effek van GPE op produktiwiteit	Hennie le Roux Fanie v Vuuren Glynnis Cook Paul Fourie
18 Mei 2012	Water Research Commission	Opvolgvergadering	Teunis Vahrmeijer Hennie le Roux Dr Backeberg
21 Mei 2012	Bayer vergadering in Isando	Bespreek aangeleenthede rakende die 7de Sitru navorsingsimposium	Hennie le Roux Henry Skinner Jean de Gasperi Dirk Uys Willem vd Pypekamp
22 Mei 2012	Exporters Technical Panel	Agenda	Dawid Groenewald

	Meeting		Keith Lesar Sean Moore Hannes Bester
23 Mei 2012	Swellendam Pakhuise: Thornlands, Swellenfruit en Suiderland	Besoeke/konsultasie	Keith Lesar
	Bacterocera invadens vergadering in Hectorspruit	Bespreking van "delimiting surveys"	Aruna Manrakhan Hennie le Roux
24 Mei 2012	Piketberg: Piketco pakhuis	Besoek/konsultasie	Keith Lesar
24 Mei 2012	Letsitele plaasbesoeke: Mahela Boerdery Christie Landman	Ondersoek Phytophthora problem op Juvells & kommersiele snoei	Hannes Bester Hennie le Roux
28 Mei 2012	Brits: Beplande Snoei-DVD	Opname van Snoei-DVD - (Gekanselleer) Boordbesoeke	Hannes Bester Hennie le Roux
	CBS proef van Elma Carstens	Monitering	Elma Carstens Hannes Bester Hennie le Roux
28 Mei 2012	Schoeman Boerdery	Ngodwana Stackraft proef	Dawid Groenewald
29 Mei 2012	Nelspruit Studiegroep	Snoei Kultivar-opsies P्रेसisie Boerdery Bemesting	Hannes Bester Hennie le Roux
29 Mei 2012	Schoeman Boerdery	Ngodwana Stackraft proef	Dawid Groenewald
30 Mei 2012	Croc Valley Pakhuis	Skil problem op nawels	Keith Lesar
31 Mei 2012	SASCCON Jaarvergadering	Agenda	Hennie le Roux Hannes Bester
5 Jun 2012	Clanwilliam Packhouses: Clanfresh (Dirkie Mouton), Namaqualand Sitrus (Tobias Basson), Groot Patrysvlei (Renshia Visser) en Oudam (Braum Marais)	Besoeke/konsultasie	Keith Lesar
5 Jun 2012	Arysta vergadering in Nelspruit	Bespreek aspekte rakende borgskap vir simposium	Hennie le Roux Henry Skinner Jean de Gasperi
6 Jun 2012	Citrusdal Pakhuise: Noordhoek (Hardy vd Merwe), ALG (Gerrit vd Merwe), Citrus Select (Jan Coetzer), New Season (Martin Esterhuizen, Patrysborg (Jannie Toerien), Groenkloof en Goedehoop	Besoeke/konsultasie	Keith Lesar
	PlusNet vergadering in Nelspruit	Bespreek aangeleenthede rakende die simposium & borgskap	Hennie le Roux Henry Skinner Jean de Gasperi
7 Jun 2012	Ashton/Robertson Pakhuise: Unipack, Sonskyn (Rabie Broers), Rosedale (Britz Broers) en Bon Courage	Besoeke/konsultasie	Keith Lesar
7 Jun 2012	Durban koelkamers	Laai van Ngodwana Stackraft proef	Dawid Groenewald.
	Esselen Kwekery Malelane	Onderstam & Kultivar dag	Johan Joubert Hennie le Roux Chris Kellerman
8 Jun 2012	Transformasieverg in Hillcrest	Bespreking van CGA se betrokkenheid by	Hennie le Roux Justin Chadwick

		Empangisweni met ADA & KZNDA	Lukhanyo
11 Jun 2012	Sondagsriviervallei	CRI Werkswinkels: Ken Niewenhuizen Dave Gerber Pieter Nortje	Hannes Bester
	Besoek Mahela en Laeveld Sitrus. Besoek Indigo	Ondersoek "Blossom End Clearing" Ondersoek gomsakkies op Nadorcotts	Paul Cronje Hennie le Roux
12 Jun 2012	Crates for Africa, Benoni	Navorsingswerk op Plastiese verpakking	Dawid Groenewald
	Besoek Wolkberg Sitrus in Vivo Besoek Zebediela	Ondersoek kraakskil op nawels	Paul Cronje Hennie le Roux
14 Jun 2012	Dole, Sitco en Farmsecure, Bellville.	Inligting vesameling en voorbereiding vir karton gewig/ kodes vergadering	Dawid Groenewald
15 Jun 2012	Carton Weight Comitee meeting	Carton weights Carton codes	Dawid Groenewald Keith Lesar Hannes Bester
18 Jun 2012	Patensie Studiegroep	Bemarkingsuitdagings Kultivars SVS Phytophthora River Bioscience	Hannes Bester Hannes Bester Hannes Bester Hannes Bester Jacques Fouché
	Baviaans Studiegroep	Bemarkingsuitdagings Kultivars SVS Phytophthora River Bioscience	Hannes Bester Hannes Bester Hannes Bester Hannes Bester Jacques Fouché
	CCCF	Vergadering om op hoogte van vordering te kom	Dawid Groenewald Hennie le Roux
19 Jun 2012	Sondagsrivier Studie-groep	Bemarkingsuitdagings Kultivars SVS Phytophthora River Bioscience	Hannes Bester Jonny Roberts Hannes Bester Hannes Bester Jacques Fouché Keith Dankwerts
19 Jun 2012	Letsitele Pakhuise: Laeveld Sitrus en Mahela	Besoeke/konsultasie	Keith Lesar
	CRI Raadsvergadering in Kempton park	Jaarlikse CRI Raadsvergadering	Vaughan Hattingh Hennie le Roux Tim Grout
20 Jun 2012	Letsitele Pakhuise: Groep 91, Rooister en Merite	Besoeke/konsultasie	Keith Lesar
20 Jun 2012	Katrivier Studiegroep	Marketing challenges Cultivars CIS Phytophthora River Bioscience	Hannes Bester Jonny Roberts Hannes Bester Hannes Bester Jacques Fouché
21 Jun 2012	Hoedspruit Pakhuise: BJ Blyde, Richmond en Canyon	Besoeke/konsultasie	Keith Lesar
23 Jun 2012	Rustenburg Studie-groep	Sitrusproduksie Bemesting Besproeiing Snoei Finansies	Hennie le Roux Hannes Coetzee Chris Barnard Hannes Bester Afgri Capital
28 Jun 2012	Sappi Technology Centre	Akkrediasie van karton vervaardigers.	Dawid Groenewald.

Opsomming van Voorligtings aktiwiteite: Julie – September 2012

Datum	Studiegroep/ Aktiwiteit	Onderwerpe/Aksies	Betrokkeses/ Sprekers
4 Jul 12	Burgersfort pakhuis: Morone, Naranja, Waterval Sitrus en PLM Boerdery	Pakhuisbesoeke en konsultasies	Keith Lesar
5 Jul 12	STC - Nampak Rosslyn Fluting	Toets van kartonne wat met Nampak "fluting" vervaardig is	Dawid Groenewald
	Bloempoot	Kultivars	Hennie le Roux Johan Joubert
6 Jul 12	CLF, KZN Universiteit	CLF Werkswinkel	Dawid Groenewald
11 Jul 12	Pretoria Mark..ETP versoek	Voorlopige werk op korter hoekstukke.	Dawid Groenewald
17 Jul 12	IOCV	Beplanningsvergadering vir 2013 se internasionale kongress	Gerhard Pietersen Hennie le Roux Paul Fourie Glynnis Cook Fanie van Vuuren MC Pretorius Ethne cameron
9-12 Jul 12	Benede-Oranjerivier	Besoeke aan individuele produsente rakende kultivars en produksie-praktyke	Hannes Bester Jonny Roberts
17 Jul 12	Nelspruit pakhuis: Joubert en seuns, Karino en Croc Valley	Pakhuisbesoeke en konsultasies	Keith Lesar
18 Jul 12	STC/Houers Akkreditasie	Ontleding van toets resultate en sny van prototipe karton op rekenaar tafel.	Dawid Groenewald. Frikkie van Wyk
18 Jul 12	Ohrigstad Studiegroep	Snoei-werkswinkel	Hennie le Roux Hannes Bester
19 Jul 12	Voorligtingvergadering	Voorligting	Vaughan Hattingh Hennie le Roux Hannes Bester
20 Jul 12	Menlyn, Pta. Mpack Paper	Vergadering met Mpack Paper oor Bay Plex fluting vir sitrus	Dawid Groenewald Karin Kruger
23 Jul 12	STC Pretoria.	Ontwerp en sny van Pallet Template	Dawid Groenewald Francois Stein
23 Jul 12	Citrusdal	Produsentebesoeke: At Venter (GHS) Johan Mouton Jaco Olivier	Hannes Bester
	Stellenbosch	Corporate Guarantee: Selfversekering in Landbou Simposium borgskap	Hannes Bester
25 Jul 12	CFB	CFB Evaluasie	Hannes Bester Res van komitee
25 Jul 12	Schoeman Boerdery en Engelbrecht Trust	Korter hoekstuk proef en werk "spot gluing"	Dawid Groenewald Frikkie van Wyk
25 Jul 12	Letsitele	Syngenta HLB	Hennie le Roux
26 Jul 12	Uitenhage	SVS Jaarvergadering	Vaughan Hattingh Paul Fourie Hennie le Roux Thys du Toit
27 Jul 2012	Pretoria Universiteit	Prof Stroh	Hennie le Roux
30 Jul 12	Nelspruit	MBB vergadering re Champagne Landgoed	Hennie le Roux Andrew Mbedzi
30-31 Jul 12	Nelspruit	Villa	Vaughan hattingh Tim Grout

			Hennie le Roux Hannes Bester Tian Schutte MC Pretorius Arno Erasmus
31 Jul 12	Simposium beplan- ningsvergadering	Simposium	Hennie le Roux Henry Skinner Jean De Gasperi Hannes Bester
31 Jul 12	Groblersdal/MarbleHall pakhuis: Boschkrans, Diphale Sitrus, Schoonbee en Roslé	Pakhuisbesoeke en konsultasies	Keith Lesar
1 Aug 12	Groblersdal/MarbleHall pakhuis: Schoeman Boerdery, Tambuti en Piet Engelbrecht Trust	Pakhuisbesoeke en konsultasies	Keith Lesar
2 Aug 12	Groblersdal pakhuis: Roslé MarbleHall pakhuis: MarbleHall Sitrus, Gert Kruger Boerdery en Piet Engelbrecht Trust (opvolg)	Opvolg besoek aan Roslé ivm. ongeregisteerde chemikalie en pakhuis- besoeke en konsultasies	Keith Lesar
2 Aug 12	Mpumalanga DA, Nelspruit	MoU between MDA, CGA & CRI	Lukhanyo Andrew Mbedzi Hennie le Roux
7 Aug 12	Willow Park Lodge, Benoni	Bywoning van die CMF vergadering	Hennie le Roux Dawid Groenewald
8 Aug 12	Nampak Rosslyn	Samesprekings oor tipiese waardes van Nampak Fluting	Raymond Lund Johan Nell Dawid Groenewald
8 Aug 12	Brits	Besoek Sanddrif BEE projek saam met DAFF	Andrew Mbedzi Hennie le Roux
9 Aug 12	Kroondal	Angola vergadering re invoer van bome uit Brasilië	Hennie le Roux Japie Krynauw
11 Aug 12	Brits	Magalies re Simposium borgskap	Hennie le Roux
14 Aug 12	Groblersdal/MarbleHall pakhuis: Roslé en Gert Kruger pakhuis	Opvolg van toets en verslag oor ongeregisteerde middel by Roslé pakhuis en swamdoder oorskrydings en "audit" van titrasies wat in Gert Kruger pakhuis uitgevoer is.	Keith Lesar
16 Aug 12	Houers, Letsitele	Samesprekings oor "goedkoop" karton wat vir produsente aangebied word en opstel van aksieplan	Wimpie Mostert Frikkie van Wyk Dawid Groenewald
16 Aug 12	Charlene Jewell	Post Harvest innovations	Arno Erasmus Hennie le Roux
17 Aug 12	STC Pretoria	Toets van prototipe "goedkoop" kartonne	Francois Stein Jacques v Schalkwyk Dawid Groenewald
17-23 Aug 12	Sitrusnavorsing-simposium	Program	Hennie le Roux Dawid Groenewald Keith Lesar Hannes Bester CRI
27 Aug 12	Nelspruit	Wys dr Tim Gottwald vergroeningbesmette boorde	Glynnis Cook Fanie van Vuuren Hennie le Roux
29 Aug 12	Sondagsrivier: Andre Combrinck	Boordbesoeke Pakhuisprobleme	Hannes Bester

	Cecil Brummer Pieter Nortje	Navorsingsbehoefes Simposium CRI Werkswinkels	
30 Aug 12	CFB	Paul Fourie: SVS	Hannes Bester
30 Aug 12	Brits	Monsterneming vir CBS projek van Elma Carstens	Hennie le Roux
4-5 Sept 12	Mpumalanga CRI Voorligtings Werks-winkel	Simposium terugvoer Lenteplaagkompleks Swartvlek	Hennie le Roux Hannes Bester Sean Moore Arno Erasmus Tian Schutte Johan Joubert
6-7 Sept 12	Limpopo CRI Voorligtingswerks winkel	Simposium terugvoer Lenteplaagkompleks Swartvlek	Hennie le Roux Hannes Bester Sean Moore Arno Erasmus Tian Schutte Johan Joubert
7 Sept 12	STC Pretoria.	Ontleding en toets van Zebediela kartonne	Francois Stein Dawid Groenewald.
12 Sept 12	Pretoria	CRI-PTF vergadering	Hennie le Roux Dawid Groenewald Hannes Bester
13 Sept 12	NAFCO: Groblersdal	Simposium terugvoer	Hennie le Roux Hannes Bester
17 Aug 12	Ohrigstad TOG	Navorsingsprioriteite	Hennie le Roux
	Hoedspruit TOG	Navorsingsprioriteite	Hennie le Roux
18 Aug 12	Tshipise	Navorsingsprioriteite	Hennie le Roux
	Weipe	Navorsingsprioriteite	Hennie le Roux
20 Sept 12	STC, Pretoria	Vergadering met "Paper Scientists" oor tipiese waardes van papier.	Francois Stein Francois Wolfaart Frans Matfield Jason Knock Dawid Groenewald
	Waterberg TOG	Navorsingsprioriteite	Hennie le Roux
	Groblersdal	Navorsingsprioriteit	Hennie le Roux
21 Sept 12	Midnight TOG	Navorsingsprioriteite	Hennie le Roux
25 Sept 12	Nelspruit TOG	Navorsingsprioriteite	Hennie le Roux
26 Sept 12	Ndandwa	Ondersoek profielgate	Hennie le Roux
27 Sept 12	DAFF Citrus Coordina-ting Meeting	Agenda	Hannes Bester
	Loudie Groenewald	Pakhuis 'Benchmarking'	Hannes Bester
27 Sept 12	Burgersfort	Navorsingsprioriteite	Hennie le Roux
29 Sept 12	Franse Ambassade Johannesburg	Schengen Visum	Hennie le Roux

Opsomming van Voorligtings aktiwiteite: Oktober – Desember 2012

Datum	Studiegroep/ Aktiwiteit	Onderwerpe/Aksies	Betrokkes/ Sprekers
2-3 Okt 12	Oos-Kaap CRI Voorligtings Werks-winkel	Simposium terugvoer Lenteplaagkompleks Swartvlek	Hennie le Roux Hannes Bester Sean Moore Arno Erasmus Paul Cronje Tian Schutte Richard Fenwick
4-5 Okt 12	Wes-Kaap CRI Voorligtings Werks-winkel	Simposium terugvoer Lenteplaagkompleks Swartvlek	Hennie le Roux Hannes Bester Sean Moore Arno Erasmus

			Paul Cronje Paul Fourie Richard Fenwick
8 Okt 12	Karino	CBS terugskouing	Tian Schutte Hennie le Roux James Warrington
8 Okt 12	Bandit Chippers	Vergadering op die moontlike aanwending van kommersiële chippers vir die sitrusbedryf te ondersoek	Hennie le Roux
9 Okt 12	IPM	Navorsingsprojek evaluerings	Tim Grout Hennie le Roux Kommittee
10 Okt 12	Na-oes Postmortem	Terugvoer oor seisoen Beplanning van CRI Na-oes werksinkels	Hennie le Roux Hannes Bester Dawid Groenewald Keith Lesar Paul Fourie Paul Cronje Arno Erasmus Steve Turner Deon Joubert
11 Okt 12	Mpact, Nelspruit. Flip Welman	Voorbereiding vir verpakkingswerkgroep vergadering.	Dawid Groenewald.
11 Okt 12	Siektebestuurs- vergadering	Navorsingsprojek evaluerings	Tim Grout Paul Fourie Hennie le Roux Kommittee
15 Okt 12	Piet Smit: Wes-Kaap CGA direkteur	Wes-Kaap aangeleent-hede	Hannes Bester
16 Okt 12	CFQM Meeting	Navorsingsprojekte	Hennie le Roux Tim Grout Hannes Bester Paul Cronje Kommittee
17 Okt 12	Kultivarvergadering	Navorsingsprojek evaluerings	Tim Grout Hennie le Roux Johan Joubert Richard Fenwick Kommittee
17 Okt 12	Sappi Technology Centre	Houers resultate en Ngodwana SK en Tugela Liner proewe.	Dawid Groenewald
21-22 Okt 12	Tshwane University of Technology	Terugvoering aanbieding oor PHI befondsing vir CRI kontrak projekte, en besoek/konsultasie aan nuwe pakhuis in die Brits gebied	Keith Lesar Hennie le Roux Arno Erasmus
25 Okt 12	Glenfair Shopping Centre	Vergadering met Hennie le Roux. Vorderingsverslag en kontrak tot einde Feb 2013	Hennie le Roux Dawid Groenewald.
26 Okt 12	Kultivarvergadering met Deon Joubert	Kultivarproewe	Hannes Bester Thys Du Toit Johan Joubert Richard Fenwick
30 Okt 12	Paarl	Een op een vergaderings met Roché Kenny, APL en Adriaan Du Bussion, Nampak	Dawid Groenewald
31 Okt 12	Market Access Meeting	Agenda	Vaughan Hattingh

			Hannes Bester Elma Carstens Dawid Groenewald
	CMF Meeting	Agenda	Vaughan Hattings Hannes Bester Elma Carstens Dawid Groenewald
1 Nov 12	SACIS	Vergadering	Paul Fourie Thys du Toit Fanie van Vuuren MC Pretorius Glynnis Cook Hennie le Roux
6 Nov 12	Burgersfort/Ohrigstad Studiegroep	Simposiumterugvoer & lente- plaagkompleks	Hennie le Roux Hannes Bester
6 Nov 12	DOW	Mancozeb	Tian Schutte Tim Grout Hennie le Roux Paul Hardman (telcon)
7 Nov 12	Packaging Working Group Meeting	Agenda	Dawid Groenewald Hennie le Roux Hannes Bester
	Midnight Studiegroep	CRI Voorligting IPM Kultivars	Hannes Bester Sean Moore Jonny Roberts Andy Lee
8 Nov 12	Oro Agri	Wetsit	Tian Schutte Tim Grout Hennie le Roux
13-24 Nov 12	Spanje	ICC	Vaughan Hattings Hennie le Roux Hannes Bester Tim Grout Paul Fourie Paul Cronje Elma Carstens Arno Erasmus Sean Moore Teunis Vahrmeijer Tian Schutte MC Pretorius
16 Nov 12	LNR Roodeplaat.	R14 Proewe – swamgroeï op palette.	Dawid Groenewald
20-22 Nov 12	Dole Belville	ETP vergadering, en vergaderings met ICA en Advantage Agri	Keith Lesar
21 Nov 12	Dole Bellville	ETP vergadering	Dawid Groenewald
23 Nov 12	Polokwane	Voorlegging by Limpopo Sitrus Boeredag	Dawid Groenewald
26-30 Nov 12	Spanje / Frankryk	Prof Bove & Nuria	Hennie le Roux
29&30 Nov 12	Houers, Letsitele en Wimpie Mostert, Tzaneen	Voorlegging by Houers se Alg, Jaarvergadering en vergadering met Wimpie Mostert en Frikkie van Wyk oor program vir 2013	Dawid Groenewald
30 Nov 12	Patensie	Phillip Dempsey: CGA Direkteur	Hannes Bester
6 Des 12	Menlyn Pta	Reven Naidoo van Mpact Paper. Nouer samewerking met hulle en proewe met Bayplex Fluting.	Dawid Groenewald

7 Des 12	Sappi Technology Centre.	Vergadering met Sappi met Finansiële en Bemerkings mense oor Sappi se markaandeel	Dawid Groenewald
18 Des 12	Sappi Technology Centre	Sny van bord vir proef met spasiëring van bo-dek planke op palet,	Dawid Groenewald.
13 Des 12	DAFF Meeting	CBS Protocol na EU	Hannes Bester Tian Schutte Elma Carstens

Opsomming van Voorligtings aktiwiteite: Januarie – Maart 2013

Datum	Studiegroep/ Aktiwiteit	Onderwerpe/Aksies	Betrokkeses/ Sprekers
10 Jan 13	Sappi Tech Centre	Accreditation Process for 2013 and Prima Box tests	Dawid Groenewald Francois Stein
11 Jan 13	Compendium van Sitrus Siektes	Ontvang "Preface" en volgende "draft" van Mulberry drukkers	Keith Lesar
14 Jan 13	CBS besmette boorde	Versamel monsters vir DAFF opleiding	Hennie le Roux
15-16 Jan 13	CRI Management Meeting - Addo	Agenda	Hennie le Roux Hannes Bester
16-17 Jan 13	Bi inspeksies	Inspekteur Bi valletjies vanaf Brits - Gaberones	Aruna Manrakhan Hennie le Roux
17 Jan 13	Mulberry Drukkers	Werk deur die dokument met Nicolene van die drukkers	Keith Lesar Nicolene Koorts
18 Jan 13	Mulberry Drukkers	Document finaal deurgewerk	Keith Lesar Nicolene Koorts
21 Jan 13	DARDLA	IOCV vergadering	Hennie le Roux Glynnis Cook
21 Jan 13	Mulberry Drukkers	Finale document geteken en afgelewer vir druk	Keith Lesar
21 Jan 13	Gerber Paper.	Meeting to discuss imported paper.	Dawid Groenewald Klaus Thieltges
21-25 Jan 13	CRI Na-oes Werkswinkel	Aanbiedinge voorberei	Keith Lesar
23 Jan 13	Addo: Sun Citrus	CRI Na-oes Wersinkels: Clint MacAleer en Cecil Brummer	Hannes Bester
24 Jan 13	CBS Proewe	Monsterneming	E. Carstens Hennie le Roux
25 Jan 13	Mulberry Drukkers	1000 afskrifte van "Compendium" by CRI afgelewer	Keith Lesar
25 Jan 13	Sanddrift boord besoek	Evalueer BEE projek om versoek van NWDA.	Hennie le Roux
28-30 Jan 13	Limpopo CRI Na-oes Werkswinkel Polokwane	Agenda	Hennie le Roux Hannes Bester Dawid Groenewald Keith Lesar Paul Cronje Arno Erasmus Sean Moore
30 Jan 13	Zebedela Citrus Packhouse	Packhouse consultation	Keith Lesar
31 Jan – 1 Feb 2013	Mpumalanga CRI Na-oes Werkswinkel Loskopdam	Agenda	Hennie le Roux Hannes Bester Dawid Groenewald Keith Lesar Paul Cronje Arno Erasmus Sean Moore

1 Feb 13	OR Tambo	IDC vergadering	Hennie le Roux Johan Marshall
4 Feb 13	Citrusdal	XSIT: Sampie Groenewald Wenkem: Jaco Olivier Jannie Toerien	Hannes Bester
5 Feb 13	Stellenbosch: SU	Studente mondelinge	Hannes Bester
5 Febr 13	Hoedspruit Letsitele	Ondersoek chimera probleem op Ou Kloon valencias Constantia studiegroep Bi vergadering	Hennie le Roux Aruna Manrakhan
6 Feb 13	Letsitele Hoedspruit	QMS CBS vergadering Hoedspruit Studiegroep Bi vergadering	Hennie le Roux Aruna Manrakhan Tom vd Meulen
7-8 Feb 13	Tamboti Landgoed	Swaziland Sitrus studiegroep. Bi vergadering	Aruna Manrakhan Hennie le Roux Chris Kellerman
7 Feb 13	Unifrutti: Kirkwood	Neem data van proefblok met verskillende bronne van plantmateriaal/virusse	Hannes Bester Glynnis Cook
	CFB	Paul Fourie Thys Du Toit	Hannes Bester Glynnis Cook
8 Feb 13	Johnson Agriculture Machinery Silverton, Pta	Development of Machine to test pallets	Mauritz Johnson Dawid Groenewald
12-13 Feb 13	KZN & Swaziland CRI Postharvest Workshop Durban	Agenda	Hennie le Roux Hannes Bester Dawid Groenewald Keith Lesar Paul Cronje Arno Erasmus Sean Moore
14-15 Feb 13	Oos-Kaap CRI Na-oes Werkswinkel PE	Agenda	Hennie le Roux Hannes Bester Dawid Groenewald Keith Lesar Paul Cronje Arno Erasmus Sean Moore
18 Feb 13	CGA Roadshow	Beitbridge	Hennie le Roux Paul Hardman Micheal Brooks
19 Feb 13	CGA Roadshow	Weipe	Hennie le Roux Paul Hardman M. Brooks
19-20 Feb 13	Wes-Kaap CRI Na-oes Werkswinkel	Agenda	Hennie le Roux Hannes Bester Dawid Groenewald Keith Lesar Paul Cronje Arno Erasmus Sean Moore
25-26 Feb 13	CGA Roadshow	Boland (Stellenbosch) Citrusdal	Hennie le Roux Paul Hardman M. Brooks
25 Feb 13	Menlyn Retail Park	Nampak Corrugated. Paper imports.	Johan Nel Dawid Groenewald.
26 Feb 13	Patensie CGA Meeting	Agenda	Hannes Bester CGA
26 Feb 13	Tzaneen	Meeting with Houers to discuss Sappi paper trials	Wimpie Mostert Dawid Groenewald.
27 Feb 13	Kirkwood: Marius Ferreira	CRI en Oos-Kaap CTA	Hannes Bester
	Kirkwood CGA Meeting	Agenda	Hannes Bester
27 Feb 13	CGA Roadshow	Kakamas	Hennie le Roux

			Paul Hardman M. Brooks
27-28 Feb 13	Houers Letsitele	Trials with Sappi Tugela Liners and Ngodwana Spray Starch Stackraft. Packaging Material Specs	Brian Percival Wimpie Mostert Phillip Potgieter Dawid Groenewald. Frikkie van Wyk.
28 Feb 13	CGA Roadshow	Vaalharts	Hennie le Roux Paul Hardman M. Brooks
1 Mrt 13	Sappi Tech Centre	Start lab tests. Tugela Liners and Spray Starch Stackraft	Francois Stein Dawid Groenewald.
5 Mrt 13	Constantia studiegroep	Bi	Aruna Manrakhan Hennie le Roux
6 Mrt 13	Glenfair Centre	Meeting to discuss Tugela White Liner future trials	Wimpie Mostert Phillip Potgieter Dawid Groenewald
6 Mrt 13	SACIS Grondgedraagde siektes DC	Nelspruit	Hennie le Roux Paul Fourie Patoloeë
7 Mrt 13	Sappi Tech Centre	Tugela Liner and Spray Starch test results	Frikkie van Wyk Francois Stein Dawid Groenewald
7 Mrt 13	GOGO Studiegroep Groblersdal	Na-oes Werkswinkel aanbiedinge	Keith Lesar
8 Mrt 13	GOGO Studiegroep	Besoek aan koelkamers, ontgroenings opset en pakhuis	Keith Lesar
12 Mrt 13	JHB Airport	360 Quality Audits and Seatrade presentation	Wout van Huijstee Arnold Wentzel Dawid Groenewald
12-13 Mrt 13	CRI Nelspruit	Job Appraisals CRI Werkswinkels beplanning	Hennie le Roux Hannes Bester Keith Lesar
14 Mrt 13	CMF vergadering	Agenda	Hennie le Roux Dawid Groenewald Hannes Bester
18 Mrt 13	Burgersfort	Bi vergadering	Aruna Manrakhan Hennie le Roux
19 Mrt 13	Oos-Kaap CTA	Agenda	Hannes Bester Wayne Kirkman Sean Moore Paul Fourie Jacques Fouché
22 Mrt 13	Magalies	Winterveldt BEE	Hennie le Roux
26 Mrt 13	Boord besoek	Winterveldt BEE	Hennie le Roux
27 Mrt 13	Agri-Kirkwood Gala Aand	Program	Hannes Bester Sean Moore Wayne Kirkman Paul Fourie
27 Mrt 13	Malelane	Bi	Aruna Manrakhan Hennie le Roux Chris Kellerman
28 Mrt 13	Sappi Tech Centre	Testing of imported wrappers/SRCC	Francois Stein Dawid Groenewald

8.1 TRANSFORMATION: EXTENSION COORDINATORS' REPORT

Extension Officers Training Workshop. Due to financial constraints the Limpopo Department of Agriculture would not make money available for the training of the extension officers who are coordinating citrus in the Limpopo province for the 2012/2013 season. This is unacceptable and not in line with the MoU between the CGA and the Limpopo Department of Agriculture.

Citrus Growers Training Workshop. The Transformation desk organized a training workshop on strategic partners, joint ventures and mentors for the citrus growers after the growers have requested more information about these structures. The workshops were conducted in different provinces as follows;

Provinces	Districts	Date	Venue	Attendance	Facilitators
Kwazulu Natal	Uthungulu	25/07/2012	Nkwaleni Hall, Nkwaleni Valley	9	Peter Green & Kathy Pitout
Eastern Cape	Cacadu	13/08/2012	Luthando Farm, Sundays River Valley	18	Peter Green & Kathy Pitout
		14/08/2012	Tobacco Office, Patensie	15	Peter Green & Kathy Pitout
	Amathole	15/08/2012	Cape College, KAT River Valley	16	Peter Green & Kathy Pitout
Limpopo	Vhembe	03/09/2012	ARC Hall, Levubu	11	Peter Green & Kathy Pitout
	Mopani	04/09/2012	Tzaneen Country Lodge. Letsitele	11	Peter Green & Kathy Pitout
	Waterberg, Sekhukhune & Capricorn	05/09/2012	Zebediela Citrus Estate, Zebediela	11	Peter Green & Kathy Pitout
Gauteng & North West	Pretoria & Bojanala	06/09/2012	Deja-vu', Pretoria	6	Peter Green & Kathy Pitout
Mpumalanga	Ehlanzeni, & Gert Sibande	12/09/2012	CRI Boardroom, Nelspruit	8	Peter Green & Deidre Rankin

CRI Packhouse Workshops. One farmer from Mariveni farm in the Limpopo province was sponsored (only registration fee) by the Greater Tzaneen local municipality to attend the Packhouse workshop that was held on 29 and 30 January 2013 at the Ranch Hotel. No developing farmers attended the Packhouse workshop that was held on 31 January and 1 February 2013 at the Loskop Dam in Mpumalanga. KZN and Eastern Cape farmers attended the Packhouse workshops at Durban and Summerstrand Hotel respectively. Four farmers in KZN and three farmers from the Eastern Cape attended the CRI pack-house workshops. The CGA sponsored their accommodation.

The Citrus Field and Growers Days. The Citrus Field Day has become an annual event for the citrus growers in the Limpopo and Eastern Cape Provinces. The event rotates in Limpopo between the five districts namely; Vhembe, Mopani, Waterberg, Capricorn and Sekhukhune. The event will rotate in the Eastern Cape Province amongst the two districts that grow citrus.

This year (2012) the Citrus Field day in Limpopo was held on 4 October at the Tzaneen Country Lodge. The event was attended by 103 delegates and represented 1.5 million export cartons. The theme of the citrus field day was "Marketing of the Citrus Fruit". The following presentations were covered; BEE Marketing Agents, Crop Production Support Services, Exporter and Producer Contract, Transport and Export logistics, Producer and Export Agents Relationship, Export Protocols, Recapitalization Flow Process and Skills Development and Bursary opportunities for the students who are interested in the citrus industry.

In the Eastern Cape the grower day was organized by CRI, the CGA and Eastern Cape Department of Rural Development and Agrarian Reform. A total of 96 delegates attended the grower day. The topics discussed were:

- Citrus Spring Pests Complex as major core of the season by Dr. Sean Moore from CRI
- Export Market Access by Dr Sean Moore on behalf of Mr. Hannes Bester from CRI,
- Citrus Black Spot by Andiswa from DAFF PE Office;
- PPECB Standards by Mrs. Nokulunga Mngqeta;
- Local Market Access by Mrs. Boitumelo Legadimana (JHB Market);
- ARC Cultivar Development by Nikki Combrink from ARC;
- Citrus Growers Association Cultivar Company by Mr. Jonathan Roberts from CGACC;
- Citrogold Cultivars by Mr Bryan Offer from Citrogold;
- Disposal of Empty Containers by Tom Mabesa from AVCASA;
- Recapitalisation by Mrs. Peliwe Njemla;
- Financial Support by Mrs. Ntsingi Tabata;

- Bursary Funds by Jacomien de Klerk from Citrus Academy;
- Farm Machinery (All Cut Power Products) by Mike Cowie from PE and
- Words of Encouragement by Mrs. N.A. Gxasheka Manager Extension Services from Bisho DRDAR on behalf of Ms. P.N. Tamban Senior Manger Extension Services

Citrus Grower Day was sponsored by the:

- CGA sponsored catering by Transformation Manager Mr.. Lukhanyo Nkombisa
- Dow AgroScience sponsored Caps and Stationery by Technical Manager Mr. Johan Van Rensburg
- Mpact East London Boxes sponsored catering by Mr Kevin
- River Bioscience sponsored catering by General Manager Keith Danckwertts
- Susan Herman Transport sponsored water bottles by Ms. Susan Herman
- Citrus Academy sponsored DVD to Citrus Technical Committee members by General Manager Jacomien de Klerk
- PPECB sponsored Pens by Executive Director Mrs. Nokulunga Mnqeta
- Department of Agriculture Nkonkobe sponsored with Venue and Programmes by Mrs. Mbere N
- Riverside Display and Coordination by chief Executive Officer Mr. Sieg

The Study Groups. The conducting of the study group sessions went well during the 2012/2013 season in Limpopo, Eastern Cape and Kwazulu-Natal provinces. The following table indicate 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
07/06/2012	Vhembe Study group	Mulaudzi's farm, Klein Tshipise	70	Mafa	Irrigation of citrus trees
				Nkhumeleni L.	Fertilization of citrus trees
				Mbedzi M.A.	Pruning of citrus trees
13/06/2012	Patensie Study Group	Tobacco offices	11	M. Odenndaal and Melton	Pruning ,irrigation and fertilization
14/06/2012	SRV Study Group	Luthando farm	12	Andre Combrink SRCC Tech	Pruning and irrigation
				Melton	Fertilization
5/07/2012	KAT Study Group	Naudeshoek farm, Peddi	23	Susan Herman	Irrigation Scheduling
				Melton	Pruning and Fertilization
11/07/2012	KZN Study Group	Nkwaleni Community hall	8	Melton	Irrigation, fertilization and pruning of citrus
18/09/2012	Waterberg Study group	Gillimburg Farm, Mokopane	13	Mbedzi M.A.	Citrus Spring Pest Complex
19/09/2012	Mopani Study group	Moletele Farm	14	Mbedzi M.A.	Citrus Spring Pest Complex
18/10/2012	Vhembe Study group	Chauke's Farm	43	Mafa	Importance of irrigating citrus
				Dube H.P.	Fertilization of citrus
				Mbedzi M.A.	Citrus pests and Diseases
6/11/2012	SRV Study Group	Willow Tree Farm	12	Melton	FCM, CBS, and DVD on integrated pest management
14/11/2012	KZN Study Group	Thulwane farm	9	Mulaudzi M.P.	CBS,FCM and DVD on pest management
15/11/2012	Vhembe Study group	Ratombo Farm, Levubu	37	Mbedzi M.A	Overview of the citrus industry
				Nkhumeleni L.	Citrus packaging materials Citrus rust mites and red scale
27/11/2012	KAT Study Group	Riverside Training centre	12	Melton	CBS,FCM control and DVD
05/12/2012	Waterberg Study group	Morajomo Farm, Radium	15	Mbedzi M.A.	Citrus rootstocks and cultivars
					Fruit quality
					Leaf roller and citrus thrips
21/01/2013	SRV V. Study Group	Willowtree	12	Melton	Fruit Fly and Activity plan 2013
23/01/2013	KAT Study Group	Cape College	18	Melton	Fruit Fly, FCM and activity plan 2013
				Ntuli Z	Addressing DAFF policy on

					working with Strategic partner
13/02/2013	Patensie Study Group	Tobacco Office	11	Melton	Fruit Fly, FCM and Activities plan 2013
20/02/2013	KZN	Thulwane farm	8	Melton	Fruitfly, FCM and Activities plan 2013
19/03/2013	KAT Study group	Riverside Training centre	14	Llew Rebert	Soil and Leaf analysis
				Lukhanyo	LRED application and CGDC
				Candice	Citrus academy bursary fund
				Lawrence Mgadle	Importance of the new citrus cultivars
25/03/2013	SRV Study Group	Luthando farm	18	Nikki Combrik	Soil and leaf analysis
				Melton	Woolly fly and B.I
21/01/2013	SRV V. Study Group	Willowtree	12	Melton	Fruit Fly and Activity plan 2013

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 mentorship funding was sourced from Agriseta and the Department of Rural Development and Agrarian Reform (EC-DRDAR) as follows;

- AgriSETA – November 2011 to April 2012.
- Eastern Cape Department of Rural Development and Agrarian Reform (EC-DRDAR) – July 2011 to June 2012.

AgriSETA provided a total of R210,000.00 for the provision of mentorship for:

- KwaZulu-Natal – Thulwane farm.
- Mpumalanga – Sibonelo Lemon Project, Champagne Citrus Estate and Sobabili farm.
- Limpopo – Easy Farm.

EC-DRDAR provided a total of R667 945-00 for the provision of mentorship for the following Eastern Cape citrus projects:

- Battlesden – Huduza.
- Letas Farm.
- Lidell (Zanentlutha) Farm.
- Torties Farm.
- Lovers Retreat Farm.
- Oakdene Farm.
- Gatyena – Orange Grange Farm.
- Topkat Farm.
- Gonzana Farm.
- Ripplemead Farm.
- Katoo Family Trust – Three Pence Farm.

Launch of Fruit of Success Publication (NC and WC). The CRI extension coordinator Melton Mulaudzi, the CGA Transformation Manager Lukhanyo and Louise Brodie (a freelance journalist) visited the farms in the Northern Cape and Western Cape to conduct interviews and the reason for conducting such interview was to find out how these farmers became successful as there is no land claims in their areas. The main purpose of the visit was also to compile another transformation magazine and also to find out the support they received from government programme like recapitalisation or CASP and also to engage the beneficiaries so that they could become members of the Citrus Growers Development Chamber.

Interviews were conducted with BEE beneficiaries from the Equity scheme from different farms in the Northern Cape namely:

- Mosplaas citrus project Upington
- Rekopane Estates Kakamas
- Berekisang empowerment trust
- Swart Booiberg Kakamas
- Thusano trust Kakamas

as well as in the Western Cape in the Citrusdal area namely:

- Bergendal Project
- De Oude Rondegat project

- Paardekop project
- Boontjies Rivier
- ALG Estates/ Cedar citrus
- Mouton citrus/ Emgro and Ruige Rivier

The Citrus Growers Development Chamber (CGDC). The CGDC nominated the Chamber Executive Committee members during the meeting that was held on 7 August 2012 at the Willow Park Conference Centre in Johannesburg. The composition of the new Citrus Growers Development Chamber Executive Committee was as follows;

Name:	Responsibility:
Mzo Makhanya	Chairperson of the Chamber Executive
Tompson Mankhili	Deputy Chairperson of the Chamber Executive
Luyanda Kutta	Chamber Executive member
Hannes Hobbs	Director of the Southern Region
Israel Nemaorani	Director of the Northern Region
Lukhanyo Nkombisa	CGA Transformation manager
Justin Chadwick	CGA CEO
Jacomien de Klerk	CA GM
Melton Mulaudzi	CRI Extension Officer Southern Region
Andrew Mbedzi	CRI Extension Officer Northern Region

The first mandate of the Chamber Executive Committee was to formulate the Terms of Reference (ToR) for the Chamber. Two meetings were held at the Garden Court hotel on 23 October 2012 and Willow Park Conference Centre on the 21st of November 2012 respectively. The first meeting looked into the ToR and the second meeting focused on the Master plan for the chamber. These two meetings were facilitated by Mr. Peter Green from Lima consultants.

Terms of Reference (ToR) for the Chamber:

- Recommendations or decision making. It was felt that the CGDC was a body that would provide guidance to the CGA board on transformation issues but would also be empowered to make decisions on issues directly relating to transformation. These decisions would need to fall in line with a budget and strategic plan approved by the CGA Board. The CGDC is an advisory arm of the CGA board with certain decision making powers relating to transformation issues.
- The CGDC is a vehicle through which emerging growers could address their concerns and have issues addressed.
- The CGDC needs to have a set of operating guidelines that would address issues such as term of office of office bearers, conflict resolution etc. In terms of the Chairman of the CGDC it was proposed that the Vice Chairman serves for a year before taking on the chairman position for a year and then again becomes vice chairman to the incoming chairman.
- The CGDC (20 members) currently meets quarterly and so does the executive (8 members) of the CGDC. It was felt that going forward it would be sufficient for the executive to meet quarterly and for the full chamber to meet twice-annually.
- Terms of office. It was suggested that the CGDC members serve a 5 year term of office with a maximum of 2 terms. The executive would serve a 3 year term of office with a maximum of 2 terms.
- Employees would have permanent seats on the CGDC executive but would not have voting rights.

Rules of Engagement between the CGA Board and the CGDC:

- There should be a standing agenda item on the CGA board for board members to report on the activities of the CGDC.
- The board currently allows 2 seats for CGDC members – one from the north and one from the south. It is proposed that a third seat for the chairman of the CGDC be made available.
- There should be access to information from the board and the CGA. Language to be taken into consideration when addressing meetings.
- The transformation budget for the year should be tabled and approved by the CGDC.
- The CGDC should submit a strategic plan annually which would be aligned to its budget.
- Point of contact with government officials and outside agencies would be jointly with officials and chamber members.

- MOU's to be negotiated jointly by officials and CGDC and signed off by Transformation Manager

Formation of Committees:

- Four committees were formed, namely; Market Access, Logistic, Skills Development and Consumer Assurance.
- Each committee will be comprised of three chamber members.
- The chairperson of each of these committees will represent the chamber in the main CGA committees

Committee	Brief	Members
Market access	<ul style="list-style-type: none"> • Local market access • Support to government with addressing tariffs and other trade barriers • Issues on marketing channel • Export agents • Export market access • New market development 	Hannes Hobbs M.J. Matlou Celestina Tlolane
Logistics	<ul style="list-style-type: none"> • Rail transport • Ports (Maputu, Durban logistics) • Roads 	Israel Nemaorani Phelelani Duma Samson Qomondi
Skills development	<ul style="list-style-type: none"> • BEE bursary support • Learning programmes • Capacity building workshops 	Luyanda Kutta Eric Nohamba Khaya Katoo
Consumer assurance	<ul style="list-style-type: none"> • Ethical trade programme • Social compact • MRLs, market standards (Globalgap etc.) • Climate change 	Nokwanele Mzamo Petros Shiba M.P. Madidimalo

CGDC Master Plan:

- It was felt that a situational analysis should be conducted on 130 developing farms and Lukhanyo Nkombisa was tasked to draft tender briefing for the situational analysis. The aim and objectives of the situational analysis are to:
- Establish and analyse what are the current state of affairs on emerging citrus grower farms in South Africa and to identify what issues and challenges are being faced by these farmers.
- Analyse the identified challenges and needs towards establishing key interventions that could address and overcome them. Interventions must be developed for each enterprise as well as to address common challenges per region and per target group categories.
- Provide sufficient information to assist the Citrus Growers Development Chamber (CGDC) in developing a programme of action to implement the proposed interventions.

MoU between CGA and DRDLR. The Chamber Executive committee met the Department of Rural Development and Land Reform (DRDLR) on 7 February 2013 at the Garden Court Hotel to finalize the draft MoU between CGA and DRDLR. The main aim of these two organizations to enter into a MoU is to try and facilitate the implementation of the recapitalization programme. The meeting then agreed that the MoU should be packed in such a way that it clarifies the multiple enterprises and that dealing with CGA will minimize the presence of fly by night service providers that have ripped the DRDLR. It was further indicated that the MoU between DRDLR and CGA should clarify ways of communication between the two parties and it should also clearly pronounce the roles of the two organizations.

The following has been agreed by the meeting as the way forward:

- The present draft MoU to be amended by DRDLR and then sent to CGA for CGA to look at it and make corrections and then send it back to DRDLR after consultation the CGA Board and the legal department CGA.
- Policy of DRDLR regarding the recapitalization programme to be sent to CGA
- CGA to email the exact support to DRDLR that they will provide to farmers that will receive the recapitalization funding.
- Provincial offices to submit a list of farmers that are on the recapitalization programme to the CGA office

- CGA to send the list of farmers that they have prioritized to benefit from the recapitalization to the DRDLR

Recapitalization and Development Programme. The citrus farms in the Eastern Cape and Mpumalanga provinces have benefited from the recapitalization funding. No citrus farms in Limpopo, Gauteng and North West Provinces benefited from the recapitalization funding.

Mpumalanga: Champagne farm located in the Bushbuckridge area has benefited from the recapitalization funding. They are using some of the grant money to renovate the packhouse.

Sibonelo (Eilandshoek) Lemon Farm: Sibonelo lemon farm located at Eilandshoek also benefited from the recapitalization funding. They have bought a small truck and they are on the process of planning to build an office.

Eastern Cape: The recapitalisation funding has been approved for the following farms in the Eastern Cape Province;

- Ripplemead Farm: irrigation system has been installed for 5Ha and M7 Navels have been planted. Mentorship is under Shaun Brown of Eden Agric Services.
- Greenwood Farm: The farmer has already done soil preparation and ridging to the area intending to plant Lemon, Lane Late and M7 trees for 10 ha. She has also bought a new white bakkie ISUZU, one Landini tractor, new herbicide boom spray and new tractor mower. Mentorship is under Shaun Brown of Eden Agric Services
- Liddel farm: Recap received, the farmer bought new tractor, new tractor mower machines and herbicides boom spray. Mentorship or strategic partner is Riverside.
- Oakdene farm: is still waiting for new financial year approval budget from recap.
- Jordan farm: he has already done soil preparation and ridging to the area intending to plant Washington Navel, Miho wase, Sonet and M7 trees for 5 ha. He also bought a new white bakkie ISUZU, one Landini tractor, new herbicide boom spray and new tractor mower. Mentorship is under Shaun Brown of Eden Agric Services.

Citrus Academy (BEEBS) Bursary Support Approval for 2011/12. The following farms received the confirmation letters from the Citrus Academy for the BEEBS Bursary approval to support them during the 2012/2013 season.

Name of farm	Beneficiary Student
1.Ripplemead Citrus	1.Mihlali Mgadle 2.Somila Mgadle 3.Yonela Mgadle
2.White citrus Farm	1.Lwazi Mpukane 2.Zibuko Marali
3.Letas farm	Andiswa Dyonase
4.Zanentlutha farm/Lidell	1.Chwayita Mfecane 2.Khanyisa Thisani
5.Topkat farm	Nontembeko Metula
6.Oakdene farm	Sigcine Manyonta
7. Lovers Retreat	Unathi Yeko
8.Naudeshoek farm	1.Nomfundo Mpahla 2.Vuyolwethu Mpahla
9.Konzi farm	1.Sinovuyo Nohamba 2.Siphiwokuhle Nohamba 3.Siyolise Nohamba
Total of 9 Farms	16 Students

Challenges

- The slow progress on the issue of signing of Memorandum of Understanding (MoU) between CGA, CRI and provincial departments of agriculture (except for the Limpopo province).
- Growers in some parts of the southern regions (i.e., KZN and Eastern Cape) and northern region (i.e., Limpopo and Mpumalanga) were hit by floods and hails storms.
- Lack of funds to finance the training of the Limpopo Department of Agriculture extension officers who are coordinating citrus in the province.
- Nkwaleni Valley and Klein Hoewe Boer are faced with a challenge of citrus black spot

- Most of the upcoming citrus growers are faced with a financial challenge.
- Poor communication between the government officials and citrus growers
- Shortage of credible mentors to assist in the CGA mentorship programme

Summary of Extension Coordinators' Activities (April 2012 – March 2013).

Date	Venue/Place	Activity/Topics	Speakers/Extension Officer
04/04/2012	Humansdorp, PE	Attending recapitalization meeting with farmers from Kouga and Mr Screech from Kouga LED	Melton Mulaudzi
02/04/2012	Greenwood	Doing demonstration on pegging for the planting of new citrus trees.	Melton Mulaudzi
09/04/2012	Northern Cape	Visiting Equity Scheme to compile an article for the Fruit of Success Publication in NC region BEE farmers.	Melton Mulaudzi
10/04/2012	Mosplaas Citrus Farm, Upington.	Visiting Equity Scheme to compile an article for the Fruit of Success Publication in NC region BEE farmers.	Melton Mulaudzi
11/04/2012	Rekopane Farm Kakamas	Visiting Equity Scheme to compile an article for the Fruit of Success Publication in NC region BEE farmers.	Melton Mulaudzi
	Swart Booisberg Kakamas	Visiting Equity Scheme to compile an article for the Fruit of Success Publication in NC region BEE farmers.	Melton Mulaudzi
	Berekisanang Empowerment Trust	Visiting Equity Scheme to compile an article for the Fruit of Success Publication in NC region BEE farmers.	Melton Mulaudzi
12/04/2012	Thusano trust/ Groenheuwel farm	Visiting Equity Scheme to compile an article for the Fruit of Success Publication in NC region BEE farmers.	Melton Mulaudzi
17/04/2012	Temo Tower, Polokwane	Attending the mentorship programme meeting with CGA and DAFF officials.	Andrew Mbedzi
18/04/2012	Harvest House, Pretoria	Attending the Plant Production Working Group (PPWG) Meeting.	Andrew Mbedzi
	Fort Beaufort –East London Blue Waters Great Kei	Discussions on sanitation, weed control and local marketing.	Melton Mulaudzi
19/04/2012	Dante Farm, Moritele	Inspect new citrus plantings at Dante citrus farm in North West	Andrew Mbedzi
23/04/2012	Jerico, Eden, Torties	Assist farmers with the hanging of Fruit fly traps	Melton Mulaudzi
24/04/2012	Champagne Farm, Bushbuckridge	Mentorship Programme Assessment	Andrew Mbedzi
	Susan Herman's Offices	Interview growers in connection with financial assistance from Standard Bank and CASP funding from Government	Melton Mulaudzi
25/04/2012	Easy Farm, Sibasa	Mentorship Programme Assessment	Andrew Mbedzi
	Peddie, Fort Beaufort	Interview growers in connection with Financial assistance from Standards Bank, CASP funding from Government	Melton Mulaudzi
26/04/2012	Riverside Packhouse	Mentorship Programme Assessment	Melton Mulaudzi
	Eden Agric Services	Mentorship Programme Assessment	Melton Mulaudzi
27/04/2012	Oakden farm	Mentorship Programme Assessment	Melton Mulaudzi
	Eden Agric. Services	Mentorship Programme Assessment	Melton Mulaudzi
	Torties farm	Mentorship Programme Assessment	Melton Mulaudzi

	Baddaford farm	Giving Riverside management feedback on the assessment of the mentorship programme	Melton Muaudzi
03/05/2012	First Avenue, PE	Attending Citrus Growers Development Chamber meeting	Andrew Mbedzi Melton Mulaudzi
	Port of Ngqura	Tour of the Port of Ngqura in PE with Chamber members, EC farmers and Govt. Ext. Officers.	Andrew Mbedzi Melton Mulaudzi
07/05/2012	Citrusdal, WC	Presentations (with CGA and Louise Brodie) for the growers and Govt. Officials on the role of the CGA and CRI extension services.	Melton Mulaudzi
08/05/2012	De Kamp farm Citrusdal, WC	Visiting Equity Scheme to compile an article for the Fruit of Success Publication in Western Cape region BEE farmers.	Melton Mulaudzi
	ALG Estates/ Ceda Citrus	Visiting Equity Scheme to compile an article for the Fruit of Success Publication in Western Cape region BEE farmers.	Melton Mulaudzi
	Mouton Citrus, Emgro and Ruiger Rivier	Visiting Equity Scheme to compile an article for the Fruit of Success Publication in Western Cape region BEE farmers.	Melton Mulaudzi
10/05/2012	Cape College, Fort Beaufort	Attending meeting with CGA, Mrs Nokulunga, Shaun Brown, Government Officials from Amathole and Nkokobe together with farmers who are in partnership with Shaun Brown	Melton Mulaudzi
11/05/2012	East London, EC	Accompany Lukhanyo to the Airport after meeting at Cape College	Melton Mulaudzi
14/05/2012	Town Lodge, PE	Preparation for attending commodity meeting with Provincial and DAFF officials	Melton Mulaudzi
15/05/2012	Khangela, Addo	Discussing the Recap programme	Melton Mulaudzi
	Santa Clara	Inspect progress: None	Melton Mulaudzi
	Dept. of Agric. and Agrarian Reform PE office	Attending Commodity meeting with DAFF and Provincial officers	Melton Mulaudzi
16/05/2012	CGA Office, Sandton	Attending the mentorship programme meeting on SLA with CGA, Dr. Bates and DAFF Officials.	Andrew Mbedzi
	Sunland farm	Monitoring government support from Recapitalisation programme	Melton Mulaudzi
	Mbuyiselo farm	Monitoring government support from Recapitalisation programme	Melton Mulaudzi
	Willow Tree	Monitoring government support from Recapitalisation programme	Melton Mulaudzi
	Gree Gables	Monitoring government support from Recapitalisation programme	Melton Mulaudzi
17/05/2012	Agric. Offices, Giyani	Planning of the Citrus Field Day to be held in Greater Tzaneen local municipality.	Andrew Mbedzi
	Konzi, Eden Jerico, Torties & Topkat farms	Monitoring fruit maturity and advising Mandla and Susan Herman to start harvesting Satsuma at Jerico Farm	Melton Mulaudzi
18/05/2012	ARC, Levubu	Looking for the venue for the Levubu citrus growers' meeting	Andrew Mbedzi
	Orange Grange	Monitoring Orchards sanitation and fruit maturity.	Melton Mulaudzi
	Greenwood	Monitoring Sanitations and CBS	Melton Mulaudzi

21/05/2012	Blue Waters, East London	Organizing tools for pruning and demonstrating pruning on lemons.	Melton Mulaudzi
22/05/2012	Jerico, Lidell, O/Grange	Monitoring orchards sanitation for the control of fruit fly and navel maturity.	Melton Mulaudzi
24/05/2012	Ripplemead, Peddi	Monitoring picking of oranges and Clementine's at both farmers.	Melton Mulaudzi
	Rallyvale farm	Picking Clementine's and delivering the fruits to Riverside pack house for packing	Melton Mulaudzi
	Naudeshoek farm	Picking Clementine's and delivering the fruits to Riverside pack house for packing	Melton Mulaudzi
28/05/2012	Mopani Offices	FPEF Initiatives meeting with Arend and the X-Goup	Andrew Mbedzi
29/05/2012	Gonzana, Oakdene, Torties	Monitoring colour break and advising the farmers to pick the fruit samples for maturity testing.	Melton Mulaudzi
30/05/2012	ARC, Levubu	Levubu Citrus Growers meeting	Andrew Mbedzi
	Ngqushwa Local Municipality/Peddie	Attending ASPIRE master plan and local spatial development plan workshop.	Melton Mulaudzi
31/05/2012	CRI office, Roadlodge, PE	Attending Executive Chamber meeting at OR Tambo	Melton Mulaudzi
01/06/2012	Makwarela, Sibasa	Vhembe Citrus Technical Committee meeting	Andrew Mbedzi
	OR Tambo Air Port JHB	Attending Citrus Growers Development chamber first executive meeting at OR Tambo	Melton Mulaudzi
04/06/2012	Agric. Offices, Giyani	Mopani Citrus Technical Committee meeting	Andrew Mbedzi
06/06/2012	Western Cape Citrusdal	Attending Western Cape Citrus Producer Forum summer citrus harvest meeting with DTI, USA Embassy and Commercial farmers from the area.	Melton Mulaudzi
07/06/2012	Klein Tshipise, Vhembe	Vhembe Citrus study Group session 1.	Andrew Mbedzi
	East London, Fort Beaufort	Attend DTI, DAFF, USA Embassy and NDRDLR meetings in Pretoria.	Melton Mulaudzi
08/06/2012	Riverside, KAT River Valley	Doing Presentation on pruning and demonstration of pruning at Jerico Satsuma block (owari)	Melton Mulaudzi
13/06/2012	Patensie	Attend Citrus study group on pruning, irrigation and fertilization. Pruning was done by Martina Odendaal.	Melton Mulaudzi
14/06/2012	Luthando Farm Sundays River Valley Kirkwood	Attend Citrus study group on irrigation, fertilisation and pruning with Andre Combrink from SRCC's Technical division	Melton Mulaudzi
	Patensie-SRV-Fort Beaufort	Monitoring Picking. Some trees were showing cracks and the manager was advised to take roots sample to CRI for analysis	Melton Mulaudzi
15/06/2012	East London, JHB, Pretoria	Preparation to attend DTI, DAFF, NDRDLR and USA Embassy meetings in Pretoria.	Melton Mulaudzi
18/06/2012	DTI, Pretoria	Attend DTI meeting on export market with Melton and Lukhanyo	Andrew Mbedzi Melon Mulaudzi
19/06/2012	DAFF, Pretoria	CRI presented a talk on the background of the citrus industry, the CRI extension model and the MoU between LDA, CGA and CRI.	Andrew Mbedzi Melton Mulaudzi

	USA Embassy	CGA presented a talk on the role of CGA on emerging citrus growers. The USA Embassy was willing to assist the small growers (example-CBS in Tshipise)	Andrew Mbedzi Melton Mulaudzi
	NDRDLR, Pretoria	Meeting with Mr Alwyn Prinsloo on recapitalization funding in the Eastern Cape.	Andrew Mbedzi Melton Mulaudzi
21/06/2012	Polokwane	Bi Awareness Workshop	Andrew Mbedzi
22/06/2012	Makwarela, Sibasa	Farmers meeting with Granopassi marketer processing	Andrew Mbedzi
27/06/2012	Tswinga, Vhembe	Citrus orchard site visit.	Andrew Mbedzi
28/06/2012	Easy Farm	Inspect the establishment of new citrus packhouse	Andrew Mbedzi
28/06/2012	Jerico, Konzi, Eden farms	Monitoring picking at both farms.	Melton Mulaudzi
03/07/2012	Lidell Farm	Monitor picking	Melton Mulaudzi
	Greenwood Farm	Assist with Global gap and monitoring the interval for GF 120, trichlorfon 950 and Cryptex	Melton Mulaudzi
04/07/2012	Battleden	Inspect harvesting.	Melton Mulaudzi
05/07/2012	Peddie Naudeshoek	Attending BEE citrus study group refertilisation, pruning, irrigation scheduling and feedback from CGDC	Melton Mulaudzi
10/7/2012	KZN Nkweleni	Attending BEE Citrus study group re fertilisation , pruning , irrigation scheduling and Feedback from CGDC.	Melton Mulaudzi
16/07/2012	Uitenhage Agricultural Office	Attending meeting with Government Extension Officers from PE and Sundays River Valley and CGA about the activities plan and planning the Citrus growers day	Melton Mulaudzi
18/07/2012	Fort Beaufort Riverside Packhouse Eden Agric. Service Cape College East London	Attending meeting with Government Extension Officers from Nkonkobe and Great Kei	Melton Mulaudzi
19/07/2012	Granorpassi, Polokwane	Buying of shares from Granorpassi cooperative by the small citrus growers	Andrew Mbedzi
23/07/2012	Intaba Lodge	Attending Fertilizer meeting with Dr Hannes Coetzee , Shaun Brown and BEE farmers who are operating under Eden Services	Melton Mulaudzi
24/07/2012	Fort Beaufort Sihlangule Komoshe Secondary Coop	Attending meeting with Government Extension officials and cooperative management about developing a proper business plan for the farm	Melton Mulaudzi
	Dept. of Agric., Giyani	Planning of citrus study group sessions by Mopani Citrus study group	Andrew Mbedzi
25/07/2012	Levubu	Discussions on challenges facing the Levubu citrus growers	Andrew Mbedzi
26/07/2012	Thabazimbi	Removal of the citrus trees.	Andrew Mbedzi
	Riverside Packhouse Heal Town	Attending water committee meeting with prof Obi and L Lew from Riverside and Introducing Prof Obi to Government Extension officer Mrs N. Mbere	Melton Mulaudzi
27/07/2012	Naboomspruit	Monitoring of the irrigation of the citrus trees with government extension officers.	Andrew Mbedzi
	Alice KAT Gonzana,	Inspect picking and advice on pruning	Melton Mulaudzi

	Konzi	Advice on pruning	Melton Mulaudzi
	Eden	Advice on LB urea sprays for flower	Melton Mulaudzi
	Jerico	Advice on pruning on Satsuma and LB urea sprays	Melton Mulaudzi
	Torties	Advice on pruning of Nules clementines	Melton Mulaudzi
	Jordan	Advice on pruning of mature trees and skirting with brush cutter	Melton Mulaudzi
30/07/2012	Fort Beaufort Uitehage Foundation Block, SRV and ARC in Addo	Visiting the CRI Foundation Block and the SRCC P/house and Addo ARC with Jonathan Roberts, Justin Chadwick, SRCC Management and Peddie Farmers.	Melton Mulaudzi
	Fort Beaufort Uitehage Foundation Block Sundays River and ARC in Addo	Attending meeting with SRCC and Safe beneficiaries, Dr Vuyisile Phehane and Anati Canca of ARC, Jonathan Roberts, Justin Chadwick, SRCC Management and Peddie Farmers.	Melton Mulaudzi
02/08/2012	Dept. of Agric., Mpumalanga	MoU discussion between CGA, DARDLA and Dr. Hennie le Roux	Andrew Mbedzi
06/08/2012	Winterveldt farm, Pretoria	Discussions on irrigation challenges	Andrew Mbedzi
07/08/2012	Willow Park, JHB	Attending the Citrus Growers Development Chamber meeting	Andrew Mbedzi Melton Mulaudzi
08/08/2012	Sanddrift, Brits, North West	Visit Sanddrift with Dr Hennie Le Roux, DAFF officials (Rose and Vhengani).	Andrew Mbedzi Melton Mulaudzi
10/08/2012	Chauke's Farm, Malamulele	Advising the producer on water resource planning before expansion of orchards	Andrew Mbedzi
13/08/2012	Sundays River Valley Luthando	Attending Lima training on how to work with strategic Partners or Mentors	Melton Mulaudzi
14/08/2012	Patensie Tobacco office	Attending training with Lima on the strategic partners. Aviwe from DAFF addressing Bi cmonitoring in the area. Discussions on CBS and citrus psylla at Patensie with Dr Paul Fourie and Mrs Makhosini, Zanele Buhlungu and Mbaso Bawuti from DAFF.	Melton Mulaudzi
	Dept. of Agric. Makwarela	Vhembe Citrus Technical Committee meeting on selling of Granorpassi shares to small growers	Andrew Mbedzi
15/08/2012	KAT River Valley Riverside Pack house Cape College	Attending mentorship meeting with Riverside regarding DRDLR mentorship programme also attending strategic workshop with Lima and Kat River farmers.	Melton Mulaudzi
19 to 23/08/2012	KZN Drakensburg	Attending the 7 th CRI Citrus Research Symposium in KZN	Andrew Mbedzi Melton Mulaudzi
30/08/2012	Letaba Citrus Letsitele	Negotiating venue for citrus field day at Letaba Citrus Estate	Andrew Mbedzi
31/08/2012	ARC Hall, Levubu	Negotiating venue for the citrus growers workshop	Andrew Mbedzi
03/09/2012	ARC Hall, Levubu	CGA workshop on Strategic Partner/Joint Venture/Mentors	Andrew Mbedzi
04/09/2012	Tzaneen Country Lodge	CGA workshop on Strategic Partner/Joint Venture/Mentors	Andrew Mbedzi
	Marine Hotel PE	Attending the commercialisation of 5 University of California seedless Mandrins: Shasta Gold, Tahoe Gold, Yosemite Gold, Gold Nugget and Tango	Melton Mulaudzi

05/09/2012	Zebediela Citrus Estate	CGA workshop on Strategic Partner/Joint Venture/Mentors	Andrew Mbedzi
	Osner Hotel East London	Attending Agribusiness workshop with CGA, DAFF and other stakeholders.	Melton Mulaudzi
06/09/2012	Deja-vu, Pretoria	CGA workshop on Strategic Partner/Joint Venture/Mentors	Andrew Mbedzi
	DRDLR, Pretoria	MoU meeting between CGA and DRDLR held in DRDLR offices in Pretoria	Andrew Mbedzi Melton Mulaudzi
12/09/2012	CRI Boardroom, Nelspruit	CGA workshop on Strategic Partner/Joint Venture/Mentors	Andrew Mbedzi
13/09/2012	Alice Kat	Discussing the issue of citrus grower day with Bani Skortile.	Melton Mulaudzi
	Tories	Monitoring pruning of citrus orchards and wind breaks.	Melton Mulaudzi
14/09/2012	Pretoria	Attending workshop with CGA	Melton Mulaudzi
18/09/2012	Gillimburg, Mokopane	Waterberg Citrus Study group on Citrus Spring Pests Complex	Andrew Mbedzi
19/09/2012	Moletele, Hoedspruit	Waterberg Citrus Study group on Citrus Spring Pests Complex	Andrew Mbedzi
20/09/2012	Nkwaleni Community Hall KZN	Attending Lima training at Nkwaleni Community Hall on developing the business plan and production activities for Umhabawethu	Melton Mulaudzi
21/09/2012	Dept. of Agric. Makwarela	Vhembe Citrus Technical Committee meeting on the Citrus Field Day to be hosted by Mopani District	Andrew Mbedzi
25/09/2012	Vincent East London ECSECC Office	Attending meeting with Sihlangule Nkomose in East London on funding and development of a business plan.	Melton Mulaudzi
26/09/2012	Cape College, Fort Beaufort	Grower day organized by CRI, CGA and Eastern Cape DRDLR.	Melton Mulaudzi
04/10/2012	Tzaneen Country Lodge	Citrus Field Day on "Marketing of the Citrus Fruit".	Andrew Mbedzi
08/10/2012	Christian Centre, East London	DAFF Extension Policy Workshop. Present were; Phuhlisani Solution, DRDAR officials, CGA and other stakeholders.	Melton Mulaudzi
10/10/2012	Du Roi QMS, Letsitels	Deliver some CBS samples on my way to Vhembe district.	Andrew Mbedzi
11/10/2012	Temo Towers, Polokwane	Fresh Produce Exporters Forum Initiatives for BEE Marketing Agents	Andrew Mbedzi
	Ripplemead Farm	Inspect bollworm control. Plan planting of new trees with Recap funding	Melton Mulaudzi
	Rally Valley Farm	Inspect bollworm control	Melton Mulaudzi
	Naudehoek Farm	Discuss spraying programmes and maintenance of the spray machines and the tractors.	Melton Mulaudzi
12/10/2012	Eden Agric. Services/White Citrus Farm	Monitoring soil preparation (ripping) and ridging	Melton Mulaudzi
	Jordan farm	Monitoring application of potash fertilizer and inspect bollworm control.	Melton Mulaudzi
15/10/2012	Dept. of Agric. Makwarela	Vhembe Citrus Technical Committee Meeting on the Planning of Session 2 Citrus Study Group.	Andrew Mbedzi
18/10/2012	Chaukes's Farm, Malamulele	Citrus Study Group on Citrus Pests and Disease and Importance of Irrigating Citrus.	Andrew Mbedzi
22/10/2012	DRDLR Offices, Pretoria	Meeting between DRDRL and CGDC Executive discussing the draft MoU.	Melton Mulaudzi

	DRDLR Offices, Pretoria	Discussion of the Draft MoU between CGA and DRDLR.	Andrew Mbedzi
23/10/2012	Garden Court Hotel, Isando, Johannesburg	The Chamber Executive Committee Strategic Planning meeting 1.	Andrew Mbedzi
	Garden Court Hotel, Isando, JHB	CGDC Executive Committee Strategic Planning Meeting facilitated by Mr. Peter Green of Lima.	Melton Mulaudzi
24 to 26/10/2012	Tala Valley, Pietermaritzburg	Meeting between ADA and CGA to sign MoU for funding and monitoring the Tala Valley Project.	Melton Mulaudzi
31/10/2012	Kirkwood, Sundays River Valley	Attend launching of 38 beneficiaries who bought 117 ha through SRCC Farming Trust	Melton Mulaudzi
06/11/2012	LDA Shoprite Building Board room, Polokwane	Attend Stakeholder meeting with CGA in Polokwane	Andrew Mbedzi
	Willow Tree Farm, Kirkwood, SRV	Attending Citrus study group on CBS and FCM control.	Melton Mulaudzi
08 to 09/11/2012			
	Boplaas	Visit new 54ha citrus BEE Farm assisted by SRCC at Patensie.	Melton Mulaudzi
	Ubone trading	Visit farm and consult on pruning of dead twigs	Melton Mulaudzi
	Dankbaar	Collecting records of harvested Mandarin and also consulting on light pruning	Melton Mulaudzi
	Peter Family trust	Inspecting orchards and giving advise on controlling snails using snail baits	Melton Mulaudzi
	Klein However boer	Inspect pruning and new irrigation.	Melton Mulaudzi
12/11/2012	Gonzana	Monitoring bollworm on mature trees and orange dog caterpillar on newly planted trees.	Melton Mulaudzi
	Greenwood	Monitoring orange dog caterpillar on newly planted trees and discussions on Recapitalisation funding approval	Melton Mulaudzi
13/11/2012	Sihlangule Nkomashe Coop, East London	Meeting with Mentor (Mr Percy), Coop Members, the person appointed by the ECDC (Eastern Cape Development cooperation) to develop a business plan for the farm.	Melton Mulaudzi
14/11/2012	Thulwane Farm, Nkwaleni Valley	Attending citrus study group meeting at Nkwaleni valley on FCM, CBS control	Melton Mulaudzi
15/11/2012	Ratombo CPA, Levubu	Citrus Study Group on Packing Material, Citrus Rust mite and Thrips and Application of Pesticides and Fungicides	Andrew Mbedzi
16/11/2012	Oakdene	Visit farms and discuss the fertilization of newly planted trees and collecting tree census.	Melton Mulaudzi
	Torties	Monitoring planting of new soft citrus	Melton Mulaudzi
19/11/2012	Lovers Retreat farm	Monitoring scales and placement of FCM trap.	Melton Mulaudzi
	Lettas farm	Advise on fertiliser applications and weed control	Melton Mulaudzi
	Easy Farm, Thsivhilwi	Inspect new citrus packhouse	Andrew Mbedzi
20/11/2012	Dept. of Agric. Nylstroom	Planning of 2 nd Session of Citrus Study group	Andrew Mbedzi
21/11/2012	Willow Park Centre, Johannesburg	The Chamber Executive Committee Strategic Planning meeting 2.	Andrew Mbedzi Melton Mulaudzi

22/11/2012	Willow Park Centre, Johannesburg	4 th Chamber Meeting on the Executive Committee Strategic Planning meeting	Andrew Mbedzi Melton Mulaudzi
23/11/2012	DAFF Offices, Pretoria	The Reference Group Meeting for the Development of National Extension Policy	Andrew Mbedzi
28/11/2012	King Shaka Airport. KZN	Mentorship Programme Meeting on the centralization of mentors	Andrew Mbedzi Melton Mulaudzi
29/11/2012	Ripplemead Farm, Peddie	Attending Recapitilization meeting farmers and CGA.	Melton Mulaudzi
30/11/2012	Seloane, Phalaborwa	Discussion on Rootstocks and Cultivars for the re-planting of Seloane Farm.	Andrew Mbedzi
04/12/2012	Battlesden	Inspect for red scale consult on fertilizer applications	Melton Mulaudzi
05/12/2012	SRCC OFFICE and Sun Land farm, Sundays River Valley	Attend meeting with operational Manager, Mr Frikkie Olivier from SRCC and Alan Operational Manager of Safe Farm to discuss progress of recapitilization.	Melton Mulaudzi
	Morajomo Farm, Radium, Warmbath	Citrus Study Group on Citrus Pests and Disease and Rootstocks and Cultivars and Fruit Quality.	Andrew Mbedzi
06/12/2012	Makhado Show Ground, LTT.	Workshops on Sectorial Determination-Discussion on the Minimum Wages for Farm Workers.	Andrew Mbedzi
	Jerico, Eden, Konzi	Discuss spraying programme for FCM and CBS.	Melton Mulaudzi
07/12/2012	Greenwood	Assisting with irrigation.	Melton Mulaudzi
	Orange Grange	Monitoring of 3:0:1 fertilizer application.	Melton Mulaudzi
	Easy Farm., Tshilwi	Visit orchards and see progress of the establishment of new citrus packhouse	Andrew Mbedzi
10/12/2012	Ripplemead	Monitor soil preparation and conducting fruit sizing on clementines to determine the need to apply Corasil-P	Melton Mulaudzi
11/12/2012	Lowveld College, Nelspruit	Fresh Produce Market Forum meeting at the Lowveld College of Agriculture.	Andrew Mbedzi
	Agric. Economics office. East London	Attending partnership policy meeting with CGA and other Government Officials.	Melton Mulaudzi
21/01/2013	Sundays River Addo Willowtree	Fruit fly control and developing 2013 activities plan	Melton Mulaudzi
22/01/2013	Sihlangule Komashe co-op at Great Kei East London	Appointment of Colin Painter as the Mentor for Sishangule-Nkomoshe project and to assist the project to develop a business plan for the farm	Melton Mulaudzi
23/01/2013	Cape College Fort Beaufort	Fruit fly control, developing activities plan for 2013 and Government policy regarding how to access funding from Government and how to work with strategic partners.	Melton Mulaudzi
	OR Tambo City Lodge Hotel	Chamber Executive Committee meeting on the Situational Analysis received proposals from service providers.	Andrew Mbedzi
24/01/2013	Eden Agri – Services Blinkwater	Attending meeting with Mr Shaun Brown regarding the role of Extension service and the mentorship programme	Melton Mulaudzi
25/01/2013	Mariveni	Discuss marketing challenges facing Mariveni farm.	Andrew Mbedzi

28/01/2013	Makwarela Dept of Agric	Vhembe citrus technical committee meeting and the CGA Roadshows.	Andrew Mbedzi
29/01/2013	The Ranch Hotel, Polokwane	Pack-house workshop held at the Ranch Hotel in Limpopo	Andrew Mbedzi
30/01/2013	The Ranch Hotel, Polokwane	Pack-house workshop held at the Ranch Hotel in Limpopo	Andrew Mbedzi
31/01/2013	Loskop Dam, Lydenburg	Pack-house workshop held at the Loskop Dam in Mpumalanga	Andrew Mbedzi
	Sihlangule co-op Great Kei East London	Finalizing the development of a business plan to be submitted to Rural Development and Land Reform for accessing Recap.	Melton Mulaudzi
01/02/2013	Loskop Dam, Lydenburg	Pack-house workshop held at the Loskop Dam in Mpumalanga	Andrew Mbedzi
05/02/2013	White Citrus Farm	Monitoring Weed control and also to fertilize applications of newly planted trees according to recommendations done by Dr Hannes Coetzee	Melton Mulaudzi
	Oakdene	Inspect weed control	Melton Mulaudzi
06/02/2013	Lidell farm	Inspect weed control	Melton Mulaudzi
	Letas farm	Inspect weed control	Melton Mulaudzi
07/02/2013	DRDLR Offices, Pretoria	Meeting between Chamber Executive Committee and DRDLR on the draft MoU	Andrew Mbedzi
13/02/2013	Patensie Tobacco office	Fruit fly control, developing activity plan for 2013, feedback from CDGC meeting and the way forward regarding Recap.	Melton Mulaudzi
14 to 15/02/2013	Summerstrand Hotel PE	Production costs by Hannes Bester Export standards by Cyril Julius FCM Dr Sean Moore Physiological rind disorders by Paul Harvesting by Keith Leasar	Melton Mulaudzi
15/02/2013	Mariveni	Assess hail damage to the citrus fruit crop.	Andrew Mbedzi
	Greenwood	Monitoring weeds control , fruit fly, FCM ,Bi. and orchard sanitation	Melton Mulaudzi
19/02/2013	Lovers Retreat	Monitoring weeds control , fruit fly , FCM ,Bi. and orchard sanitation.	Melton Mulaudzi
	Thulwane Farm, Nkwaleni Valley	Fruit fly control, developing 2013 activity plan and the CBS	Melton Mulaudzi
20/02/2013	Noordgrens Lapa, Weipe	CGA Roadshow held at the Noordgrens Lapa, Weipe in Limpopo	Andrew Mbedzi
	The Junction, Letsitele	CGA Roadshow held at the Junction, Letsitele in Limpopo	Andrew Mbedzi
21/02/2013	Nulandis	Vhembe study group plan for 2013/2014 season	Andrew Mbedzi
22/02/2013	Chauke's Farm	Farm visit and to check on the control of orange dog caterpillars	Andrew Mbedzi
26/02/2013	Ripplemead	Monitoring the damage caused by hail towards December 2012. Discussing the development of Recap.	Melton Mulaudzi
	Rallyvally	Inspect hail damage	Melton Mulaudzi
	Naudeshoek	Inspect hail damage	Melton Mulaudzi
27/02/2013	Lidell farm	Monitor new tractor, herbicides and mower machine bought by recap funding.	Melton Mulaudzi
	Jordan Farm	Monitor soil preparation and ridging done by Pringle contractor using recap funds.	Melton Mulaudzi

28/02/2012	Fort Beaufort Katco	Attending CGA Roadshow with farmers, the Citrus Academy, the CCGA, the CGA and River Bioscience	Melton Mulaudzi
07/03/2013	Gonzana farm	Monitor FCM, Fruit fly and B.i traps	Melton Mulaudzi
	Eden farm	Monitor B.i traps	Melton Mulaudzi
	Jerusalem farm	Inspect weed control.	Melton Mulaudzi
08/03/2013	Makwarena Dept of Agric	Vhembe Citrus Technical Committee meeting on the Planning of Citrus Study Groups and the Citrus Field Day.	Andrew Mbedzi
11/03/2013	Willow Park Centre, Johannesburg	Attend the first Citrus Growers Development Chamber meeting.	Andrew Mbedzi
12/03/2013	Sefala Building, Pretoria	Attend the DAFF Extension Policy Reference Group meeting	Andrew Mbedzi
	EL Road Lodge to PE Newton Park	Attend PPECB Eastern Cape pre-season workshop	Melton Mulaudzi
15/03/2013	Greenwood	Farm visit. Monitoring new tractor, herbicides and mower machine bought by recap funding. Also monitoring soil preparation and ridging done by Pringle contractor using recap funds.	Melton Mulaudzi
	Jerico	Monitoring FCM, Fruit fly and B.i traps	Melton Mulaudzi
	Orange Grange	Monitoring FCM, Fruit fly and B.i traps	Melton Mulaudzi
18/03/2013	Tzaneen Country Lodge, Taretaaland	Accompany the Taiwanese delegation, the DAFF Asia Desk, and GTEDA.	Andrew Mbedzi
	PE CRI Offices	Attending Job appraisal with line Manager Mr Hannes Bester.	Melton Mulaudzi
19/03/2013	Bosveld Sitrus, GTEDA offices	Visit Bosveld Sitrus farm and GTEDA offices with the Taiwanese Delegation	Andrew Mbedzi
20/03/2013	Tours farm, Nkowankowa	Visit Tours farm and leather making GTEDA project at Nkowankowa with the Taiwanese Delegation	Andrew Mbedzi
22/03/2013	Easy farm	Farm visit and check on the progress of the packhouse.	Andrew Mbedzi
25/03/2013	Fort Beaufort to Sundays River Valley Luthando Farm	Study group meeting with Mrs Nikki Combrink from ARC doing presentation on how to take soil and leaf analysis, woolly white fly and B.i was done by CRI Extension Coordinator	Melton Mulaudzi
26/03/2012	African Sand B&B, PE	Meeting organized by Rural development and farmers to discuss the farmers' debts with Land Bank.	Melton Mulaudzi

8.2 RESEARCH PRIORITIES 2012-2013

The research priorities for 2013 were determined during September 2012. A communication was sent to all the citrus producers who are on CRI's Technology Transfer Group (TTG) list, listing all the research approved for 2012. This provided a means for growers to assess which of their previous requests were being addressed. Growers were requested to study these research projects and indicate any additional research required as a priority. They were advised to weight requirements from 1-3, with 3 the higher priority. Once the TTG's Technical Committee had received all the research requests from the area they compiled a summary and a meeting was held with the Area Extension Manager and the Technical committees. The Area Extension Managers from the North and the South compiled the resultant research priorities and forwarded these to the Manager Research and Technical.

The research priorities were also determined at the five CRI Post Harvest Workshops held in Limpopo, Mpumalanga, KwaZulu-Natal, the Eastern Cape and the Western Cape in February 2012 as well as the Exporters Technical Panel and the growers involved in the Transformation process. The majority of the research priorities are the same as last year. In the Crop and Fruit Quality programme a number of new priorities were added.

The research priorities were basically the same as the previous year with the exception of research requested with regard to netting. This is new but several areas asked what the influence would be on flower, fruit set, maturity, internal quality, *Alternaria* and the pest complexes. The resultant Research Priorities can be summarized as follows:

1. DISEASE MANAGEMENT

1.1 Citrus Black spot

- The growers experienced enormous problems with DAFF during the latter part of the 2012 season when all PUCs that were sending fruit to the EU had to be inspected for CBS. DAFF could not get round to do these inspections in time and fruit had to be kept for days before the necessary documents could be obtained to ship these fruit. It is thus of the utmost importance that the status of CBS should be changed from a phytosanitary to a cosmetic disease in order to get rid of this disruption. This requires that all the research that was completed on CBS should be published in refereed journals. 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. (Replacement for mancozeb.) 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.
- Convince the chemical companies selling strobilurens to change the labels in order to be able to spray these products without mancozeb as there is no threat of CBS resistance.
- Get the USDA to acknowledge the Weipe & Tshipise areas as areas of low pest prevalence.

1.2 *Alternaria*

- Alternative spray programmes to control *Alternaria* more effectively with fewer 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.
- Determine the effect of shade netting on *Alternaria* on susceptible cultivars.
- Confirm the possibility of *Alternaria* resistance against the strobilurens

1.3 *Botrytis*

- Spray programmes to control *Botrytis* on lemons during flowering.
- Determine the effect of *Botrytis* on lemons in the Sundays River Valley.

1.4 *Phytophthora citrophthora*

- More effective control programmes.
- Screening of all new cultivars against *P. citrophthora*.

1.5 Post-Harvest diseases

- Optimisation of the flooder to allow commercial use in packhouses
- Optimisation of fungicide treatments in the packhouse to be the most effective to protect the fruit and to prevent resistance developing.
- Optimising 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*.

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 KwaZulu-Natal for a possible introduction of Asian HLB.
- 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.
- Confirm if there are any alternative hosts for Asian HLB in South Africa.

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 eg. Fruit set, maturity, colour.

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 pack line.
- 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 SADEC countries together to deal with *Bactrocera* together. Get Zimbabwe to acknowledge the existence of the pest in that country.
- Registration of Hym lure/Prolure + Spinosad

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/Prolure.

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 piercing moths

- Monitoring and control of fruit piercing 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 @ 30ml/100l 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. HORTICULTURE 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.

3.1.2 Chilling injury

- Develop post harvest treatments to control chilling injury.

3.1.3 Rind breakdown

- Develop better methods to predict and control rind breakdown.
- Need alternatives for TBZ.

3.1.4 Blossom end clearing

- Develop a better understanding of the problem and ways to prevent it.

3.1.5 Cold damage

- Develop techniques to protect fruit and trees against frost damage

3.1.6 Shelf life

- Develop techniques to extend the shelf life of citrus fruit.
- Method to quantify over ripeness and puffiness.

3.1.7 Tear staining

- Determine the cause of tear staining on Nadorcotts and how to prevent it.

3.1.8 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 Morris, orrs and Nadorcotts a problem.
- Effect of netting on flower

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.
- Effect of netting on fruit colour development.

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.
- Effect of shade nets on internal quality

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.
- Effect of netting on cold damage

3.2.10 Sunburn

- Methods to reduce sun burn.
- Alternatives for oil to reduce sun burn on grapefruit.
- Effect of netting on sunburn

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.
- Effect of netting on fruit colour

3.2.12 Fruit size

- Methods to increase fruit size on Deltas, Clementines and Rustenburg navels.

3.2.13 Blossom end clearing on grapefruit

- Causes and control

3.2.14 Evaluation of biostimulants

- Biostimulants such as Alexin, Citrox, CropBiolife, Cilic, Messenger, Mannitol, Sorbitol and GA14 should be evaluated.

3.2.15 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 & 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 CO₂-levels during shipping.

3.3.2 Packaging and Palletizing

- Evaluate new pallets and 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.

4. CULTIVAR EVALUATION

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 with a better internal quality.
- Earlier and later Clementines.
- Late mandarins of which the plantings are 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 sheepnose.
- A better tasting red grapefruit.
- Earlier and later Valencia selections.
- Olinda Valencia to be reintroduced to the Foundation Block.

SUMMARY: HOW WELL DO THE RESEARCH PROPOSALS ADDRESS THE RESEARCH NEEDS?

Disease Management Portfolio

- This programme covers most of the requests put forward by the industry. The most pressing issue in this programme is CBS market access. It does however have the full attention of the CEO (CRI), the Disease Management Portfolio Manager and the researchers. They are involved with researchers from the USA and the current market access threat into the EU should be solved within the foreseeable future. Ironically the CBS threat into the EU could reach its height this coming season. An aspect that is too long outstanding is the registration of a phosphonate which can safely be used against *Phytophthora* brown rot.

Integrated Pest Management Portfolio

- As is the case with Disease Management the research proposals cover the producer's research needs. As was stated last year: There are no other citrus producing country where so much research is conducted by so few to cover so many pest problems which threaten the future existence of a citrus industry.
- FCM is by far the industry's largest market access threat. It does receive the majority of the research funding and CRI is doing whatever possible to prevent the industry being forced into cold sterilisation for all exports.
- *Bactrocera invadens* is the second biggest IPM threat to the industry. Between CRI and DAFF they have done wonders in eradicating each invasion of this fly so far. This threat will however keep on coming and eventually the industry will have to deal with it on a scale where eradication is no longer possible. Producers will have to realise that each PUC will have to monitor the status of Bi to be able to prove that it is free of this fly. Research has put in place the means to control it.

Crop and Fruit Quality Management

- The status quo are the same as last year with many of the priorities not being addressed because of a lack of research capacity: 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 a lack of capacity and nobody available to fill that capacity. 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 sheep nose which is also not adequately addressed. Pruning of the late mandarins and lemons has not been addressed and sunburn is still an issue. Cold damage in the orchard is not addressed properly. There has also been a new priority viz. research into the use of netting. This research need did not exist in the past. This year several areas requested research with one area making it its number 1 priority

CRI-PTF (CCCF)

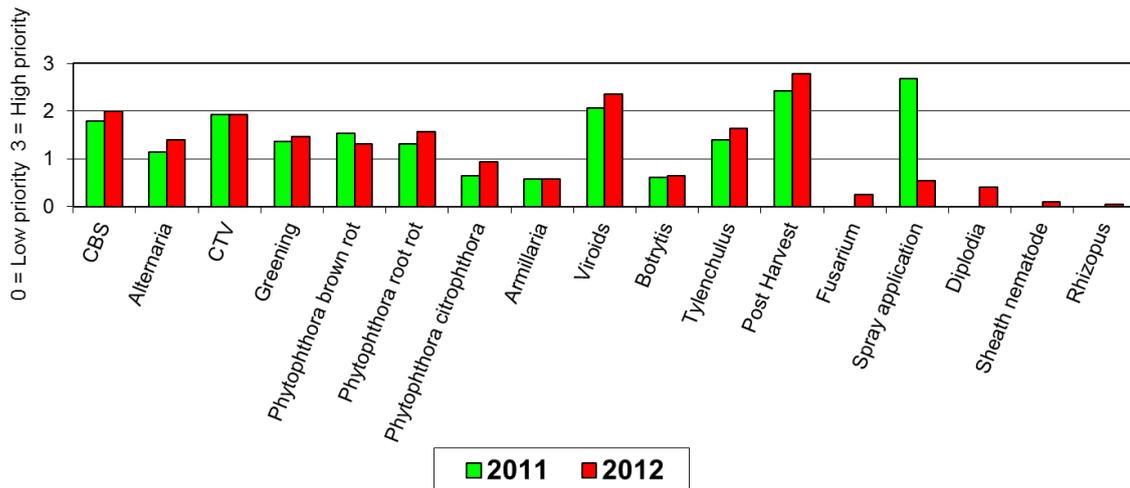
- In the past the CRI-PTF was the one area where most of the issues which were not addressed could be found. The appointment of Dawid Groenewald has helped to solve this. Many of these issues have since been addressed. Because of the accreditation of carton manufactures and carton

standards no problems were experienced during this past season with carton collapse. Pallet manufacturers have not been accredited which can be seen in the number of complaints received with regard to substandard pallets. This will be addressed.

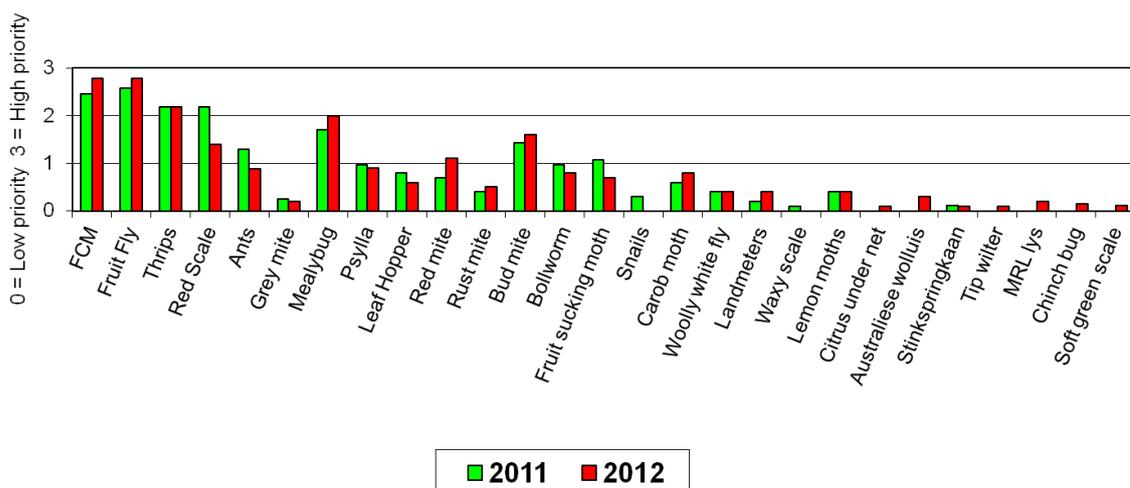
Cultivar Evaluations

- As new cultivars become available they are included in the Cultivar Evaluation programme and the results are available to growers in the form of the Cultivar Fact Sheets. Unfortunately Richard Fenwick has resigned and will need urgent replacement in order for CRI to deal with the cultivar evaluations in the south.

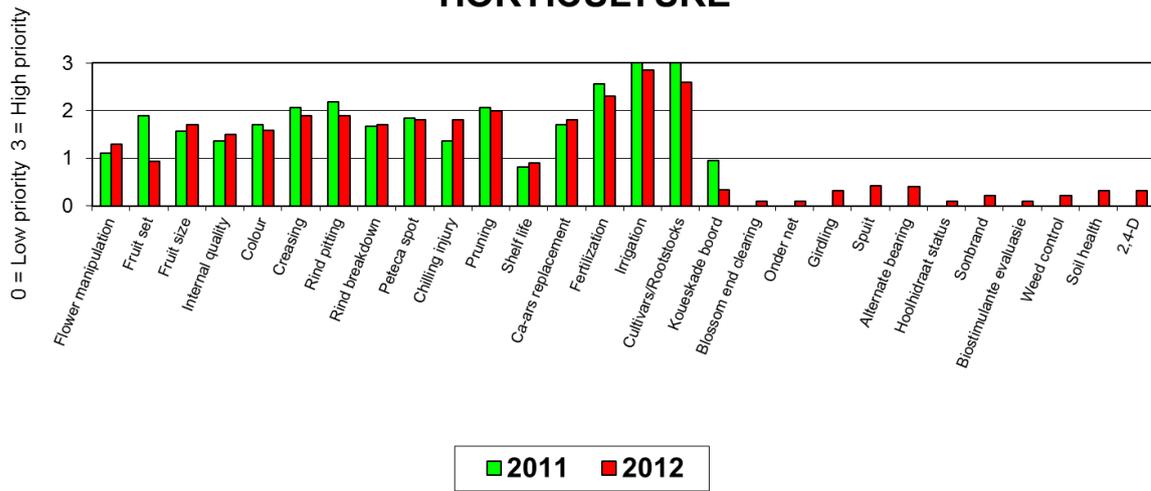
AVERAGE PRIORITY RATINGS FOR DISEASE MANAGEMENT



AVERAGE PRIORITY RATINGS FOR INTEGRATED PEST MANAGEMENT



AVERAGE PRIORITY RATINGS FOR HORTICULTURE



RESEARCH PRIORITIES – NORTHERN & SOUTHERN AREAS - 2012-13

TABLE 1.

DISEASE MANAGEMENT

Citrus Area	CBS		Alternaria		CTV		Greening		Phytophthora brown rot		Phytophthora root rot		Phytophthora citrophthora		Armillaria		Viroids		Botrytis		Tylenchulus		Post Harvest		Fusarium		Spray application		Diplodia		Sheath nematode		Rhizopus			
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012		
Baviaans	2	2	1	1	3	3	2	2	2	2	1	1	1	1	3	3	3	3	0	0	2	2	3	3	0	1	3	0	0	1	0	2	0	0		
Beitbridge	2	2	0	0	2	2	1	1	1	1	1	1	0	0	0	0	1	1	0	0	1	1	2	2	0	0	3	3	0	0	0	0	0	0		
Breederivier	0	0	2	2	2	2	3	3	1	1	1	1	2	2	3	3	3	3	0	0	3	3	3	3	0	1	3	0	0	1	0	0	0	0		
Burgersfort	2	3	2	3	0	0	2	3	2	0	1	2	0	3	0	0	3	3	0	0	2	1	3	3	0	0	3	0	0	0	0	0	0	0		
Citrusdal	0	0	1	2	3	3	0	0	1	1	1	2	2	3	0	0	3	3	1	1	3	3	3	3	0	1	3	0	0	1	0	0	0	0		
Constantia	3	3	2	0	2	3	1	1	1	3	2	3	0	0	0	0	2	3	0	0	2	3	3	3	0	0	3	3	0	0	0	0	0	0		
Hoedspruit	3	3	1	3	3	3	0	2	1	0	1	1	0	0	1	1	1	1	1	1	1	3	3	3	3	0	0	3	3	0	0	0	0	0	0	
Katrivier	2	2	2	2	2	2	3	3	1	1	3	3	1	1	2	2	3	3	1	1	3	3	0	3	0	0	3	0	0	0	0	0	0	0	0	
Knysna	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Malelane/Komat	3	3	1	1	3	3	1	1	1	1	1	1	0	0	0	0	1	1	0	0	2	2	3	3	0	0	3	0	0	0	0	0	0	0	0	
Marble Hall	3	3	2	3	1	0	2	3	3	3	2	3	0	0	0	0	3	3	1	1	2	2	3	3	0	0	3	0	0	2	0	0	0	0	0	
Midnight	0	3	0	2	0	0	0	2	0	1	0	2	0	0	0	0	3		0	0	0	2	0	3	0	0	0	0	0	0	0	0	0	0	0	
Nelspruit	3	3	1	3	1	0	3	3	2	0	2	2	1	2	0	0	3	3	3	2	2	2	2	3	3	0	0	3	0	0	0	0	0	0	0	
Nkwaleni	3	3	0	0	2	2	0	0	2	2	1	1	1	1	1	1	3	2	1	1	1	1	3	3	0	1	3	0	0	0	0	0	0	0	0	
Ohrigstad	1	3	2	3	0	0	2	3	1	0	1	3	0	3	0	0	3	3	0	0	0	1	3	3	0	0	3	0	0	2	0	0	0	0	0	
Oranjerivier	0	0	1	1	3	3	0	0	2	2	3	3	2	2	0	0	3	3	0	0	1	1	2	2	0	1	3	3	0	0	0	0	0	0	0	
Patensie	2	2	1	1	3	3	2	2	2	2	1	1	1	1	3	3	3	3	0	0	2	2	3	3	0	1	3	0	0	1	0	0	0	0	0	
Pongola	3	3	1	1	3	3	2	2	2	2	1	1	0	0	0	0	1	1	1	1	1	1	3	3	0	0	3	0	0	0	0	0	0	0	0	0
Rustenburg	3	3	2	2	0	0	3	3	2	2	1	1	0	0	0	0	1	1	0	0	3	3	3	3	0	0	3	0	0	0	0	0	0	0	0	0
Sondagsrivier	3	3	2	2	3	3	3	3	3	3	1	1	1	1	1	1	3	3	3	3	1	1	3	3	0	0	3	0	0	0	0	0	0	0	0	0
Stellenbosch Paarl Swartland	0	0	1	1	3	3	2	2	3	3	2	2	2	2	0	0	3	3	1	1	1	1	3	3	0	0	3	0	0	0	0	0	0	0	0	1
S. Natal	3	3	1	1	3	3	2	2	2	2	3	3	1	1	0	0	3	3	1	1	1	1	3	3	0	1	3	0	0	0	0	0	0	0	0	0
Swaziland	3	3	2	2	3	3	2		2		1	1	0		2	2	1	1	1		1	1	3	3	0	0	3	0	0	0	0	0	0	0	0	0
Swellendam	0	0	2	2	2	2	2	2	2	2	1	1	3	3	0	0	3	3	0	0	0	0	3	3	0	0	3	0	0	0	0	0	0	0	0	0
Tshipise	3	3	0	0	2	2	0	0	1	1	1	1	0	0	0	0	1	1	0	0	1	1	2	2	0	0	3	3	0	3	0	0	0	0	0	0
Vaalharts	0	0	1	1	3	3	0	0	1	1	1	1	0	0	0	0	3	3	2	2	1	1	0	3	0	0	3	0	0	0	0	0	0	0	0	0
Waterberg	2	3	3	3	0	0	2	1	1	0	2	2	0	0	0	0	1	3	0	3	2	3	3	3	0	0	3	0	0	0	0	0	0	0	0	0
Weipe	3	3	0	0	2	3	0	0	1	1	1	0	0	0	0	0	0	3	0	0	0	1	2	3	0	0		0	0	0	0	0	0	0	0	0
Weight	50	56	32	39	54	54	38	41	43	37	37	44	18	26	16	16	58	66	17	18	39	46	68	78	0	7	75	15		11		2		1		
Average	1.79	2	1.14	1.39	1.93	1.93	1.36	1.46	1.536	1.32	1.32	1.57	0.64	0.929	0.57	0.57	2.07	2.36	0.607	0.643	1.3929	1.643	2.429	2.786	0	0.3	2.679	0.536	0	0.4	0	0.07	0	0.04		

CULTIVAR & ROOTSTOCK DEVELOPMENT

TABLE 4. Citrus Area	Research Priorities/Navorsingsprioriteite 2012-13		
	Cultivars & Rootstocks		
	2012	2013	
Baviaans	3	3	Kry onderstam wat meer weerstand het teen Armillaria. Kyk voortdurend na verbeterde nawelseleksies vir die area om bestaande seleksies te vervang, ook later nawels. Enige niskultivars vir die area belangrik.
Beitbridge	3	3	
Breederivier	3	3	Soek nuwe niskultivars. Kry manderyn wat ferm is met goeie kleur en vruggrootte.
Burgersfort	3	3	Goeie vroeë sagtesitrus kultivars in plek van Satsuma
Citrusdal	3	3	Nuwe kultivars altyd belangrik. Evalueer alle nuwe kultivars in area. Kry uitsluitel oor onverenigbaarheid met Fokumoto en Mor t.o.v. Trifoliaat-onderstamme (Troyer, Carrizo, C35 en Swingle).
Constantia	3	3	Watter onderstamme om te gebruik op swaar gronde. Sagtesitruskultivarproewe.
Hoedspruit	3	2	Sagtesitruskultivars vir Hoedspruit. Proewe moet biede by Ambrosia (drup) onder net en by Hannes Meintjies onder micros gedoen word.
Katrivier	3	3	Find alternative rootstocks for replant soils. Find niche varieties for area, especially mandarin/soft citrus types.
Knysna	0	0	
Malelane/Komati.	3	3	
Marble Hall	3	2	Evalueer kultivars vir koue gevoeligheid.
Midnight	0	3	C35 en ander verdwergde onderstamme
Nelspruit	3	3	
Nkwaleni	3	3	This is ongoing research to find better varieties for the area. Need early grapefruit and other varieties to extend the season.
Ohrigstad	3	0	Voorligting
Oranjerivier	3	3	Evalueer verskillende kultivars per sitrustipe en onderstam om mees geskikte opsies vir area te vind.
Patensie	3	3	Kry onderstam wat meer weerstand het teen Armillaria. Kyk voortdurend na verbeterde nawelseleksies vir die area om bestaande seleksies te vervang, ook later nawels. Enige niskultivars vir die area belangrik.
Pongola	3	3	
Rustenburg	3	3	
SRCC	3	3	Kry RL onderstam vir suurlemoene wat verdraagsaam is teen Phytophthora. Vind uit of Esselen Kwekery een het. Kyk ook na ander onderstamme vir suurlemoene wat minder groeikragtig is as RL, maarmeer as X639. Kry niskultivars vir area. Stel bestuurder vir nuwe kultivarmaatskappy aan. Kry fermere en beter nawelseleksies. Kry goeie vroeë nawel.
Stellenbosch Paarl Swartland	3	0	Kry vroeë Valencia met beter interne gehalte vir VSA – laer suur en effens beter suiker.
S. Natal	3	3	Need early and late navels to spread packing season. Need replacement for Rustenburg and something later. Also need soft citrus varieties for these areas.

Swaziland	3	3	
Swellendam	3	3	Onderstamme: Soek onderstam wat fisiologiese skildefekte verbeter sonder om kwaliteit in te boet. Kultivars: Laat Manderyne steeds groot behoefte. Meer deeglike evaluasie van nuwe kultivars voor vrystelling benodig.
Tshipise	3	3	
Vaalharts	3	3	Benodig alternatiewe nawelseleksies vroeg, met beter kleur.
Waterberg	3	3	Sagte siturs vir hierdie spesifieke area. Vroeë Valencias.
Weipe	0	0	
Weight	75	70	Highest priority = 3 in red.
Average	2.68	2.5	

8.3 **STUDY GROUP CHAIRMEN FOR 2012-13.** The list of contact numbers of the chairmen of the different Technology Transfer Groups, also known amongst the growers as Citrus Study groups, are the following.

TTG	Name	Tel. no	Email
Baviaans	Phillip Dempsey	082 498 2778	phillipdempsey@southernfruit.co.za
Beitbridge	Paul Bristow	072 701 9227	pbristow@iwayafrica.com
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	Albert Winterbach		waterval@bfwisp.com
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	Isabel Sparks	071 415 0288	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	Theuns Nieuwoudt	082 559 2992	sneht@ctecg.co.za
Nelspruit	Willem Kieviet	082 490 2991	wkieviet@vodamail.co.za
Nkwalini	Mike Wafer	083 278 6150	michaelwafer@yahoo.com
Ohrigstad	Clive Pountney	082 772 4608	citrusc@telkomsa.net
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	mhlati@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
Swaziland	Gerd Höppner	09268-3232311	ghoppner@rhodesfoodgroup.Com
Swellendam	Sarel Neethling	082 551 2357	sarel@thornlands.net
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

8.4 THE RELATIVE FUNDING SUPPORT FOR RESEARCH PORTFOLIOS AND PROGRAMMES FOR 2012-13

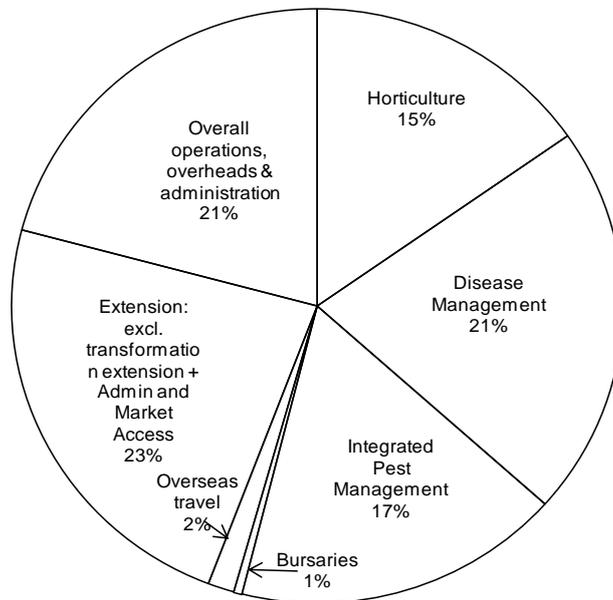


Fig. 8.4.1. Percentage funding in each CRI Portfolio and the rest of the budget for 2012-13.

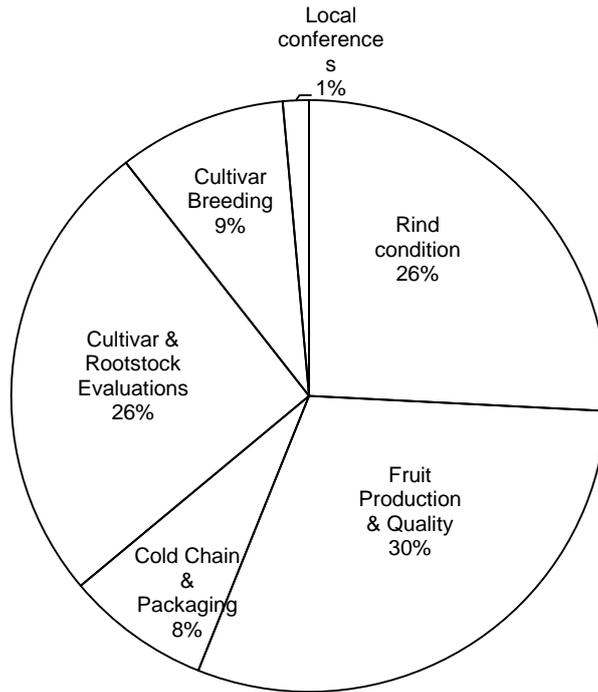


Fig. 8.4.2. Percentage funding to projects in the CRI Research Portfolio: Horticulture for 2012-13.

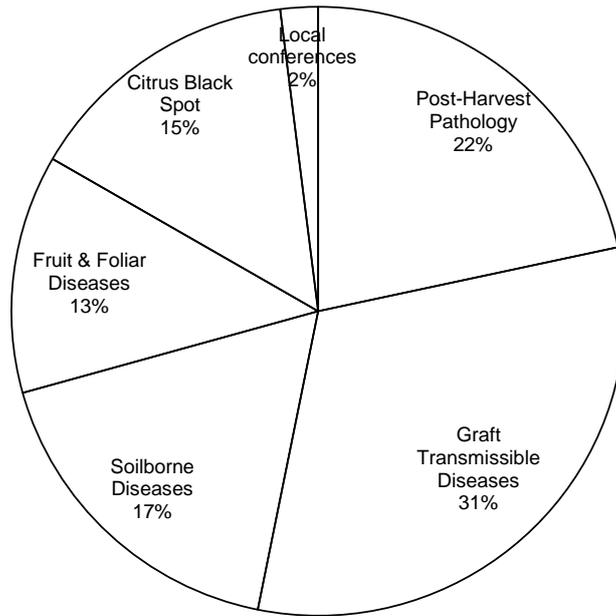


Fig. 8.4.3. Percentage funding to projects in the CRI Research Portfolio: Disease Management for 2012-13.

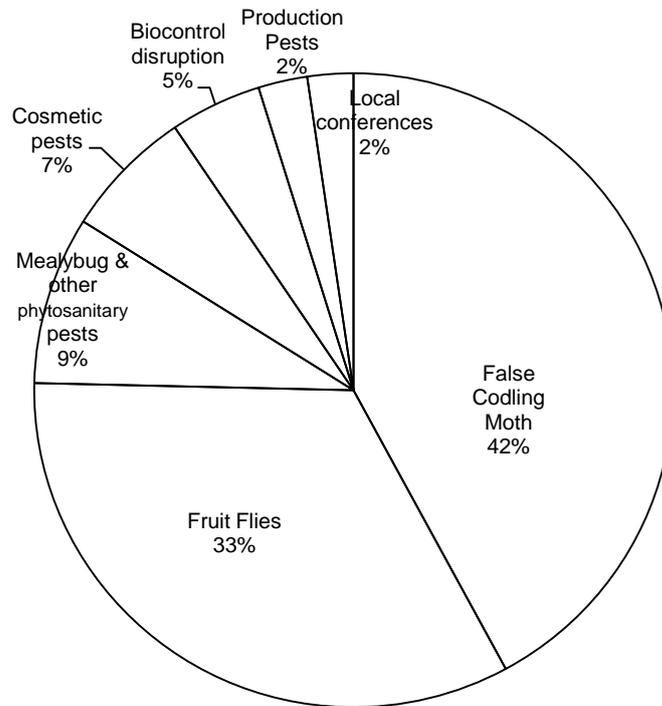


Fig. 8.4.4. Percentage funding to projects in the CRI Research Portfolio: Integrated Pest Management for 2012-13.

8.5 EXTENSION PRESENTATIONS BY CRI RESEARCHERS IN 2012-13

Name	Date	Place	Topic
Basson, E.	7 th Citrus Research Symposium 19-22 Aug 2012	Champagne Sports Resort, Drakensberg	Poster: The Diagnostic Centre: A diagnostic service to the citrus industry
Breytenbach, J.H.J.	7 th Citrus Research Symposium 19-22 Aug 2012	Champagne Sports Resort, Drakensberg	<i>Citrus Tristeza Virus</i> cross-protection of Star Ruby grapefruit: field trial results
Carstens, E.	7 th Citrus Research Symposium 19-22 Aug 2012	Champagne Sports Resort, Drakensberg	CBS-free production areas in South Africa
Cook, G.	7 th Citrus Research Symposium 19-22 Aug 2012	Champagne Sports Resort, Drakensberg	Investigation into the seasonal population fluctuation of <i>Trioza erytreae</i> and infection with the greening organism, <i>Candidatus Liberibacter africanus</i>
Cronjé, P.	February 2013	Packhouse Workshops: Polokwane Loskopdam Port Elizabeth Stellenbosch	Rind Condition Poster: Differential susceptibility to peel pitting in fruit from citrus cultivars with different albedo thickness
	7 th Citrus Research Symposium 19-22 Aug 2012	Champagne Sports Resort, Drakensberg	Could ethylene metabolism in lemon fruit influence peteca spot incidence? (2) Postharvest rind disorders of 'hardorcott' mandarin are affected by rootstock in addition to postharvest treatments Poster: Differential susceptibility to peel pitting in fruit from citrus cultivars with different albedo thickness
Erasmus, Arno	7 th Citrus Research Symposium 19-22 Aug 2012	Champagne Sports Resort, Drakensberg	Practical impact of imazalil resistance on control of postharvest citrus green and blue mould
	February 2013	Packhouse Workshops: Polokwane Loskopdam Port Elizabeth Stellenbosch	Management of critical control points; (2) Fungicide options & resistance management; (3) Fungicide application & residue loading
Groenewald, D.	February 2013	Packhouse Workshops: Polokwane Loskopdam Port Elizabeth Stellenbosch	Packaging material specifications for 2013
Fenwick, R.	7 th Citrus Research Symposium	Champagne Sports Resort, Drakensberg	Cultivar maturity charts for specific citrus production regions (southern areas)
Fourie, P.H.	7 th Citrus Research	Champagne Sports	Modelling of G.

	Symposium 19-22 Aug 2012	Resort, Drakensberg	<i>pseudothecium</i> maturation and ascospore dispersal in citrus orchards Optimal use of pyrimethanil, a new postharvest fungicide, for the control of green mould on citrus in SA
Grout, T.G.	24 Apr 2012	University of Pretoria	Discussion on water usage on citrus farms
	17 May 2012	Bee-Eaters, Nelspruit	Discussion with Villa Crop Protection on products required in citrus.
	18 May 2012	Water Research Commission, Pretoria	Workshop for research on citrus water use
	4 Jul 2012	CRI, Nelspruit	Nulandis citrus workshop
	19-22 Aug 2012	Champagne Sports Resort, Drakensberg	<i>Bactrocer</i> <i>invadens</i> control; residues on citrus under shadecloth; development of ant bait; response of FCM to low temperatures and controlled atmospheres.
	30 Jan 2013	CRI, Nelspruit	Present workshop for DAFF inspectors.
	17-22 Feb 2013	KZN, Swaziland, Mpumalanga	CGA road show
Hattingh, V.	7 th Citrus Research Symposium 19-22 Aug 2012	Champagne Sports Resort, Drakensberg	Market Access, Biosecurity and the SA citrus industry
Joubert, J.	7 th Citrus Research Symposium 19-22 Aug 2012	Champagne Sports Resort, Drakensberg	Cultivar maturity charts for specific citrus production regions (northern areas)
Kirkman, W.	7 th Citrus Research Symposium 19-22 Aug 2012	Champagne Sports Resort, Drakensberg	Post-harvest false codling moth risk mitigation through cold treatment
	21 November 2012	ETP, Cape Town	FCM systems approach for EU
Le Roux, H.F.	7 th Citrus Research Symposium 19-22 Aug 2012	Champagne Sports Resort, Drakensberg	Poster: Improvements to the CRI extension model
Lesar, K.H.	7 th Citrus Research Symposium 19-22 Aug 2012	Champagne Sports Resort, Drakensberg	Poster: Improvements to the CRI Extension model
	February 2013	Packhouse Workshops: Polokwane Loskopdam Port Elizabeth Stellenbosch	(1) Overview of postharvest diseases; (2) Degreening Guidelines; (3) Pre- & Postharvest handling & sanitation for decay control
	4 Apr 2012	Naranja, Burgersfort	Imazalil en TBZ residue oorskrydings
	17 Apr 2012	Karino Co-op	Konsultasie oor chloor en swamdoder aanwendings
	3 May 2013	Schalk Visser, Wenkem	Citrosol citrus waxes, Citrosol & Fortisol
	7 May 2012	Katrivier/Riverside	Consultation & visit

		Packhouses: Katco,Riverside & Eden Agri	
Manrakhan, A.	7 th Citrus Research Symposium 19-22 Aug 2012	Champagne Sports Resort, Drakensberg	Fruit Flies: Are we winning the war?
Moore, S.D.	3 Apr 2012	BEE grower meeting, Fort Beaufort	Fruit fly and WWF
	19 July 2012	Area-wide strategic planning meeting, Kakamas	FCM and fruit fly
	4-5 Sep 2012	Grower meeting, White River	FCM and symposium feedback
	6-7 Sep 2012	Grower meeting, Polokwane	FCM and symposium feedback
	26 Sep 2012	BEE grower meeting, Fort Beaufort	Spring complex and FCM
	2-3 Sep 2012	Grower meeting, Port Elizabeth	FCM and symposium feedback
	4-5 Sep 2012	Grower meeting, Goudini	FCM and symposium feedback
	31 Jan-1 Feb 2012	Packhouse Workshop, Loskop	FCM systems approach for EU
	12-13 Feb 2012	Packhouse Workshop, Durban	FCM systems approach for EU
	14-15 Feb 2012	Packhouse Workshop, Port Elizabeth	FCM systems approach for EU
	19-20 Feb 2012	Packhouse Workshop, Stellenbosch	FCM systems approach for EU
	12 March	PPECB meeting, Port Elizabeth	FCM systems approach for EU
	7 th Citrus Research Symposium 19-22 Aug 2012	Champagne Sports Resort, Drakensberg	Can imidacloprid cause false codling moth repercussions? Poster: Controlling FCM and Fruit Fly with entomopathogenic nematodes
Pretorius, M.C.	7 th Citrus Research Symposium 19-22 Aug 2012	Champagne Sports Resort, Drakensberg	Holistic approach for the control of soilborne diseases on citrus
Schutte, G.C.	7 th Citrus Research Symposium 19-22 Aug 2012	Champagne Sports Resort, Drakensberg	Spray programmes for the control of fruit and foliar diseases in SA
Skinner, H.R.	7 th Citrus Research Symposium 19-22 Aug 2012	Champagne Sports Resort, Drakensberg	CRI Organogram incorporating the CRI group
Stephen, P.R.	7 th Citrus Research Symposium 19-22 Aug 2012	Champagne Sports Resort, Drakensberg	Poster: Growing citrus under shade cloth can result in higher pesticide residues at harvest
Vahrmeijer, J.T.	7 th Citrus Research Symposium 19-22 Aug 2012	Champagne Sports Resort, Drakensberg	Citrus water use: Quo Vadis?
Van Vuuren, S.P.	7 th Citrus Research Symposium 19-22 Aug 2012	Champagne Sports Resort, Drakensberg	Poster: Evaluating sweet orange clones for greening resistance

8.6 OTHER MEANS OF TECHNOLOGY TRANSFER

8.6.1 SA Fruit Journal by Tim G Grout (CRI)

Summary

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 2012/3 are listed in Table 8.6.1.1. Due to the lag time of two months between submission of the articles and circulation of the journal, urgent information is circulated to growers as Cutting Edge or Snykant articles via CRIInet and emails to the technology transfer groups.

Opsomming

Alle produsente wat uitvoer, ontvang die SA Vrugte Joernaal. Daarom is dit een van die beste maniere om tegnologiese rakende tegniese aspekte oor te dra. Twee maandelikse Voorligtingsinligtingstukke word deur Hennie le Roux en Hannes Bester geredigeer. Alle navorsers dra op 'n gereelde basis hiertoe by. In diepte, semi-wetenskaplike navorsingsartikels word ook voorsien wat gewoonlik van 'n praktiese aard is en ander aktuele of nuusartikels word ook soms ingesluit. Die sitrusartikels wat in die SA Vrugte Joernaal gedurende 2012/13 gepubliseer is, is in Table 8.6.11 gelys. Weens die tydsverloop van die twee maande tussen die indiening van die artikels en die sirkulasie van die joernaal, word dringende inligting aan produsente as CRI Cutting Edge of CRI Snykant artikels via CRIInet gesirkuleer en as e-posse aan die tegnologiese oordragsgroepe.

Table 8.6.1.1. SA Fruit Journal articles by CRI Group members during 2012/3.

Issue	Article	Author
April/May	Akkreditasie van kartonvervaardigers Accreditation of carton manufacturers	D. Groenewald
	Citrus Research International's 10 year celebration and history	T.G. Grout
	Factors influencing the distribution of Medfly and Natal fly in South Africa: Current research status	J. Terblanche, P. Addison, C. Nyamukondiwa & A. Manrakhan
June/July	Pesticide mixtures and incompatibility	T.G. Grout
Aug/Sept	A Review of current pre-harvest control options for False Codling Moth in citrus in southern Africa	S. Moore & V. Hattingh
	Monitoring attraction of fruit-feeding moths in citrus orchards to different fruit baits in the Eastern Cape Province, SA	C.G. Robinson, T. Pretorius, S.D. Moore & M.P. Hill
Oct/Nov	Woolly whitefly, <i>Aleurothrixus floccosus</i> , on citrus in South Africa	T.G. Grout, W. Kirkman & S. Moore
Dec/Jan	CRI Postharvest Technical Forum – A critical component for competitive participation in world markets CRI Na-oes Tegniiese Forum – Kritiese component vir kompeterende mededinging in wêreldmarkte	J.J. Bester, D. Groenewald & H.F. le Roux
	7 th CRI Citrus Research Symposium	T.G. Grout & H.F. le Roux
Feb/Mar	CRI's award at the 2012 Eskom International Science Fair	T.G. Grout
	Performance of Star Ruby grapefruit on various rootstocks at Letaba Estates, Letsitele (2003-2007)	J. Joubert, A. Lee, R. Fenwick

8.6.2 CRI website by Tim G Grout (CRI)

Summary

The CRI website was redesigned in September 2012 and since then the average number of hits per month has increased. Most visits were from dot-net domains followed by South African IP addresses, German IP addresses and dot-com. There was also interest from Sweden, Indonesia, Poland and American dot-edu sites. Statistics on usage are shown in Table 8.6.2.1.

Opsomming

Vanaf CRI se webtuiste herontwerp is in September 2012 het die aantal “hits” per maand toegeneem. Meeste besoeke was van dot-net adresse gevolg deur Suid Afrikaanse IP adresse, Duitse IP-adresse en dot-com. Daar was ook besoeke vanaf Swede, Indonesië, Poland en Amerika dot-edu. Statistieke oor gebruik word in Tabel 8.6.2.1 getoon.

Table 8.6.2.1. Visits and page requests on www.cri.co.za since April 2012.

Month	Unique visitors	Number of visits	Pages	Hits	Bandwidth
Apr 2012	891	1324	7988	18007	231.98 MB
May 2012	1077	1722	9697	21166	299.90 MB
Jun 2012	862	1333	8273	18354	229.92 MB
Jul 2012	916	1473	8946	18545	268.63 MB
Aug 2012	871	1329	15189	44977	334.81 MB
Sep 2012	939	1401	15965	52553	660.05 MB
Oct 2012	786	1349	7585	29855	387.48 MB
Nov 2012	917	1551	7592	29363	432.50 MB
Dec 2012	757	1262	5771	21597	242.74 MB
Jan 2013	903	1560	10275	35910	434.69 MB
Feb 2013	901	1608	8997	37599	493.79 MB
Mar 2013	965	1894	10486	34651	425.94 MB
Total	10785	17806	116764	362577	4.44 GB

8.6.3 CRInet by Tim G Grout (CRI)

Summary

Usage of CRInet during the report period was close to the average of around 50 messages per annum for the last 6 years (Table 8.6.3.1). It provides a good opportunity for growers to share opinions on any technical citrus topic but it is mostly being used for dissemination of information from CRI or CGA. Membership has now passed 470.

Opsomming

Gebruik van CRInet gedurende die verslagperiode was naby aan 50 boodskappe per jaar vir die laaste ses jaar (Tabel 8.6.3.1). Dit verskaf 'n goeie geleentheid vir produsente om opinies oor enige tegniese sitrusonderwerp te deel en is meer gebruik vir die verspreiding van informasie vanaf CRI of CGA. CRInet het nou meer as 470 lede.

Table 8.6.3.1. Numbers of messages circulated per month on CRInet.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
2013	1	15	0										
2012	5	1	19	4	5	2	4	3	1	0	2	0	46
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

8.6.4 Cutting Edge by Tim G Grout (CRI)

Summary

During 2012/3 the Cutting Edge passed the 150th issue. 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 2012/3 are given in Table 8.6.4.1.

Opsomming

Gedurende 2012/13 het die CRI Snykant die 150^{de} uitgawe oorskry. Party produsente beskou dit as CRI se waardevolste manier van kommunikasie, moontlik omdat dit altyd dringende inligting bevat (kort en kragtig). Vorige uitgawes van die CRI Snykant kan van die lede-afdeling van CRI se webwerf afgelaai word. Onderwerpe wat in 2012/12 behandel is, word in Tabel 8.6.4.1 aangedui.

Table 8.6.4.1. Cutting Edge issues during 2012-13.

No.	Title	Issue	Author/s
135	Food Safety Update	April	Paul Hardman
136	Recommendations for the pre-packline drench	April	Arno Erasmus, Keith Lesar & Paul Fourie
137	Control recommendations in areas with high fruit fly population pressure	May	Sean Moore & Aruna Manrakhan
138	The use of blocks impregnated with methyl eugenol and malathion for control of <i>Bactrocera invadens</i> in South Africa	May	Aruna Manrakhan & Vaughan Hattingh o.b.o. <i>B. invadens</i> steering committee
139	Identification of insect larvae infesting citrus fruit	May	Sean Moore & Aruna Manrakhan
140	Food Safety Update	May	Paul Hardman
141	Procedures to be implemented after the detection of <i>Bactrocera invadens</i> in a specific area	June	<i>B. invadens</i> Steering Committee
142	Update: Eradication of <i>Bactrocera invadens</i> at incursion sites in Limpopo and Mpumalanga Provinces, SA	June	Aruna Manrakhan & Vaughan Hattingh o.b.o. <i>B. invadens</i> steering committee
143	Update on the QAC situation in the EU	July	Paul Hardman
144	Regulation of disinfectants intended for use in the citrus industry	July	Paul Hardman, Paul Fourie, Keith Lesar & Arno Erasmus
144a	Regulasie van ontsmettingsmiddels bestem vir gebruik in die sitrusbedryf	Julie	Paul Hardman, Paul Fourie, Keith Lesar & Arno Erasmus
145	Update on the Quaternary Ammonium Compound (QAC) situation in the EU	July	Paul Hardman
146	Imidacloprid can result in increased levels of false codling moth	August	Sean Moore
147	Food Safety Update	August	Paul Hardman
148	Consumer Assurance Update	August	Paul Hardman
149	SOPP update and meeting to discuss sustainable citrus supply into the EU	September	Paul Hardman
150	Urgent reminder for <i>Bactrocera invadens</i> surveillance per PUC	November	Aruna Manrakhan & Vaughan Hattingh o.b.o. <i>B. invadens</i> steering committee
151	Endoxerosis of lemon fruit: Proposed re-classification of inspection guidelines	November	Paul Cronjé, Hennie le Roux (CRI) & Cyril Julius, Jurgens Bence (PPECB)
152	Consumer Assurance Update	January	Paul Hardman
153	Phytophthora warning (for Limpopo area)	February	M.C. Pretorius

8.6.5 CRI Production Guidelines

Certain sections of the Disease Management and the IPM chapters have been updated. Production Guideline IV is being re-written in its totality but will only be available in 2014. The chapter on the Economy of Citrus Production is outdated and needs to be replaced.

Sekere dele van die Siektebestuur en die IPM hoofstukke is opgedateer. Produksie Riglyn IV word weer geskryf in sy totaliteit, maar sal eers in 2014 beskikbaar wees. Die hoofstuk oor die Ekonomie van Sitrus Produksie is verouderd en moet vervang word.

8.6.6 SASCCON interaction

There is a strong link between CRI Extension and SASCCON and both Hennie le Roux and Hannes Bester are associate members of SASCCON to ensure that the same message goes out to the producers.

Daar is 'n sterk verband tussen CRI Voorligting en SASCCON en beide Hennie le Roux en Hannes Bester is mede-lede van SASCCON om te verseker dat dieselfde boodskap na die produsente uitgaan.

9 PUBLICATIONS IN 2012-13

9.1 REFEREED PUBLICATIONS (OR ISI RANKED JOURNALS)

Boardman, L., T.G. Grout and J.S. Terblanche. 2012. False codling moth *Thaumatotibia leucotreta* (Lepidoptera, Tortricidae) larvae are chill-susceptible. *Insect Science* 19: 315-328.

Carstens, E., H.F. le Roux, M.A. Holtzhausen, L. van Rooyen, J. Coetzee, R. Wentzel, W. Laubscher, Z. Dawood, E. Venter, G.C. Schutte, P.H. Fourie, V. Hattingh. 2012. Citrus black spot is absent in the Western Cape, Northern Cape and Free State Provinces. *S. Afr. J. Sci.* 108(7/8): 56-61.

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 (note)* 96(5): 772.

De Villiers, M., V. Hattingh and D.J. Kriticos. 2013. Combining field phenological observations with distribution data to model the potential distribution of the fruit fly *Ceratitis rosa* Karsch (Diptera: Tephritidae). *Bull. Entomol. Res.* 103: 60-73.

Erasmus, A., C.L. Lennox, J.L. Smilanick, K. Lesar, P.H. Fourie 2012. Imazalil residue loading and green mould control on citrus fruit as affected by formulation, solution pH and exposure time in aqueous dip treatments. *Postharvest Biology & Tech* 77: 43-49.

Magwaza, L.S., U.L. Opara, L.A. Terry, S. Landahl, P.J. Cronje, H. Nieuwoudt, A.M. Mouazene, W. Saeysf, B.M. Nicolaï. 2012. Prediction of 'nules Clementine' mandarin susceptibility to rind breakdown disorder using Vis/NIR spectroscopy. *Postharvest Biology and Technology* 74: 1–10.

Magwaza, L.S., U.L. Opara, H. Nieuwoudt, P.J.R. Cronje, W. Saeys, B. Nicolaï. 2012. NIR Spectroscopy Applications for Internal and External Quality Analysis of Citrus Fruit - A Review. *Food Bioprocess Technol.* 5: 425-444.

Manrakhan, A., C. Kotze, J.H. Daneel, P.R. Stephen, R.R. Beck. 2013. Investigating a replacement for malathion in bait sprays for fruit fly control in South African citrus orchards. *Crop Protection* 43: 45-53.

Njombolwana, N.S., A. Erasmus, P.H. Fourie. 2013. Evaluation of curative and protective control of *Penicillium digitatum* following imazalil application in wax coating. *Postharvest Biology & Technology* 77: 102-110.

Opoku-Debrah, M.P. Hill., C. Knox, S.D. Moore. 2013. Overcrowding of false codling moth, *Thaumatotibia leucotreta* (Meyrick) leads to the isolation of five new *Cryptophlebia leucotreta* granulovirus (CrleGV-SA) isolates. *Journal of Invertebrate Pathology* 112: 219–228.

Pereira-da-Conceicao, L.L., M.P. Hill & S. Moore. 2012. Development of a peroral, droplet-dose bioassay laboratory technique and its application on a granulovirus for *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae). *African Entomol.* 20(1): 187-190.

Schutte, G.C., C. Kotze, J.G. van Zyl, P.H. Fourie. 2012. Assessment of retention and persistence of copper fungicides on orange fruit and leaves using fluorometry and copper residue analyses. *Crop Protection* 42: 1-9.

Scott, K.A., Q. Hlela, O. Zablocki, D. Read, S. van Vuuren, G. Pietersen. 2013. Genotype composition of populations of grapefruit-cross-protecting citrus tristeza virus strain GFMS12 in different host plants and aphid-transmitted sub-isolates. *Arch. Virol.* 158(1): 27-37.

van Zyl, J.G., P.H. Fourie, G.C. Schutte. 2013. Spray deposition assessment and benchmarks for control of *Alternaria* brown spot on mandarin leaves with copper oxychloride. *Crop Protection* 46: 80-87.

Yonow, T., V. Hattingh & M. de Villiers. 2013. CLIMEX modelling of the potential global distribution of the citrus black spot disease caused by *Guignardia citricarpa* and the risk posed to Europe. *Crop Protection* 44: 18-28.

9.2 SEMI-SCIENTIFIC PUBLICATIONS OTHER THAN SA FRUIT JOURNAL

Pretorius, M.C. and H.F. le Roux, 2012. In: Nematode Pests of Citrus in South Africa. Nematology in southern Africa by D.P. Keetch (book chapter).

10 PRESENTATIONS AT SOCIETAL AND INTERNATIONAL CONGRESSES

The following presentations were all made at the International Society of Citriculture Congress in Valencia, Spain in November 2012.

Assessment of retention and persistence of copper fungicides on orange fruit and leaves using fluorometry and copper residue analyses. Schutte G.C., Kotze C., Van Zyl J.G., and Fourie P.H.

Association and interaction of edaphic factors with root disease related citrus decline. Pretorius M.C., Labuschagne N., Kotze C., and McLeod A.

Can imidacloprid cause lepidopteran pest repercussions? by Sean Moore, Rachel van der Walt, Wayne Kirkman and Derek Du Preez.

Citrus water use in South Africa. Vahrmeijer J.T., Annandale J.G., Gush M.B., and Taylor N.J.

Combating *B. invadens* in South Africa, delivered at the TEAM Second International Symposium 2012-13 July 2012, Kolymbari, Crete, Greece. Manrakhan, A., Hattingh, V., Grout, T.G., De Villiers, M. and Venter, J.H.

Could ethylene influence peteca spot incidence of lemon fruit? Cronjé P.J.R.

Curative and protective control of *Penicillium digitatum* following imazalil application in aqueous dip and wax coating. Njombolwana N.S., Erasmus A., and Fourie P.H.

Influence of light on carotenoid accumulation in Star Ruby grapefruit. Lado Lindner J., Cronje P.J.R., Rodrigo M.J., and Zacarías L.

Modelling of *Guignardia pseudothecium* maturation and ascospore dispersal in citrus orchards. Fourie P.H., Schutte G.C., Serfontein S., and Swart S.H.

Population Genetics of *Guignardia citricarpa* in South Africa. Carstens E., Linde C.C., Slabber R., Langenhoven S., Schutte G.C., Fourie P.H., and McLeod A.

Practical impact of imazalil resistance on control of postharvest citrus green and blue mould. Erasmus A., Rikhotso V., Lesar K.H., Lennox C.L., and Fourie P.H.

Silicon uptake in citrus and the validation of an analytical method. Vahrmeijer J.T., Asanzi N.M., and Taylor N.J.

Spray deposition benchmarks for control of *Alternaria* brown spot and evaluation of adjuvants to improve fungicide spray deposition in citrus orchards. Van Zyl J.G., Schutte G.C., and Fourie P.H.

The importance of research and technical services in the recent growth of the Southern African citrus export industry. Hattingh V.

The status of citrus IPM in South Africa. Grout T.G.

Other presentations

Boardman, L., Sørensen, J.G., Grout, T.G., Terblanche, J.S. 2012. Heat shock protein 70 response to low temperature and elevated carbon dioxide in the false codling moth, *Thaumotobia leucotreta*. Society of Experimental Biologists Annual Meeting, Salzburg, Austria.

Erasmus, A., V. Rikhotso, C.L. Lennox, K. Lesar and P.H. Fourie. 2012. Practical impact of imazalil resistance on control of postharvest citrus green and blue mould. Oral presentation at the 7th CIGR International Technical Symposium, 25-29 November 2012, Stellenbosch, South Africa.

Kellerman, M., N.S. Njombolwana, A. Erasmus, P.J.R. Cronje and P.H. Fourie. 2012. Thiabendazole residue loading for control of green mould and chilling injury on citrus. Oral presentation at the 48th Congress of the South African Society for Plant Pathology, ATKV Klein Kariba, 20-24 January 2013.

Njombolwana, N.S., A. Erasmus, P. Cronje, W. du Plooy and P.H. Fourie. 2013. The effect of wax coating and brush type on imazalil residue loading and citrus green mould control. Poster presentation at the 48th Congress of the South African Society for Plant Pathology, ATKV Klein Kariba, 20-24 January 2013.

Van der Merwe, I.S., K.I. Theron, J.S. Verreyne and P.J.R. Cronjé. 2012. Phenology of alternate bearing 'nadorcott' Mandarin trees. 2nd All Africa Horticultural Congress, 15-20 Jan, Skukuza, Mpumalanga, South Africa.

Van Zyl, J.G., P.H. Fourie and G.C. Schutte. Improvement of spray deposition and control *Alternaria* brown spot on mandarin leaves following sprays with copper oxychloride and selected adjuvants. Poster presentation at 48th SASPP congress 2013.

Viljoen, R., Steenkamp, E.T., and Pietersen, G., 2012. Alternative indigenous Rutaceous hosts of '*Candidatus Liberibacter africanus*' and '*Candidatus Liberibacter africanus* subsp. *capensis*'. South African Association of Botanists, January, 2012.

Viljoen, R., Steenkamp, E.T., and Pietersen, 2013. Alternative hosts of "*Candidatus Liberibacter africanus*" amongst indigenous members of the Rutaceae in South Africa. 48th Conference of the Southern African Society for Plant Pathology, 20-24 January, 2013, Bela-Bela.

Viljoen, R., Steenkamp, E.T., and Pietersen, 2013. "*Candidatus Liberibacter*" in four indigenous Rutaceous species from South Africa. 3rd International Research Conference on Huanglongbing. 4-7 February, 2013 Orlando, Florida, USA