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1 MARKET ACCESS TECHNICAL COORDINATION

By Vaughan Hattingh and Elma Carstens (CRI)

1.1 SUMMARY

The level of CBS interceptions in exports to the EU was low and a delegation from the EU who inspected the SA internal controls reported that SA is implementing a robust CBS risk management system. CRI successfully provided extensive inputs in support of ensuring that new EU regulations specific to FCM, include pragmatic risk mitigation options. The EU made the new draft regulations available through the WTO for comment by trading partner countries. It is expected that the new regulations will apply to the 2018 citrus export season. Improved conditions for access to Japan remain long outstanding, despite CRI having provided all the necessary supportive technical information. The cold treatment conditions for export to USA reverted back to the 22d cold treatment and the mandatory fruit cutting during pre-shipment inspections was waived. Technical inputs were provided in anticipation of the USA publishing the final rule to allow the import of citrus fruit from SA production areas outside of the officially recognised CBS free areas. Publication of this final rule by USA remained pending. There were further technical exchanges between SA and China in pursuit of allowing bulk shipping and exemption of lemons from the cold treatment requirements. Both issues remained pending a response from China. Conditions of access to India were disrupted by changes in the way in which India implements their import requirements, specifically requiring that land-based cold treatment be applied as opposed to in-transit cold treatment. A trial shipment of pears was successfully completed to demonstrate SA's ability to implement in-transit cold treatment in anticipation of being able to do so for future citrus exports. Good progress was made towards finalising an export protocol with Vietnam, with decisions pending from Vietnam at the end of the report period. The PRA process to gain access to the Philippines made little progress due to the Philippines continuing to insist on listing quarantine pests that are not associated with citrus from SA or do not follow the citrus fruit pathway, but engagement is ongoing. Technical information was supplied to Myanmar in support of opening access to this market.

OPSOMMING

Daar was min SSV onderskeppings na die EU en 'n afvaardiging van die EU wat SA se interne beheermaatreëls geïnspekteer het, het gerapporteer dat SA ŉ robuuste SSV risikobestuurstelsel implementeer. CRI het met sukses intensiewe insette gelewer om te verseker dat nuwe EU-regulasies, spesifiek vir VKM, pragmatiese risiko bestuursopsies insluit. Die EU het die nuwe konsep-regulasies deur die WHO vir kommentaar deur die handelsvennootlande beskikbaar gestel. Daar word verwag dat die nuwe regulasies op die 2018 sitrusuitvoer-seisoen van toepassing sal wees. Beter vereistes vir toegang na Japan is lank uitstaande, ten spyte daarvan dat CRI al die nodige ondersteunende tegniese inligting verskaf het. Die koue behandeling wat vereis word vir uitvoer na die VSA is weer ŉ 22d koue behandeling en die verpligte sny van vrugte tydens die voorverskepings-inspeksies is opgehef. Tegniese insette is gelewer in afwagting op die publisering van VSA wetgewing om die invoer van sitrusvrugte van SA se produksie-areas buite die amptelik erkende SSV vrye areas toe te laat. Publisering van die finale wetgewing deur die VSA is steeds hangende. Daar was verdere uitruiling van tegniese inligting tussen SA en China in die hoop dat "bulk reefers" toegelaat sal word en dat suurlemoene van ŉ koue behandeling vrygestel sal word. Beide sake is hangende terugvoering van China. Voorwaardes vir toegang na Indië is ontwrig deur verandering in die manier waarop Indië hul invoervereistes toepas. Indië vereis spesifiek dat landgebaseerde koue behandeling in teenstelling met in-transit koue behandeling toegepas word. ŉ Proefbesending van pere is suksesvol voltooi om SA se vermoë om in-transit koue behandeling toe te pas, te demonstreer, met die hoop dat dit op toekomstige sitrusuitvoere van toepassing sal wees. Goeie vordering is met die finalisering van 'n uitvoerprotokol na Viëtnam gemaak, met terugvoering hangende van Viëtnam teen die einde van die verslagtydperk. Daar was min vordering met die PRA proses om toegang na die Filippyne te verkry omdat die Filippyne voortgaan om kwarantynpeste te lys wat nie met sitrus vanaf SA geassosieer is nie of nie met sitrusvrugte kan versprei nie. Interaksies gaan egter voort. Tegniese inligting is aan Myanmar verskaf ter ondersteuning van verkryging van toegang tot hierdie mark.

1.2 EUROPE (EU)

In 2015, the EU recognised the Gordonia magisterial district as CBS free, but not the other 17 CBS-free magisterial districts in the Northern Cape, Free State and North West provinces of SA. During the 2016 export season, problems were encountered due to the fact that some of the registered PUCs lie in the Kenhardt magisterial district, which is not recognised by the EU as CBS free. Several discussions were held between SA-DAFF and CRI on the future handling of the situation in the Northern Cape. The Municipal Demarcation Office in Pretoria and the Department of Rural Development and Land Reform - Bloemfontein office, were also consulted. It was ascertained that some of the farms and orchards in the export production region do fall in

the Kenhardt magisterial district. This provided clarity on the requisite procedure for handling 2017 exports to the EU. However, new draft EU legislation that was released in February 2017 for WTO comment will in future enable SA to use its own demarcation of CBS free areas and EU approval of such demarcations will no longer be required. This will greatly facilitate handling of EU exports from the region.

An EU-FVO delegation visited SA to audit the SA-CBS Risk management system from 13 – 24 June 2016. Planning meetings were held with SA-DAFF and other fruit industries to agree on a combined position with regard to potential future FCM regulations in the EU. Production units, packhouses and ports in Limpopo, KZN and Eastern Cape were visited. A wrap-up meeting between the FVO delegation, CGA, CRI and SA-DAFF was held on 24 June and no further technical information was requested at this meeting by the FVO. A final report on the FVO visit was received in February 2017. They identified two areas of concern namely, the official field inspections (capacity constraints) and investigation of internal (local) CBS interceptions, but overall reported that SA was applying a robust phytosanitary control system.

By the end of the 2016 export season SA had received four notifications of CBS interceptions in the EU. A meeting was held between industry and SA-DAFF in September to agree on the CBS-RMS for 2017 and the final document was circulated on 2 April 2017. CRI developed a procedure to manage the risk of potential mixing of fruit from non CBS free areas with fruit from the Western and Northern Cape provinces (CBS free areas) during packing or re-packing for CBS sensitive markets.
In February 2017, the EU notified trading partners through the WTO that the EU is amending Annexes I to V to Council Directive 2000/29/EC (plant health regulations). The amendments include relatively minor changes in relation to CBS and a draft of the new FCM regulations. CRI arranged consultation with SA-DAFF and other industry bodies, producing consolidated inputs on the SA response to the WTO notification. SA DAFF submitted official SA comments prior to the 14 April 2017 deadline. The draft regulation provided for a 5-month period after publication, before entering into force and are hence expected to apply to the 2018 export season.

CRI provided numerous technical inputs and undertook various consultations in support of pragmatic risk management measures being included in the new EU FCM regulations. Several papers of strategic relevance in supporting a feasible set of future FCM EU regulations progressed to publication in international scientific journals. In anticipation of the new EU FCM regulations, CRI issued several interim risk mitigation recommendations for the 2017 export season. These were communicated at the CRI Regional Packhouse Workshops, the March 2017 CMF meeting in Port Elizabeth and at the grower meeting following the CGA Summit in Port Elizabeth. The relevant Variety Focus Groups were engaged and they reverted with indications of support. The recommendations were communicated in CRI Cutting Edge articles.

The EU published a new set of plant health regulations in December 2016. These new regulations replace the current plant health regime, but will only enter into full force by the end of 2019. A process was initiated by CRI to effect a comprehensive review of the implications of the new regulatory framework.

1.3 **JAPAN**

There are two long outstanding issues pertaining to this market - the request to Japan-MAFF submitted in November 2009 to allow for the importation of all South African mandarins (except Satsumas) under the current protocol for Clementines and the request to adopt a revised cold treatment condition for the export of all citrus fruit from SA. The initial request to revise the temperature protocol was submitted in 2009 and the final data package to support this request was submitted in 2014. Despite several follow up queries and re-sending of information by SA-DAFF and inquiries by Industry, responses are still pending from Japan-MAFF. SA-DAFF also sent a request to the newly appointed Attaché in Japan to follow up with Japan-MAFF on all the long outstanding citrus matters.

In September 2016, a request was submitted to Japan-MAFF to amend the current export protocol to include all navel oranges cultivars as the protocol currently only specifies Washington and Cara Cara Navel cultivars. By the end of this reporting period, no feedback had been received from Japan- MAFF despite several follow up requests by SA-DAFF and Industry.

1.4 **USA**

In April 2016, USDA-APHIS informed SA-DAFF that the cold treatment for the export of fresh citrus fruit had been adjusted back to T107-e (22-days). In August 2016, a bilateral meeting was held between SA-DAFF and USDA-APHIS. At the meeting USDA-APHIS indicated that the cutting of 200 asymptomatic fruit is no longer required, that T107-e treatment (22-days) will also apply to shipments destined for Houston, that fruit will be allowed through the ports of Newark (New Jersey), Philadelphia (Pennsylvania), Wilmington (Delaware) and Houston (Texas), but that the approval of the port in Miami (Florida) will be considered at a later stage. The

APHIS Fruits and Vegetable Manual was revised in November 2016 reflecting all the decisions taken at the bilateral meeting in August 2016.

CRI provided information to SA-DAFF to update the current workplan and the revised workplan was submitted to USDA-APHIS in May 2016. The pest list included in the workplan also needed revision and CRI provided relevant inputs to SA-DAFF who submitted the information to USDA-APHIS in November 2016.

At the bilateral meeting in August 2016, USDA-APHIS indicated that fruit from non CBS free areas, as contemplated in a draft USA rule pending publication, will need to be subjected to specific packhouse treatments. CRI compiled a draft packhouse operational manual (as a proposed annex to the workplan) and SA-DAFF submitted the information to USDA-APHIS in November 2016. SA-DAFF informed USDA-APHIS at the meeting that SA still requires recognition for CBS free places of production in an area of low pest prevalence and inclusion of other Western Cape CBS free magisterial districts in the workplan.

Discussions were held between CRI, Summer Citrus, SA-DAFF, PPECB and USDA-APHIS to address concerns about the phytosanitary inspections and movement of fruit destined for this market at times when the Fresh Fruit Terminal (FPT) reaches full capacity. A standard operational procedure (SOP) was drafted to identify the circumstances that can cause bottlenecks at FPT and procedures to follow. During these circumstances, fruit will be inspected at identified outside depots and securely transported to FPT so as to prevent reinfestation with quarantine organisms. The SOP was approved and signed by PPECB, SA-DAFF and USDA-APHIS. A memorandum of agreement was also signed between SA-DAFF and the outside depots.

By the end of this reporting period feedback on the following issues remained pending from USDA-APHIS: updated workplan, amended pest list, importation of citrus fruit from CBS affected areas (draft rule), recognition for CBS free places of production in an area of low pest prevalence, and inclusion of other Western Cape CBS free magisterial districts. A USA Presidential instruction on 20 January 2017 directed that all Federal regulations that have not been sent for publishing in the Federal Register have to be reviewed, resulting in further delays in progressing the draft rule to finalisation.

1.5 **CHINA**

Two issues remain pending for this market – a request submitted to AQSIQ to exempt lemons from the current cold treatment requirement (24-days) by accepting the non-host status of lemons for fruit flies and FCM, and a request to accept bulk shipping of fresh citrus fruit from SA. Currently citrus fruit can only be exported in containers.

In September 2016 feedback was provided by AQSIQ, in response to technical information on the temperature measurements provided by SA-DAFF in March 2016. AQSIQ requested further technical information on temperature probes and information on registration, supervision, accreditation processes for reefer vessels and the air circulation systems. CRI consulted with PPECB and the information was provided to SA-DAFF, who submitted it to AQSIQ on 25 November 2016. In March 2017, SA-DAFF received feedback from AQSIQ, again requesting further information on the temperature sensors used in different cargo spaces with different cargo loading volumes. CRI and PPECB compiled the required information and provided it to SA-DAFF in April 2017.

CRI assessed the reasons offered by AQSIQ for not accepting SA's request to exempt lemons from cold treatment on the basis of non-host status for FCM and fruit flies. It was evident that the reasons provided by AQSIQ were without technical justification and not aligned with the scientific evidence referred to. In May 2016 CRI provided a technical analysis and a proposal to SA-DAFF for submission to AQSIQ. Further meetings were held with SA-DAFF to prepare the response to AQSIQ and in November 2016 SA-DAFF submitted the information to AQSIQ. By the end of this reporting period no further feedback had been received from AQSIQ.

In June 2015 CRI requested SA-DAFF to submit a data package to AQSIQ detailing a FCM Systems Approach as an alternative to the current mandatory cold treatment mitigation option for FCM. However, in April 2016 CRI requested SA-DAFF not to proceed with submitting the Systems Approach proposal, indicating that it will at an appropriate time in the future submit a revised proposal.

In 2014, SA-DAFF received a request from AQSIQ to export citrus from China to South Africa. CRI studied the PRA information received from AQSIQ and found that the list of pests present on citrus in China was incomplete. A complete list of 445 pests indicating which of the listed pests can follow the fruit pathway and which of these pests are not present in South Africa was provided to SA-DAFF in September 2016. Further

meetings were held with SA-DAFF and the final list of pests that require more information from AQSIQ was submitted to AQSIQ by SA-DAFF in March 2017.

1.6 INDIA

In 2016 India changed their import regulations, terminating the opportunity to import into India under import permit. Consignments were previously shipped to India (since 2005), according to treatment conditions for Medfly and Natal fruit fly that entailed Methyl Bromide fumigation or cold treatment, but the import conditions only provided for land based cold treatment. However, in 2005 SA-DAFF obtained confirmation from the Indian Authorities that SA can make use of in transit cold treatment. At the end of the 2016 citrus export season, Indian authorities started to enforce the published conditions, requiring land based cold treatment. Information was provided to SA-DAFF to communicate to the Indian authorities requesting that both options of land based and in transit cold treatments be allowed. In January and February 2017, consultation took place between SA-DAFF, PPECB, Deciduous fruit exporters, HORTGRO, CRI and the Agricultural Attaché in India. A trial consignment of pears was successfully sent to India in February 2017, using an in transit cold treatment, to demonstrate SA's ability to successfully apply such a protocol. The understanding was that a successful trial would enable the further use of in transit treatment for other fruit types. In March 2017, SA Directorate - International Relations sent a letter to India to request a formal report on the trial pear shipments.

1.7 VIETNAM

In the previous reporting period, the Vietnamese authorities indicated that they will consider the removal of *Ceratitis quinaria and Aspidiotus nerii* once they receive the updated datasheets from CABI Crop Protection Compendium (CPC) via SA-DAFF. The updated datasheet on *Aspidiotus nerii* stated that although *Citrus* is a host of this pest, fresh citrus fruit from South Africa is not a pathway for this pest. The updated datasheet on *Ceratitis quinaria* stated that *Citrus* spp is not a host for this fruit fly. These updated datasheets supporting SA's position, were provided to SA-DAFF with a request to submit to the Vietnamese Authorities. In July 2016 feedback was received from Vietnam that they agreed to remove *Ceratitis quinaria* from the list but not *Aspidiotus nerii and Pseudomonas syringae pv syringae.* They also provided a draft of the phytosanitary import requirements for fresh oranges and requested a visit to South Africa to inspect and monitor orchards, packhouses and pest control programmes. In September 2016 further information was provided to SA-DAFF in support of removing *Aspidiotus nerii and Pseudomonas syringae pv syringae* from the quarantine list. Concern about requirements for pre- and post-harvest management procedures in the draft phytosanitary import requirements were highlighted and SA-DAFF was advised to indicate to Vietnam that South Africa cannot comply with some of the requirements. SA-DAFF submitted the information to Vietnam in September 2016. No feedback was received from Vietnam by the end of this reporting period.

1.8 NEW MARKETS

1.8.1 The Philippines

In the 2014 and 2015 reporting years' information had been provided to the Philippine Authorities (the BPI), to demonstrate that their list of quarantine pests needed to be revised as most of the listed pests did not follow the fresh fruit pathway. In July 2016 SA-DAFF received feedback from the Philippines that they had removed nine of the 19 pests from the quarantine pest list. They however still retained 10 of the pests on the list. Further meetings were held with SA-DAFF and scientific evidence was provided to SA-DAFF to support South Africa's request to remove the 10 pests not associated with the citrus fruit pathway. SA-DAFF submitted this information to the BPI on 25 November 2016.

In January 2017, SA-DAFF received feedback from the BPI. Although the BPI removed five of the pests, they still retained five of the ten pests, despite the fact that SA-DAFF has repeatedly provided them with the latest scientific/technical information to prove that fresh citrus fruit from South Africa is not a pathway for spreading these pests. The BPI however referred to old scientific evidence for keeping these five pests on the list. Further meetings were held with SA-DAFF to discuss feedback to BPI and the decision was taken that SA-DAFF will extend an invitation to the BPI to visit SA. This will provide an opportunity for technical officials within the BPI, who are responsible for decision making in the PRA process, to have discussions with relevant SA officials and scientists to obtain first-hand information about these five pests/diseases and their association with South African citrus fruit. A letter, extending an invitation to the technical experts from BPI to visit SA, was sent to the BPI on 10 April 2017.

1.8.2 Myanmar

In 2017 exporters were informed that plant and plant products can no longer be exported to Myanmar. The NPPO of Myanmar indicated that they wanted a Pest Information Package (PIP) for all citrus types to conduct

a PRA to determine which pests are of quarantine concern to Myanmar. At the CMF in March 2017, the producers indicated that it is an important market. The completed PIP was provided to SA-DAFF on 27 March 2017 and SA-DAFF submitted the information to the Myanmar authorities and the SA Ambassador in Bangkok on 29 March 2017.

1.9 REGULATIONS

By the end of this reporting period revision of the import conditions for Citrus vegetative propagation material remained pending. Assurance was given by SA-DAFF that permits will be issued although the import conditions are under revision/reviewing.

In this reporting period no new pest reports were sent to the IPPC on detections of *B. dorsalis* (previously *B. invadens*) incursions in South Africa. The status of the fruit fly remained the same – the pest is considered to be present in specified regions, actionable and under official control in South Africa. The Northern Cape, Western Cape, Eastern Cape and Free State provinces still remain free from *B. dorsalis*.

In November 2015 a survey was conducted in the CBS area of low pest prevalence in the far northern Limpopo province to determine whether the area still meets the requirements to be recognised as an area of low pest prevalence. A total of 203 samples were collected and the laboratory report indicated that there were no positive findings for CBS, confirming that the area still meets the requirements for an area of low pest prevalence.

An African Greening delimiting survey was conducted in 2016 in the Riebeeck Kasteel magisterial district of the Western Cape Province. In 2015, 38 samples were collected from this district from which one of the samples tested positive for African Greening (Laf). In the 2016 survey, eight samples were collected and all the samples tested negative for Laf and *Candidatus Liberibacter asiaticus*.

2 PORTFOLIO: INTEGRATED PEST MANAGEMENT

2.1 PORTFOLIO SUMMARY

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

For many years now, the research priorities within the IPM Portfolio have been determined by market access issues. This pertains particularly to retention and growth of existing markets. False codling moth continues to be the number one priority within the IPM Portfolio, as it is a regulated pest for several export markets. However, what has elevated its importance and expedited the research, is impending EU regulations for FCM. The main gist of the research is achieving consignment-freedom of FCM by means other than a standalone postharvest treatment, such as cold sterilisation. Having said this, dramatically improved cold sterilisation treatments for FCM (higher temperatures and shorter durations than the standard -0.55°C for 22 days) were published in a peer-reviewed scientific journal during the past year, which will hopefully lead to acceptance of these less severe protocols.

Other pests affecting market access that have attracted research focus and funding are fruit flies, particularly the Oriental fruit fly, which has been established in certain northern areas of the country for a few years now, carob moth, mealybug and Fuller's rose beetle. Apart from market access, biosecurity has become more important, particularly with the news that the Asian citrus psylla, *Diaphorina citri*, which is the vector of Asiatic greening disease (HLB), has been recorded in Tanzania and is moving southwards. Fortunately, HLB has not yet been recorded on the African continent outside of Ethiopia. However, this too appears to be spreading. The drive to tackle this threat was led by our first Biosecurity Manager, Dr Hennie Le Roux, who tragically past away last year. Nevertheless, his legacy lives on in the first research projects which have specifically focussed on *D. citri*.

IPM research is divided into five programmes: FCM, fruit flies, mealybug and other phytosanitary pests, key non-phytosanitary pests and minor pests and mites.

During the past year, 16 projects were conducted within the FCM programme. Ten of these projects entailed preharvest studies, five entailed postharvest studies and one covered a combination of the two. The preharvest studies covered five general research areas: mating disruption, the sterile insect technique, microbial control, chemical control and ecological studies. Some highlights from the preharvest projects within the programme include the discovery of synergism between the FCM granulovirus and the newly discovered nucleopolyhedrovirus from litchi moth, and discovery of the greater attractiveness of fruit from young trees to FCM and a greater susceptibility to FCM. Highlights from the postharvest studies on FCM include the promising efficacy of a CO_2 and cold combination treatment, and detection of volatiles specific to FCM-infested

fruit. Another highlight is a project combining pre- and postharvest practices into a Systems Approach, which was validated on a semi-commercial basis, leading to improvements being made in the system.

As has been the case during the previous few years, all of the research conducted in the fruit fly programme focussed on preharvest management of these pests. The main objectives of the fruit fly programme during the past year were understanding the use of citrus by fruit fly pests, understanding the population ecology of Oriental fruit fly, Natal fly and Cape fly (the new species now split from Natal fly), determining the efficacy of fruit fly monitoring tools and optimising pre-harvest control measures for fruit fly pests. An important breakthrough was the discovery that export class Eureka lemons were not infested by any fruit flies at harvest, despite the presence of adult fruit flies in the orchards, thus opening the door for such lemons to be categorised as a non-host for fruit fly.

This past year saw growth in the mealybug and other phytosanitary pests programme, with five projects being registered. A very comprehensive study on carob moth was completed, including the finding that carob moth is more cold susceptible than FCM, opening the door for existing cold-sterilisation protocols for FCM to also include carob moth. Projects also covered the mealybug species *Delotococcus aberiae* and postharvest fumigation of a range of phytosanitary pests, with most recent focus on FCM, using a combination of CO_2 and cold.

The final two programmes, which covered non-phytosanitary key pests and minor pests and mites, included four projects. The most important of these were those that focussed on the Asian citrus psylla, *Diaphorina citri*, for the first time. Trials in Mauritius and locally indicated that both systemically and foliar applied treatments hold potential for control of the pest, should it someday spread to South Africa.

During the past year, CRI research entomologists also played a key role in disseminating their research findings both to their constituency, the southern African citrus growers, and to the world at large, particularly through relevant scientific mouthpieces. Growers have been provided with relevant and up to date research findings in CRI's Production Guidelines, Cutting Edge and Fruit Journal articles, grower study groups and through CRI's flagship platform, the biennial Citrus Research Symposium. Communication through science, to the world at large, is extremely important, as this is the only way in which market acceptance of new research findings can be achieved and phytosanitary regulations can be changed. This has been pursued through publication of research in key international journals and dissemination of findings at international meetings such as the International Citrus Congress in Brazil and the International Congress of Entomology in Florida.

PORTEFEULJE OPSOMMING

Vir baie jare nou is die navorsingsprioriteite binne die IPM Portefeulje deur marktoegangs faktore bepaal. Hierdie verwys veral na behouding en groei van huidige markte. Valskodlingmot bly die nommer een prioriteit binne die IPM Portefeulje, omdat dit vir verskeie uitvoer markte 'n gereguleerde plaag is. Wat sy belangrikheid egter verhoog het en die navorsing versnel het, is die dreigende EU regulasies vir VKM. Die hoof doel van die navorsing is om besending-vryheid van VKM te bereik deur metodes anders as 'n alleenstaande na-oes behandeling soos koue-sterilisasie. Ondanks hierdie doel is dramaties verbeterde koue-sterilisasie behandelings vir VKM (hoër temperature en korter duur as die standaard -0.55°C vir 22 dae) nogsteeds in 'n internasionale wetenskaplike joernaal gedurende die laaste jaar gepubliseer. Hierdie sal hopelik tot aanvaarding van hierdie minder streng protokolle lei.

Ander plae wat marktoegang affekteer en wat navorsings bevondsing gelok het is vrugtevlieë, veral die Oosterse vrugtevlieg, wat nou vir 'n paar jaar in sekere noordelike streke in die land gevestig is, karobmot, witluis en Fuller se rooskewer. Afgesien van mark-toegang, het biosekuriteit ook meer belangrik geword, veral met die nuus dat die Asiatiese sitrus bladvloei, *Diaphorina citri*, die vektor van Asiatiese vergroenings siekte (HLB), in Tanzania aangeteken is en beweeg nou suidwaarts. Gelukkig is HLB nog nie in Afrika buite Ethiopia aangetken nie, maar is ook besig om te versprei. Die poging om hierdie bedreiging aan te pak is gelei deur ons eerste Biosekuriteits Bestuurder, Dr Hennie Le Roux, wat tragies verlede jaar oorlede is. Nie te min leef sy nalatenskap voort in die eerste navorsings projekte wat spesifiek op *D. citri* gefokus het.

IPM navorsing word in vyf programme verdeel: VKM, vrugtevlieë, witluis en ander fitosanitêre plae, sleutel nie-fitosanitêre plae en minder belangrike plae en myte.

Geduurende die laaste jaar is 16 projekte binne die VKM program aangepak. Tien van hierdie projekte het vooroes studies behels, vyf het na-oes studies behels en een het 'n kombinasie van die twee behels. Die vooroes studies het vyf algemene navorsings onderwerpe gedek: paringsontwrigting, die steriele insek tegniek, mikrobiese beheer, chemiese beheer en ekologiese studies. Sommige hoogtepunte vanuit die vooroes projekte binne die program sluit in die ontdekking van sinergisme tussen die VKM granulovirus en die

nuut ontdekte nukleopolihedrovirus van lietsjiemot, en die ontdekking van hoër aantreklikheid van vrugte van jong bome vir VKM en hulle hoër vatbaarheid vir VKM. Hoogtepunt van die na-oes studies op VKM sluit in die belowende werking van ŉ CO_2 en koue kombinasie behandeling, en opsporing van vlugtige stowwe wat spesifiek is vir VKM besmette vrugte. Nog ŉ hoogtepunt is ŉ projek wat voor en na-oes praktyke in ŉ Stelselsbenadering kombineer, wat op ŉ semi-kommersiële basis gevalideer is, wat tot verbeteringe in die stelsel gelei het.

Soos wat die geval gedurende die vorige paar jaar was, het al die navorsing wat in die vrugtevlieg program aangepak is, op vooroes bestuur van hierdie plae gefokus. Die hoofdoele van die vrugtevlieg program gedurende die laaste jaar is om die benutting van sitrus deur vrugtevliegplae te verstaan, die populasie ekologie van die Oosterse vrugtevlieg, Natalse vlieg en Kaapse vlieg (die nuwe spesie wat nou van Natalse vlieg geskei is) te verstaan, effektiwiteit van vrugtevlieg moniteringshulpmiddels te bepaal en om vóór-oes beheermaatreëls vir vrugtevliegplae te optimaliseer. ŉ Belangrike deurbraak is die ontdekking dat uitvoer klas Eureka suurlemoene nie teen oestyd deur enige vrugtevlieë besmet is nie, ondanks die feit dat volwasse vrugtevlieë wel in die boorde voorgekom het. Die deur is dus oopgemaak vir sulke suurlemoene om as nie-gashere vir vrugtevlieë gekategoriseer te word.

In die laaste jaar het die program op witluis en ander fitosanitêre plae gegroei, met vyf projekte wat geregistreer is. ŉ Baie omvattende studie op karobmot is voltooi. Onder andere is dit gedemonstreer dat karobmot meer koue gevoelig as VKM was, wat die deur oopmaak vir bestaande koue sterilisasie protokolle vir VKM om karobmot ook in te sluit. Projekte het ook die witluis spesie *Delotococcus aberiae* gedek en na-oes beroking van ŉ reeks fitosanitêre plae, met die fokus mees onlangs op VKM, met ŉ kombinasie van CO_2 en koue.

Die finale twee programme wat nie-fitosanitêre sleutel plae en minder belangrike plae en myte gedek het, het vier projekte ingesluit. Die belangrikste van hierdie is díe wat op Asiatiese sitrusbladvloei, *Diaphorina citri*, vir die eerste keer gefokus het. Proewe wat te Mauritius en plaaslik uitgevoer is het aangedui dat albei sistemies toegediende en gespuite behandelings belowend was vir beheer van die plaag, indien dit in die toekoms tot in Suid-Afrika versprei.

Gedurende die laaste jaar het CRI navorsings entomoloë ook ŉ sleutel rol in die verspreiding van hulle navorsings resultate gespeel, albei vir hulle eie kiesafdeling, die sitrus produsente in suidelike Afrika, en tot die wêreld in die algemeen, veral deur relevante wetenskaplike mondstukke. Produsente is voorsien met relevante en vars navorsings resultate in die CRI Produksie Riglyne, Snykant en Vrugtejoernaal artikels, studiegroepe en deur CRI se vlagskip platform, die tweejaarlikse Sitrus Navorsings Simposium. Kommunikasie deur wetenskap met die wêreld in die algemeen is besonders belangrik omdat hierdie die enigste manier is dat mark aanvaarding van nuwe navorsings bevindinge bereik kan word en fitosanitêre regulasies verander kan word. Hierdie is agtervolg deur publikasie van navorsing in sleutel internasionale joernale en verspreiding van bevindinge by internasionale vergaderings soos die Internasionale Sitrus Kongres in Brasilië en die Internasionale Kongres van Entomologie in Florida.

2.2 PROGRAMME: FALSE CODLING MOTH

Programme coordinator: Sean D Moore (CRI)

2.2.1 Programme summary

Due to impending and inevitable regulations for exporting of FCM-suseceptible fresh produced from Africa to the EU, including citrus, the FCM research programme remains the highest priority programme, with most entomological research within the IPM portfolio being directed at this pest. During the 2016/7 research cycle, 16 projects were registered within the FCM research programme. Ten of these projects entailed preharvest studies, five entailed postharvest studies and one covered a combination of the two. Additionally, a good balance was sought between long-term strategic research and projects which provided rapid answers to key problems.

One of the preharvest projects focussed on monitoring of FCM, by comparing the relative attractiveness of a range of known synthesised pheromones to FCM males (2.2.3). This included naturally occurring blends of isomers reported in the literature and blends in commercial lures. The South African blend was the most attractive, followed by the blends used in the commercial products. This project also investigated relative attractiveness of female moths from different regional populations to male moths sterilised for SIT, determinging that the male moths were significantly more attracted to females from their own population.

In another project, a basic comparison was conducted between registered treatments for FCM control (2.2.7). FCM infestation was too low to establish any significant differences between spray treatments and the untreated control. Although FCM infestation of fruit in a Mating Disruption/Attract and Kill trial was similarly

low, there were large differences in trap catches, a good indication of treatment efficacy. These were lowest in the Isomate treatment, followed by Checkmate and Last Call, all of which were lower than the untreated control.

Another two trials also focussed on pheromones for FCM control. The first examined whether application of Mating Disruption at three times the registered rate could improve control (2.2.11). This was unfortunately not the case. The other trial investigated the efficacy of a novel compound, 7-vinyl decyl acetate 1 (7-VDA), rather than the FCM pheromone. In laboratory trials, FCM pheromone, 7-VDA and 10% 7-VDA in FCM pheromone all reduced fecundity by 69%. However, the 7-VDA treatments completely eliminated fertility of eggs, whereas there was a small percentage of fertilized eggs with the FCM pheromone treatment.

Another trial investigated control of FCM in the field. However, this was with entomopathogenic fungi, targeted against the soil-borne life stage (2.2.14), achieving up to 82% reduction in infestation. However, problems were experienced with viability of a very promising *Beauveria bassiana* isolate, delaying further laboratory bioassays.

Two other trials also involved the use of entomopathogens. The first involved investigation of a novel alphabaculovirus, originating from litchi moth, namely CrpeNPV (2.2.4). In collaboration with the University of Gdansk, the virus genome was full sequenced. Laboratory trials also indicated an exciting synergy between this novel virus and the homologous baculovirus of FCM, CrleGV (i.e. Cryptogran). The next project also involved virus research, the aim being to genetically select for UV-resistant CrleGV virus particles, for improved field persistence (2.2.5). Results are still pending.

Two of the remaining preharvest trials were ecological in nature. One of them investigated the movement of false codling moth (FCM) and fruit flies (FF) in multi-crop (citrus, stone fruit, grape, pomegranate) systems (2.2.15). FCM levels peaked in all crops at the same time. However, it was concluded that citrus grown close to pomegranates could be more vulnerable to FCM and carob moth attack. Unlike FCM, increases in fruit fly levels conincided with fruit ripening in the respective crop. The other ecological project investigated the influence of orchard age on FCM levels (2.2.6). Egg parasitism was higher in established orchards than in juvenile orchards. Non-bearing orchards had the highest occurrence of EPFs in soil samples. Washington Navel fruit from a juvenile orchard were more susceptible to FCM than those from an established orchard. A dose response bioassay showed that neonate larvae on diet containing powder from fruit from mature trees were significantly less susceptible to virus than those on diet containing powder from fruit from juvenile trees.

One project looked at combining pre- and postharvest practices into a Systems Approach for FCM management (2.2.10). A validation trial for the Systems Approach was conducted in 10 Nova orchards, ending with a 2°C cold treatment for 18 days. Currently, a statistician is being consulted to improve the calulations used in the Systems Approach verificiation. Another project, which feeds into the development of the systems approach is a survey of the pest status of FCM in various regions throughout southern Africa (2.2.8). The full data set is still pending.

Two projects addressed the postharvest control of FCM – both using extreme temperatures, but on opposite sides of the spectrum. The first investigated both complete and partial cold treatments (2.2.13). It was established that 1°C for 19 d provided probit 9 efficacy against fourth and fifth instars. A replicated trial with 4°C for 16 to 26 days achieved mortalities of between 70.28% and 99.85%. At 7°C, mortality after 26 days was only 17%. The other trial studied the effect of heat treatments on mortality of FCM larvae in fruit (2.2.12). At 46°C, vapour heat for 6 h caused 100% mortality of third instars. At 44°C for 6 h, there were survivors, so this temperature and period was used to compare the susceptibility the final three instars. Mortality of fourths and fifths ranged from 37-58%.

The final two projects investigated technologies for detection of FCM infested fruit. The first project evaluated the ability of commercially available automatic sorting equipment to detect FCM infested fruit (2.2.2). Feedback was provided to the two companies, whose equipment was evaluated and improvements were pursued. None of the equipment was considered adequately sensitive. The project may therefore be resumed in a few years when improvements have been successfully made. The second project sought to identify volatile emissions associated with FCM infestation of fruit (2.2.9). Oxime and methyl eugenol were found in volatiles from larvae and infested fruit, but not in healthy fruit. The ratio of D-limonene and naphthalene was significantly different between healthy and infested fruit. No differences were found in caryophyllene levels. Additionally, an electronic nose was able to detect up to 80% of infested fruit.

Programopsomming

As gevolg van die dreigende en onvermydelike regulasies vir die uitvoer van VKM-vatbare vars produkte vanaf Afrika na die EU, insluitende sitrus, bly die VKM navorsings program die hoogste prioriteit program, met die meeste entomologiese navorsing in die IPM portefeulje wat op die plaag gerig word. Gedurende die 2016/7 navorsings siklus, was 16 projekte geregistreer binne die VKM navorsings program. Tien van hierdie projekte het voor-oes studies bevat, vyf het na-oes studies bevat en een ŉ kombinasie van die twee. Daarbenewens is ŉ goeie balans gesoek tussen langtermyn strategiese navorsing en projekte wat spoedige antwoorde lewer tot sleutel probleme.

Een van die voor-oes projekte het gefokus op die monitering van VKM, deur die relatiewe aantreklikheid van ŉ reeks bekende gesintetiseerde feromone tot VKM mannetjies te vergelyk (2.2.3). Dit het die natuurlike mengsels van isomere berig in die literatuur en die mengsels in kommersiële lokmiddels ingesluit. Die Suid-Afrikaanse mengsel was die aantreklikste, gevolg deur die mengsels gebruik in die kommersiële produkte. Die projek het ook die relatiewe aantreklikheid van wyfie motte van verskillende streeks populasies tot mannetjie motte gesteriliseer vir SIT ondersoek, daar is vasgestel dat die mannetjie motte aansienlik meer aangetrokke is tot wyfies van hulle eie populasie.

In ŉ ander projek is ŉ basiese vergelyking gedoen tussen geregistreede behandelings vir VKM beheer (2.2.7). VKM besmetting was te laag om enige beduidende verskille tussen bespuitings en die onbehandelde kontrole vas te stel. Alhoewel VKM besmetting van vrugte in ŉ Paringsontwrigting/Lok en Vrek proef net so laag was, was daar ŉ groot verskil in lokval vangste, ŉ goeie indikasie van behandelings effektiwiteit. Hierdie was die laagste in die Isomate behandeling, gevolg deur Checkmate en Last Call, almal was laer as die onbehandelde kontrole.

Nog twee proewe het ook gefokus op feromone vir beheer van VKM. Die eerste het ondersoek of die aanwending van Paringsontwrigting teen drie keer die geregistreerde dosis beheer kon verbeter (2.2.11). Dit was ongelukkig nie die geval nie. Die ander proef het die effektiwiteit van ŉ nuwe samestelling, 7-vinieldeksielasetaat 1 (7-VDA), instede van die VKM feromoon ondersoek. In laboratorium proewe het die VKM feromoon, 7-VDA en 10% 7-VDA in VKM feromone fekunditeit verlaag met 69%. Die 7-VDA-behandelings het egter die vrugbaarheid van eiers heeltemal uitgeskakel, terwyl daar 'n klein persentasie bevrugte eiers met die VKM feromoon behandeling was.

Nog ŉ proef het die beheer van VKM in die veld ondersoek. Dit was egter met entomopatogeniese swamme, geteiken teen die grondgedraagde lewensstadiums (2.2.14), wat ŉ vermindering in besmetting van tot 82% behaal het. Daar is egter probleme ondervind met die lewensvatbaarheid van 'n baie belowende *Beauveria bassiana*-isolaat, wat verdere laboratorium biotoetse vertraag het.

Twee ander proewe sluit ook die gebruik van entomopatogene in. Die eerste behels die ondersoek van ŉ nuwe alfabakulovirus, afkomstig van die lietsjie mot, naamlik CrpeNPV (2.2.4). In samewerking met die Universiteit van Gdansk, is die virus genoom volledig gesekwenseer. Laboratorium proewe het ook ŉ opwindende sinergie tussen die nuwe virus en die homologiese bakulovirus van VKM, CrleGV (d.i. Cryptogran), aangedui. Die volgende projek het ook virus navorsing behels. Die doel is om geneties te selekteer vir UV-bestande CrleGV virus partikels, vir verbeterde nawerking in die veld (2.2.5). Resultate is nog uitstaande.

Twee van die oorblywende voor-oes proewe is ekologies van aard. Een van hulle ondersoek die bewegeing van VKM en vrugtevlieë (VV) in multi-gewas (sitrus, steenvrug, druif, granaat) stelsels (2.2.15). VKM vlakke het terselfdertyd gepiek in alle gewasse. Daar is egter tot die gevolgtrekking gekom dat sitrus wat naby granaat groei meer kwesbaar vir VKM en Karob mot aanvalle is. Anders as VKM, stem die toename in vrugtevlieg vlakke ooreen met vrugte rypwording in die onderskeie gewasse. Die ander ekologiese projek ondersoek die invloed van boord ouderdom op VKM vlakke (2.2.6). Eier parasitisme was hoër in gevestigde boorde as in jong boorde. Nie-draende boorde het die hoogste aantal EPFs in grondmonsters. Washington Nawel vrugte vanaf ŉ jong boord was meer vatbaar vir VKM as díe van ŉ gevestigde boord. ŉ Dosis respons biotoets het getoon dat pasuitgebroeide larwes op dieet wat poeier bevat van vrugte van volwasse bome aansienlik minder vatbaar was tot die virus as die op dieet wat poeier van vrugte van jong bome bevat het.

Een van die projekte het gekyk na die kombinasie van voor- en na-oes praktyke in ŉ Stelsels Benadering vir VKM bestuur (2.2.10). ŉ Bevestigingsproef vir die Stelsels Benadering was uitegevoer in 10 Nova mandaryn boorde, wat met ŉ 2°C vir 18 dae behandeling gëeindig het. Tans word ŉ statistikus geraadpleeg om die berekeninge wat in die Stelsels Benadering verifikasie gebruik word te verbeter. Nog ŉ projek, wat help met die ontwikkeling van die Stelsels Benadering is ŉ opname van die plaag status van VKM in verskeie streke oor suider-Afrika (2.2.8).Die volle data stel is nog hangend.

Twee projekte adresseer die na-oes beheer van VKM – beide gebruik uiterste temperature, maar aan teenoorgestelde kante van die spektrum. Die eerste het beide algehele en deeltelike koue behandelings

ondersoek (2.2.13). Daar is vasgestel dat 1°C vir 19 dae probit 9 effektiwiteit teen vierde en vyfde instars verskaf het. ʼn Gerepliseerde proef met 4°C vir 16 tot 26 dae het mortaliteit van tussen 70.28% en 99.85% behaal. Teen 7°C was die mortakiteit na 26 dae slegs 17%. Die ander proef het die effek van hitte behandelings op die mortaliteit van VKM larwes in vrugte ondersoek (2.2.12). Teen 46°C het stoom hitte vir 6 ure 100% mortaliteit van derde instars veroorsaak. By 44°C vir 6 ure was daar oorlewendes, en hierdie temperatuur en tydperk is dus gebruik om die vatbaarheid van die laaste drie instars te vergelyk. Mortaliteit van vierde en vyfde instars het tussen 37-58% gewissel.

Die finale twee projekte het die tegnologie vir die opsporing van VKM besmette vrugte ondersoek. Die eerste projek het die vermoë van kommersieël beskikbare outomatiese toerusting om VKM besmette vrugte op te spoor ondersoek (2.2.2). Terugvoer is aan die twee maatskappye wie se toerusting geëvalueer is voorsien en verbeterings sal deur hulle ondersoek word. Geen van die toerusting is as sensitief genoeg geag nie. Die projek kan daarom in ʼn paar jaar hervat word wanneer suksesvolle verbeterings gemaak is. Die tweede projek het probeer om die vlugtigestowwe afskeidings geassosieer met VKM besmetting te identifiseer (2.2.9). Oksiem en metiel-eugenol is gevind in vlugtige stowwe van larwes en besmette vrugte, maar nie in gesonde vrugte nie. Die verhouding van D-limoneen en naftaleen was aansienlik verskillend tussen gesonde en besmette vrugte. Geen verskille is in kariofileen vlakke gevind nie. Daarbenewens kon 'n elektroniese neus tot 80% van die besmette vrugte opspoor.

2.2.2 FINAL REPORT: Developing and optimising automatic sorting equipment with focus on online detection of FCM

Project 1120 (April 2015 – March 2018): Wayne Kirkman & Sean Moore (CRI)

Summary

False codling moth (FCM) is considered a phytosanitary organism by certain markets, and there is therefore an urgent need to investigate methods for its post-harvest detection in fruit. Collaborative research with manufacturers of automatic sorting equipment, to develop systems to detect FCM infested fruit in an online grading system, has been conducted over the past few years. Progress has been made, but no systems are able to detect FCM at all stages of infestation. The companies are based in Europe, and so are unable to conduct research on FCM there. However, CRI with its expertise on the pest, can conduct the research in South Africa, using equipment provided by the companies. Discussion and planning was conducted with two leading manufacturers (Company A and B). Company A has a sorting unit which employs four different visual detection technologies, which collectively take 80 images of each fruit at full packhouse line speed. Technicians and researchers from the company were present on two occasions where several hundred fruit (laboratory and naturally infested) were run through their unit. Where visible, penetrations were marked with a permanent marker, so that the programmers could see exactly where infestation took place, and could use these images to improve algorithms to detect FCM. Several other batches of fruit were run through this system, and images were downloaded in Europe. All fruit were dissected after scanning to verify infestation, and this information was sent to the programmers. Company A technology is advanced for visual aspects, but less so for early signs of decay. Three visits were made to the laboratories of Company B, where several hundred fruit were run through their demonstration unit, and various batches of infested fruit were sent to them. Two new technologies, which are not currently part of their automated system, were also tested on each fruit, with reasonable success. These technologies have been incorporated into their systems. The project could be resumed after a few years when the efficacy of the units from both companies will be evaluated for their ability to detect FCM infested fruit.

Opsomming

Valskodlingmot (VKM) word deur sekere markte as ʼn fitosanitêre plaag beskou, en daar is dus ʼn dringende behoefte om alle na-oes metodes wat VKM moontlik kan opspoor te ondersoek. Gesamentlike navorsing met vevaardigers van outomatiese sorteringstoerusting is vir die afgelope paar jaar uitgevoer. Vordering het plaasgevind, maar geen toerusting kan VKM teen alle lewensstadiums in vrugte opspoor nie. Die maatskapye is in Europa gebaseer, en kan dus nie daar navorsing op VKM doen nie. Nietemin, weens sy kundigheid op VKM, kan CRI die navorsing in Suid-Afrika uitvoer met toerusting wat deur die vervaardigers verskaf is. Onderhandelings en beplanning is met twee van die voorste maatskapye uitgevoer (Maatskapy A en B). Maatskapy A het ʼn sorteerings eenheid wat vier visuele opsporings tegnologieë gebruik om gesamentlik 80 beelde van elke vrug te neem, teen normale pakhuislyn spoed. Tegnikuste en navorsers van die maatskapy was op twee geleenthede aanwesig waar ʼn paar honderd vrugte (natuurlik en kunsmatig besmet) deur hulle stelsel gevoer is. Waar sigbaar, is elke penetrasie
merk met ʼn permanente merker aangedui, dat die programeerders presies kon sien waar die besmetting plaasgevind het, en die beelde kon gebruik om hulle algoritmes te verbeter om VKM op te spoor. Verskeie

ander besendings vrugte is ook deur hulle sisteem gesit, en die beelde is in Europa afgelaai. Alle vrugte is na skandeering gedissekteer om besmetting te verifieer. Maatskapy A se visuële tegnologie is vêr gevorder, maar hulle spoor nie maklik vroeë tekens van verotting op nie. Drie besoeke is aan die laboratoriums van Maatskapy B gemaak. Honderde vrugte is deur hulle demonstrasie-eenheid gesit, en verskeie vragte besmette vrugte is na hulle gestuur. Twee nuwe tegnologieë wat nie tans deel van hulle eenheid is nie, is op elke vrug getoets, met redelike sukses. Hierdie tegnologieë sal vir proewe in hulle eenheid ingesluit word. Die projek kan oor ŉ paar jaar hervat word, wanneer die doeltreffendheid van die eenhede van albei maatskapye geëvalueer kan word.

Introduction

False codling moth (FCM), *Thaumatotibia leucotreta* (Meyr) (Lepidoptera: Tortricidae), is one of the most important pests on citrus (Moore *et al,* 2004). The European Union, which is by far the largest market for South African citrus, is becoming increasingly strict in its perspective on the phytosanitary risks of FCM, and cold sterilisation to these markets could be enforced, which would not be economically viable. Several effective pre-harvest control measures exist for FCM, but there is no 'silver bullet' which can ensure that no infested fruit reach the packhouse. Such control measures are inadequate for a pest for which there is zero tolerance. There is an urgent necessity to investigate methods for post-harvest detection of FCM. Collaborative research with manufacturers of automatic sorting equipment, to develop systems to detect FCM infested fruit in an online grading system, has been conducted over the past few years. Progress has been made, but no systems are able to detect FCM at all stages of infestation. Most of the leading companies are based in Europe, and so are unable to conduct research there, due to the phytosanitary status of FCM. CRI is the expert on the pest and its behaviour, and so can conduct the research in South Africa, using equipment provided by the companies.

Infra-red, sonar and X-ray technology have been used to detect internal defects and insect presence in apples and grain (Bennedsen & Peterson, 2005). Infra-red and Near-Infra-red techniques have been used to detect surface defects on apples, as well as internal core rot (Chen *et al*, 2002). This technique has also been used to detect larval infestation of grain kernels and tart cherries. However, only larger larval instars were easily detected (Xing & Guyer, 2008). Greefa manufacture units using Infra-red scanning to determine size, fruit quality and certain defects in various types of fruits. Mafroda also supplies similar products. The detection of changes in volatile emission of fruit has been used as a tool to identify infested fruit. Green walnuts infested with codling moth emitted higher levels of certain chemicals, and also certain different chemicals, than healthy nuts (Buttery *et al*, 2000). Certain wavelengths of NIR could be used to detect known volatile compounds (Diederik Pieters, personal communication).

From initial literature surveys and studies, it would appear that visible and Infra-red (Kavdir *et al*, 2007), Near infra-red (Nicolai *et al*, 2007), multispectral computer vision (Blasco *et al*, 2007) and black UV technologies seem to be the most suitable for the detection of larval penetration.

Objectives:

Objective 1: Assist the three companies to improve the ability to detect FCM in citrus fruit with automatic online grading systems

Objective 2: Evaluate the sorting equipment after development/improvements

Scope for Objective 1: By artificially infesting fruit with FCM, and running them through the grading systems at various stages after infestation, many images and much information will be acquired. The fruit will then be dissected, and the findings reported to the companies involved. By correlating the images/information to the dissection results, the companies can make programming adjustments to their equipment, in order to make them more accurate.

Scope for Objective 2: Once the development phase is over, each system will be scientifically evaluated to determine the ability to detect FCM in citrus fruit. This will allow CRI to make informed decisions about the role that automatic grading units can play in a systems approach for risk mitigation of FCM being present in fruit destined for export.

Materials and methods

Discussion and planning was conducted with two leading manufacturers (Company A and B) early in 2015. Many of the technologies used are proprietary, and so cannot be named.

Company A

Company A has a sorting unit which employs four different visual detection technologies, which collectively take 80 images of each fruit at full packhouse line speed. Technicians and researchers from the company were present on two occasions where several hundred fruit (naturally infested) were run through their unit. Where visible, penetrations were marked with a permanent marker, so that the programmers could see exactly where infestation took place, and could use these images to improve algorithms to detect FCM. Several other batches of fruit (laboratory and naturally infested) were run through this system at two packhouses in the Sundays River Valley, and images were downloaded in Europe. All fruit were dissected after scanning to verify infestation, and this information was sent to the programmers.

Company B

Company B has a demonstration unit and line at their offices. Three visits were made to them, where several hundred fruit, laboratory and naturally infested, were run through their demonstration unit. On the third visit, two new technologies, which are not currently part of their automated system, were tested in isolation on each fruit. Several batches of laboratory infested fruit were couriered to them for further evaluation. Images were taken and sent to their head office in Europe for evaluation and algorithm development. All fruit were dissected after scanning to verify infestation, and this information was sent to the programmers. In a final trial, 90 Valencia oranges were artificially infested with neonate FCM larvae, and were scanned 5, 9 and 15 days after infestation. The fruit were dissected immediately after scanning.

Results and discussion

Task table

Objective / Milestone	**Achievement**
Run fruit infested with a full range of larval instars through systems	Done
Dissect fruit and provide feedback	Done
Ongoing assistance with programming/data	Done
Evaluation of systems	Will be done in a few years' time, when the project may be revived as the required technology advances to a usable stage.

Company A

Company A's unit was a pre-sorting unit, as the technology could not be used to detect FCM once the fruit had been waxed. Company A's technology is advanced for visual aspects, as can be seen in Figure 2.2.2.1, where the infestation point of a naturally infested Navel orange is highlighted by image saturation. The system was able to detect a high percentage of naturally infested fruit. However, when laboratory infested fruit were run through the unit, only a low percentage of FCM penetrations were detected. This was due to the fact that when fruit are artificially infested off the tree, they have a different physiological response to the infestation, and do not form discoloration, as a "blush" around the infestation point, as would be the case on the tree. This indicated that the unit was highly reliant on visual aspects to detect infestation, and early signs of decay were not detected. This information was relayed to the company. Algorithms are being updated, and the unit will be evaluated towards the end of the 2016 season.

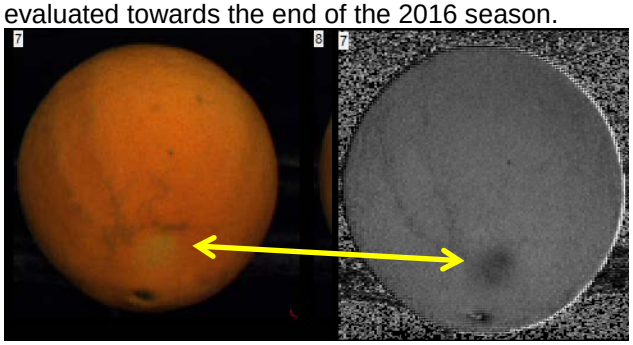

Fig. 2.2.2.1. A naturally infested Navel orange, with the FCM penetration point highlighted by image saturation

Company B

The demonstration unit of Company B was able to detect FCM penetration points on naturally and laboratory infested fruit. The two new technologies, which were tested individually, both showed much promise (Fig.

2.2.2.2). These technologies have been incorporated into their demonstration unit. In the final trial, fruit were dissected and inspected for FCM infestation immediately after scanning. The results (Table 2.2.2.1) were sent to the manufacturers. The results are being analysed, but some images were made available (Fig. 2.2.2.3).

Fig. 2.2.2.2. A laboratory infested Navel orange, with the FCM penetration point highlighted new technologies.

Table 2.2.2.1. Dissection results (infestation and larval stage (L)) of fruit infested with neonate FCM larvae, scanned 5, 9 and 15 days after infestation.

5 days		**9 days**		**15 days**	
Fruit number	Infestation description	Fruit number	Infestation description	Fruit number	Infestation description
1	Clean	31	Clean	61	Clean
2	1 X L2	32	Clean	62	1 X L2
3	1 X L2	33	Clean	63	Clean
4	1 X L2	34	Clean	64	Clean
5	Clean	35	1 x L3	65	1 X L2
6	Clean	36	1 x L3	66	Clean
7	Clean	37	1 x L3	67	Clean
8	1 X L2	38	1 x L3	68	Clean
9	1 X L2	39	Clean	69	Clean
10	1 X L2	40	Clean	70	1 X L2
11	Clean	41	1 X L2	71	1 X L2, 1 X L3
12	2 X L2	42	1 x L3	72	Clean
13	1 X L2	43	1 X L2	73	1 X L2
14	Clean	44	Clean	74	1 X L3
15	1 X L2	45	1 X L2	75	1 x L3
16	1 X L2	46	Clean	76	1 x L3
17	2 X L2	47	Clean	77	Clean
18	Clean	48	1 x L3	78	2 x L3
19	Clean	49	Clean	79	Clean
20	Clean	50	Clean	80	Clean
21	Clean	51	1 X L2	81	1 X L2, 1 X L3
22	1 X L2	52	Clean	82	1 x L3
23	1 X L2	53	Clean	83	2 x L3
24	2 X L2	54	Clean	84	1 x L3
25	1 X L2	55	Clean	85	1 x L3
26	Clean	56	Clean	86	Clean
27	1 X L2	57	Clean	87	1 X L2
28	Clean	58	1 X L2	88	1 x L3
29	2 X L2	59	Clean	89	Clean
30	2 X L2	60	Clean	90	Clean

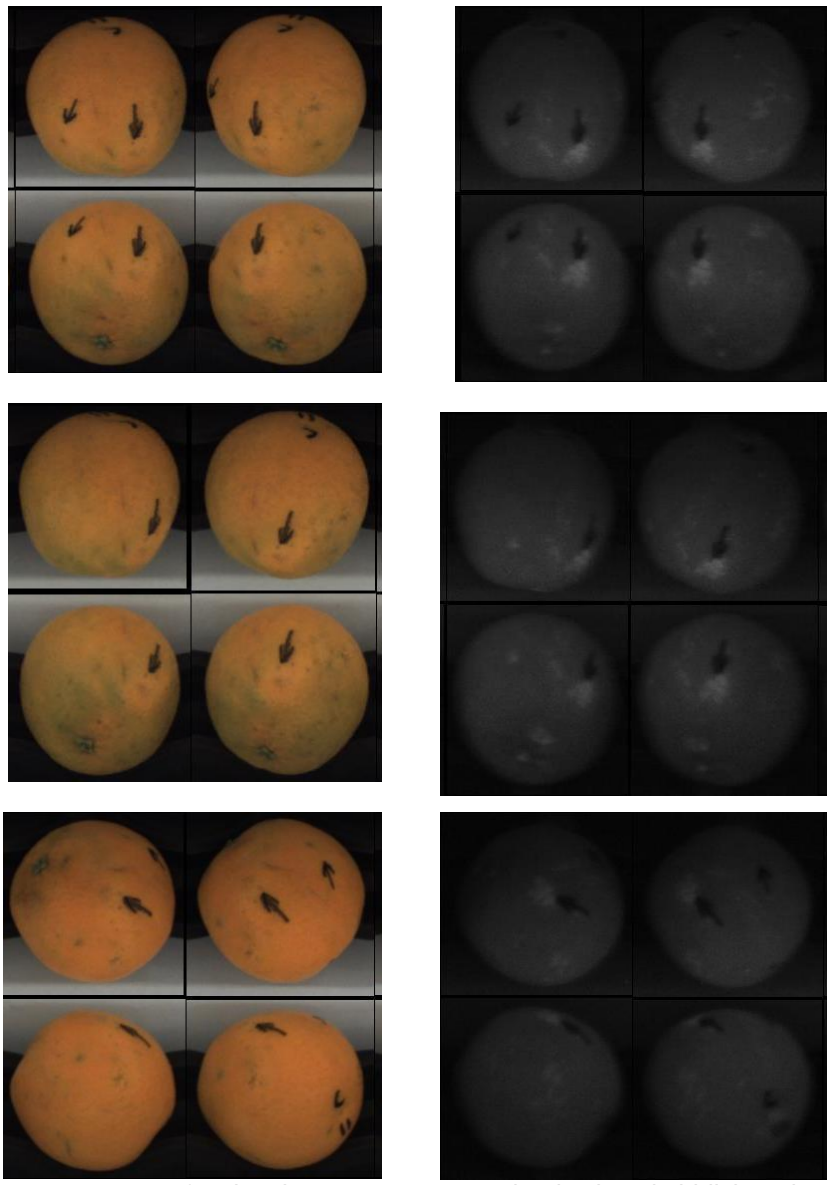

Fig. 2.2.2.3. Images of Valencia oranges scanned 5 (top), 9 (middle) and 15 (bottom) days after infestation with neonate FCM larvae.

Conclusions

Collaboration with the two companies has led to improvements in their ability to detect FCM. Company A is heavily reliant on its visual technologies, but is working on improving the ability to detect early signs of decay. Company B has added two new technologies to their demonstration unit, which should enhance its capabilities. Towards the end of the 2016 season, the efficacy of the units from both companies will be evaluated for their ability to detect FCM infested fruit. The project will then be terminated, to give the companies time to improve their equipment and programming. The project could be resumed after a few years.

Future research

The systems could be evaluated after a few years, in which time the companies can incorporate changes and improvements into their units.

Technology Transfer

Postharvest detection of false codling moth in citrus fruit: from X-ray to volatiles and beyond

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2.2.3 PROGRESS REPORT: Assessment of pheromone specificity in *Thaumatotibia leucotreta* (Meyrick) populations with focus on pest monitoring and the regional rollout of the sterile insect technique in citrus

Project 1116 (April – March 2017) by Francois Joubert, Unathi Heshula, Martin Hill (RU) and Sean Moore (CRI)

Summary

This study should have been completed during the report year. However, completion of the thesis has been slightly delayed. Submission of a final report is therefore postponed to the following research year. False codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Torticidae), is one of the most important pests of citrus in South Africa, due to its endemism to sub-Saharan Africa and the exporting of the majority of South Africa's citrus. The control of this pest is thus crucial to keep export markets open. One of the methods of control is the sterile insect technique (SIT). Xsit (Pty) Ltd runs the SIT programme for FCM in South Africa. They release sterilised males from a culture that was established using FCM collected from the Citrusdal area. The question that is addressed in this project is whether these males are able to locate female FCM from other areas in South Africa equally compared to females from their area of origin (Citrusdal) via the pheromones the females emit. The aim of this project is thus to determine if there are any differences in attractiveness between different FCM populations across South Africa and if they have an impact on female location by males. This might have major implications for the SIT programme and findings may help to improve the programme. In early 2015 a Y-tube olfactometer study was initiated to test the relative attractiveness of female FCM. Firstly, the attractiveness of Addo females to Addo males was tested i.e. males and females from the same population so that the setup of the olfactometer could be optimized. The positive reaction of males to females was up to 85%. However, the results were very inconsistent and it was therefore decided to do the experiments under complete darkness, using only red light to observe the male behaviour. During these experiments, the males did not react to the females during numerous replicates. This indicated that previous behaviour observed may have been an artefact of the artificial light in the laboratory. After these results, it was decided to move onto field experiments. The field experiments consisted of two separate trials: 1) testing the attractiveness of virgin females from five different populations (cultures maintained at Rhodes University) to sterile Citrusdal males (from the Xsit culture) and 2) testing the attractiveness of three commercially used pheromones (Checkmate, Pherolure and Isomate) and three regional blends (South Africa, Malawi and Ivory Coast) to sterile Citrusdal males (used by Xsit). A trial testing the attractiveness of the females from the five available populations to sterile Citrusdal males has been completed. The recaptures were very low and no conclusions could be made. This trial will be replicated three times in a polyethylene tunnel to ensure better control within the experiment. This will also ensure that the trials can be extended into the colder winter months. A single trial has been completed in the polyethylene tunnels and two are to follow. Three replicates of the trial with the commercial and regional pheromone blends have also been completed. It has been concluded that the South African regional blend is the most attractive followed by the commercial blends and the Ivory Coast and Malawi blends being the least attractive. This hints to the possibility that the South African blend might be a better alternative for implementation in commercial lures and mating disruption products. The results from the trials with the

virgin females will hopefully shed light on the possibility of females from areas other than a male's area of origin being less attractive. After the completion of the field trials the laboratory trials will be revisited. A flight tube system will be implemented rather than the Y-tube olfactometer method previously used. A cross-mating trial was also conducted under laboratory conditions in petri dishes with five different FCM cultures. Females produced more eggs when mated with males from the same population. This was the case for the Addo, Marble Hall, Nelspruit and Old (mixed origin) cultures. The only case in which this was found to be statistically significant was for the Marble Hall culture. All the crosses produced viable eggs and the origin of the male or female did not influence egg hatch.

Opsomming

Hierdie studie moes gedurende die verslagsjaar voltooi word maar voltooing van die tesis is effens vertraag. Daarom is die finale verslag tot volgende navorsingsjaar uitgestel. Valskodling mot (VKM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Torticidae), is een van die belangrikste plae van sitrus in Suid-Afrika omrede hy endemies vir Afrika suid van die Sahara is en die feit dat die meerderheid Suid-Afrikaanse sitrus uitgevoer word. Die beheer van die plaag is dus noodsaaklik om markte vir uitvoer oop te hou. Een van die metodes van beheer is die steriele insek tegniek (SIT). Xsit (Edms) Bpk bedryf die SIT program vir VKM in Suid-Afrika. Hulle laat steriele mannetjie motte los, vanaf 'n kultuur gevestig van motte van die Citrusdal area. Die vraag wat in die projek ontleed word is of die mannetjies in staat is om die wyfies van ander areas in Suid-Afrika net so doeltreffend op te spoor in vergelyking met hul area van herkoms (Citrusdal) deur middel van die feromone wat die wyfies afgee. Die doel van die projek is om dus vas te stel of daar enige verskil is in die aantreklikheid tussen verskillende VKM populasies deur Suid-Afrika, en of hulle 'n impak het op die opsporing van wyfies deur mannetjie motte. Dit kan belangrike implikasies hê vir die SIT program, en resultate het die potensiaal om die program te kan verbeter. Vroeg in 2015 is laboratorium werk begin deur van 'n Y-buis olfaktometer gebruik te maak om die aantreklikheid van wyfie VKM te toets. Eerstens is die aantreklikheid van Addo wyfies tot Addo mannetjies getoets dws mannetjies en wyfies vanaf dieselfde populasie omrede die olfaktometer opset te optimaliseer. Die positiewe reaksie van mannetjies tot wyfies was tot 85%. Nietemin is daar baie variasie in die resultate en daar is besluit om die eksperimente onder volkome donker te doen met net die gebruik van 'n rooi lig om die mannetjies se gedrag waar te neem. Gedurende die eksperimente, het die mannetjies gedurende talle herhalings nie op die wyfies gereageer nie. Dit dui daarop dat die gedrag wat voorheen waargeneem is, dalk deur die kunsmatige lig in die laboratorium veroorsaak is. Na hierdie resultate is besluit om aan te beweeg na veldproewe. Die veldproewe bestaan uit twee verskillende proefnemings: 1) die aantreklikheid van maagd wyfies van vyf verskillende populasies (kulture word by Rhodes Universiteit gehandhaaf) tot steriele Citrusdal mannetjies (deur Xsit aangeteel) en 2) toets die aantreklikheid van drie kommersiële feromone (Checkmate, Pherolure en Isomate) en drie plaaslike mengsels (Suid-Afrika, Malawi en Ivoorkus) tot steriele Citrusdal mannetjies. 'n Proef wat die aantreklikheid van wyfies van die vyf beskikbare populasies toets tot die steriele Citrusdal mannetjies is voltooi. Die hervangs was baie laag en geen afleidings kon gemaak word nie. Die proef sal nog drie keer in 'n poliëtileen tonnel herhaal word om beter beheer oor die eksperiment te verseker. Dit sal ook verseker dat die proewe uitgebrei kan word tot in die winter maande. Een van hierdie proewe is al voltooi. Drie proewe met die kommersiële en plaaslike feromoon mengsels is ook voltooi. Die gevolgtrekking is dat die Suid-Afrikaanse plaaslike mengsel die mees aantreklik is, gevolg deur die kommersiële mengsels, en die Ivoorkus en Malawi mengsels die minste aantreklik. Dit sinspeel tot die moontlikheid dat die Suid-Afrikaanse mengsel dalk 'n beter alternatief is vir implementering in kommersiële lokmiddels en paringsontwrigting produkte. Die resultate van die proewe met die bevrugde wyfies sal hopelik lig werp op die moontlikheid dat wyfies van areas anders as die van die mannetjies se herkoms minder aantreklik is. Na voltooiing van die veldproewe sal laboratorium proewe hervat word. 'n Vlug buis sisteem sal geïmplementeer word eerder as die Y-pyp olfaktometer metode wat voorheen gebruik is. 'n Kruis-parings studie was ook in petri bakkies in die laboratrium met VKM gedoen. Die wyfies het meer eiers geproduseer wanneer hulle gepaar het met mannetjies vanaf hulle eie populasie. Dit was die geval vir die Addo, Marble Hall, Nelspruit en Ou (gemengde oorsprong) kulture. Dit was egter net statisties relevant vir die Marble Hall kultuur. All die kruisings het larwes geproduseer en geen steriliteit tussen kruisings was opgemerk nie.

2.2.4 PROGRESS REPORT: Genetic and biological characterization of a novel nucleopolyhedrovirus from the false codling moth (FCM), *Thaumatotibia leucotreta*, for improved control of FCM

Project: RCE 1 (2015/16 – 2018/9) by Michael Jukes, Caroline Knox, Martin Hill (Rhodes University) and Sean Moore (CRI)

Summary

The use of baculoviruses as biocontrol agents has become an essential component of integrated pest management programmes for the control of a variety of agricultural pests around the world. In South Africa, the baculovirus *Cryptophlebia leucotreta* granulovirus (CrleGV) has been commercially formulated into the

biocontrol product Cryptogran for the control of *Thaumatotibia leucotreta*, more commonly known as the false codling moth (FCM). FCM is an important pest in the citrus industry in South Africa, partly due to phytosanatory concerns for export markets. A recent study at the University of Gdansk and Medical University of Gdansk in Poland on homogenates of infected FCM larvae was carried out to produce a complete genome sequence of CrleGV. During this study a second unknown baculovirus was detected in the homogenate samples alongside CrleGV, with this novel virus identified as a nucleopolyhedrovirus (NPV). The identification of a novel baculovirus which infects FCM allows for a new avenue of research regarding the production of biocontrol agents for the control of this pest. The primary aim of this project is to isolate the NPV from FCM homogenates in order to evaluate its virulence against FCM larvae. This will further be expanded to evaluate whether a combination of the NPV and CrleGV can increase the virulence of these viruses as well as determine the ratio required to achieve high levels of mortality. To date, the virus has been successfully identified in and isolated from FCM homogenate samples. A multiplex PCR assay has been developed to screen these and any future samples for the presence of either the GV or NPV. Biological assays using a surface dose method have been completed evaluating the efficacy of the NPV alone and in combination with CrleGV. Field trials have also been started to evaluate the efficacy of the NPV when used in combination with CrleGV. Furthermore, a series of laboratory experiments are also underway to study potential synergistic interactions of the two viruses with quantitative PCR assays planned to accurately quantify virus occlusion bodies recovered from larval cadavers. The results of this project will provide an indication of whether the NPV can form the primary component of a new biopesticide and whether it can be used in combination with CrleGV to achieve greater levels of FCM control in the field.

Opsomming

Die gebruik van bakulovirusse as biologiese beheer agente het ŉ fundamentele komponent van geïntegreerde plaagbestuur programme geword vir die beheer van ŉ verskeidenheid landbouplae regoor die wêreld. In Suid-Afrika, is die bakulovirus *Cryptophlebia leucotreta* granulovirus (CrleGV) kommersieel geformuleer as die biologiese beheer produk Cryptogran vir die beheer van *Thaumatotibia leucotreta*, meer algemeen bekend as valskodlingmot (VKM). VKM is ŉ belangrike plaag in die sitrusbedryf in Suid-Afrika, gedeeltelik as gevolg van sy fitosanitêre status vir sekere uitvoer markte. In ŉ onlangse studie by die Universiteit van Gdansk en die Mediese Universiteit van Gdansk in Poland is homogenates van geïnfekteerde VKM larwes gemaak om ŉ volledige genoom van CrleGV te produseer. Gedurende hierdie studie is ŉ tweede onbekende bakulovirus in die monsters saam met CrleGV ontdek, en hierdie virus is as ŉ nukleopolihedrovirus (NPV) geïdentifiseer. Die identifikasie van ŉ nuwe bakulovirus wat VKM besmet, maak voorsiening vir nuwe navorsing oor produksie van biologiese beheer agente vir die beheer van die plaag. Die primêre doel van die projek is om die NPV van VKM homogenate te isoleer en om sy virulensie teen VKM larwes te evalueer. Die studie sal verder uitgebrei word om te bepaal of ŉ kombinasie van die NPV en CrleGV die virulensie van die virusse verhoog asook om die ideale verhouding te bepaal om hoë vlakke van mortaliteit te kry. Tot op hede is die virus suksesvol in VKM geïdentifiseer en van VKM homogenate monsters geïsoleer. ŉ Multipleks PCR toets is ontwikkel om hierdie en enige toekomstige monsters vir die teenwoordigheid van óf die GV of NPV te toets. Biologiese toetse deur gebruik van 'n oppervlak-dosis metode is voltooi. Hierdie biotoetse het die doeltreffendheid van die NPV op sy eie en in kombinasie met CrleGV getoets. Veldproewe is ook begin om die doeltreffendheid van die NPV in kombinasie met CrleGV te evalueer. Verder is 'n reeks laboratorium eksperimente ook begin om moontlike sinergisme van die twee virusse te bepaal deur gebruik van PCR om virus partikels van dooie besmette larwes te kwantifiseer. Die resultate van hierdie projek sal 'n aanduiding gee of die NPV die primêre komponent van 'n nuwe bioplaagdoder kan wees en of dit in kombinasie met CrleGV gebruik kan word om hoër vlakke van VKM beheer in die veld te bereik.

2.2.5 PROGRESS REPORT: Development of UV-resistant CrleGV-SA for use as an enhanced biopesticide for FCM control on citrus

Project NMMU - 1117: Patrick Mwanza, Gill Dealtry, Mike Lee (NMMU) and Sean Moore (CRI)

Summary

The use of baculoviruses as biopesticides continues to gain popularity as they appear to provide a more advantageous approach to combat crop pests than chemical pesticides do. As such they have become important in the agricultural economy. *Cryptophlebia leucotreta* granulovirus (CrleGV) has been formulated as a biocontrol agent against the common citrus pest, the false codling moth, (FCM). One of the major setbacks with the use of baculoviruses as biopesticides has been their susceptibility to ultraviolet (UV) radiation from the sun. When exposed to UV some baculoviruses lose their activity within 24 hours. This forces farmers to have to frequently reapply the biopesticides. In this study, the existence CrleGV-SA naturally resistant to UV is being investigated. Samples of CrleGV-SA are repeatedly exposed to UV, passed through fifth instars and re-exposed to UV. The re-exposure experiment will isolate and select UV resistant strains. Presently three exposure cycles have been completed. At least five cycles are required to be able to isolate resistant occlusion

bodies (OBs). In this same study, potential UV protectants are also being investigated. The most effective UV protectant will be combined with the UV-resistant CrleGV-SA to formulate a more UV-resistant biopesticide. Structural studies using transmission electron microscopy (TEM) showed that UV damaged and destroyed the nucleocapsid. TEM images of UV-irradiated CrleGV-SA also showed a deterioration in the OB. This is a meaningful breakthrough, as UV damage can now be visually observed. Further structural evidence is being obtained using Raman analysis, which gives a molecular spectrum of the OB surface before and after UV-exposure. Molecular analysis including genome sequencing and qPCR will also be used to show the extent of the molecular damage.

Opsomming

Die gebruik van bakuloviruse as bioplaagdoders word al hoe meer gewild omdat hulle ŉ meer voordelige benadering tot die bestryding van gewasplae voorsien as wat die geval met chemiese plaagdoders is. As sulks het hulle meer belangrik in die landbou ekonomie geword. *Cryptophlebia leucotreta* granulovirus (CrleGV) is as ŉ biologiese beheer produk geformuleer vir gebruik teen die algemene sitrusplaag, die valskodlingmot (VKM). Een van die hoof nadele met die gebruik van bakuloviruse as bioplaagdoders is hulle vatbaarheid vir ultraviolet (UV) bestraling van die son. Na blootstelling aan die son verloor sekere bakuloviruse hulle aktiwiteit binne 24 ure. Hierdie veroorsaak dat boere gereeld die bioplaagdoder hoef te hertoedien. In hierdie studie word die voorkoms van CrleGV-SA wat natuurlik teen UV bestand is ondersoek. Monsters van CrleGV-SA word herhaaldelik aan UV bestraling blootgestel, deur vyfde instars deurgeloop en weer aan UV blootgestel. Die herblootstelling eksperiment sal UV-bestande virusse isoleer. Tans is drie blootstellings siklusse voltooi. Minstens vyf siklusse word benodig om UV-bestande oklussie partikels (OPs) te isoleer. In dieselfde studie word belowende UV-beskermers ook ondersoek. Die mees doeltreffende UV-bskermer sal met die UV-bestande CrleGV-SA gekombineer word om ŉ meer UV-bestande bioplaagdoder te formuleer. Strukturele studies met die gebruik van transmissie elektronmikroskopie (TEM) het gewys dat UV die nukleokapsied beskadig en vernietig het. TEM beelde van UV-bestraalde CrleGV-SA het ook ŉ agteruitgaan in die OP gewys. Hierdie is ŉ beduidende deurbraak want UV-skade kan nou visueel opgelet word. Verdere strukturele bewyse word versamel met gebruik van Raman analiese, wat ŉ molekulêre spektrum van die OP oppervlak voor en na UV-blootstelling gee. Molekulêre analiese, insluitent genoom sekwensies en qPCR sal ook gebruik word om die volle mate van molekulêre skade te wys.

2.2.6 PROGRESS REPORT: FCM population ecology in citrus orchards: the influence of orchard age

Project 1114 (2015/6 – 2017/8) by S Albertyn, M Hill (RU) and S D Moore (CRI)

Summary

Anecdotal reports have been made of high populations of false codling moth, (FCM*) Thaumatotibia leucotreta*, during the first three to five harvesting years of being citrus planted in virgin soil, after which, FCM numbers decline. The FCM population ecology of newly non-bearing, juvenile (2 - 4 years) and mature (9 years and older) orchards are being monitored to determine if and why juvenile orchards facilitate higher FCM infestation than mature orchards. Orchards were monitored weekly to determine FCM egg counts and parasitism of eggs, FCM trap catches, fruit infestation and ant presence. Egg parasitism was consistently higher in established orchards than in juvenile orchards. Non-bearing orchards had the highest occurrence (38%) of entomopathogenic fungi (EPF) in soil samples, followed by established orchards (34%). EPF occurrence was significantly lower in juvenile orchards (31%) than in non-bearing orchards. The occurrence of entomopathogenic nematodes were low in all orchards sampled. Fruit from a juvenile Washington Navel orchard were found to be significantly more susceptible to FCM than the same variety of fruit from an established orchard on the same farm. A diet dose response bioassay showed a significant response to increasing concentrations of dried citrus powder from both juvenile and mature trees. However, the dose response was higher when the various concentration of dried fruit powder from mature citrus trees were added to the diet. Nutritional analyses showed fruit from the juvenile orchards to possess substantially higher ash content, of 3.32%, than fruit from established orchards, with 0.26% ash content. A virus dose response bioassay showed that at the lowest concentration tested, neonate larvae placed on diet containing 15% fruit powder from mature Washington Navel oranges were significantly less susceptible to virus than neonate larvae placed on a diet containing 15% fruit powder from juvenile trees. No significance in dose response to EPF were found in 5th instar FCM reared on diet containing fruit powder from mature and juvenile orchards respectively.

Opsomming

Anekdotiese verslae is gemaak van hoë bevolkings van valskodlingmot, (VKM) *Thaumatotibia leucotreta*, gedurende die eerste 3-5 oes jare van sitrus geplant in onversteurde grond, waarna VKM getalle verminder. Die VKM bevolkings ekologie van nie-draende, jong (2-4 jaar) en volwasse (9 jaar en ouer) boorde word

gemonitor om te bepaal of en waarom jong boorde hoër VKM besmetting fasiliteer as gevestigde boorde. Boorde is weekliks gemonitor om VKM eiertellings en parasitisme van eiers, VKM lokval vangste, vrugbesmetting en mier teenwoordigheid te bepaal. Eier parasitisme was deurgaans hoër in gevestigde boorde as in jong boorde. Nie-draende boorde het die hoogste voorkoms (38%) van entomopatogeniese swamme (EPS) in grondmonsters gehad, gevolg deur gevestigde boorde (34%). EPS voorkoms was aansienlik laer in jong boorde (31%) as in nie-draende boorde. Die voorkoms van entomopatogeniese nematodes was laag in alle boorde. Die vatbaarheid vir VKM besmetting vir vrugte vanaf ŉ 12 jarige, volwasse Washington Nawel boord en ŉ jong 4 jarige Washington Nawel boord vanaf dieselfde plaas is vergelyk. Vrugte van jong boorde was aansienlik meer vatbaar vir VKM besmetting as vrugte vanaf volwasse boorde. 'n Dieet dosisreaksie biotoets het 'n beduidende reaksie op toenemende konsentrasies van gedroogde sitrus poeier van beide jong en volwasse bome getoon. Die dosis reaksie was egter hoër as die konsentrasie van gedroogde vrugte poeier vanaf volwasse sitrusbome verhoog word. Voedingswaarde analise het getoon vrugte van die jong boorde aansienlik hoër as inhoud besit, van 3.32%, as vrugte van gevestigde boorde, met 0.26% as inhoud. 'N virus dosisreaksie biotoets het getoon dat teen die laagste konsentrasie getoets, was pasuitgebroeide larwes geplaas op dieet met 15% vrugte poeier van volwasse Washington Nawellemoene aansienlik minder vatbaar vir die virus as pasuitgebroeide larwes geplaas op 'n dieet met 15% vrugte poeier van jong bome bevat. Geen verskil in dosis reaksie op EPS is gevind in 5de instar VKM onderskeidelik grootgemaak op dieet met vrugte poeier van volwasse en jong boorde nie.

2.2.7 PROGRESS REPORT: The efficacy of registered treatments for FCM control in Limpopo

Project 1112 (Apr 2015 – July 2016) by Sean Moore, Wayne Kirkman (CRI), Sean Thackeray (CRI/RU), Francois Joubert (RU), Jacolene Meyer and Marili Mouton (QMS Laboratories)

Summary

In this project we proposed to test and compare the efficacy of control measures that are registered for use against FCM on citrus. This is because it cannot be assumed that results generated from trials elsewhere in the country will be identical for Letsitele or any other region for that matter; and because anecdotal information indicates differences in efficacy of certain products between certain regions. During the 2015/6 season, two trials were conducted: one involving mating disruption (MD) and attract and kill (A&K) products and one involving products registered as sprays. Monitoring of traps and fruit infestation was conducted weekly until the end of March 2016, and was terminated at this time, as FCM infestation was too low to justify continuation thereof. FCM infestation was too low to establish any significant differences between spray treatments and the untreated control. Although FCM infestation of fruit in the MD and A&K trial was similarly low, there were large differences in trap catches, a good indication of treatment efficacy. Trap catches were lowest in the Isomate treatment, followed by Checkmate and Last Call, all of which were lower than the untreated control. The spray trial was to be repeated in March 2017, but low levels of FCM necessitated delaying the application of the trial. The final report of this project will therefore be submitted next year.

Opsomming

In hierdie projek beplan ons om die werking van produkte wat op sitrus teen VKM geregistreer is te toets en met mekaar te vergelyk. Hierdie is omdat dit nie sommer aanvaar moet word nie dat restultate van proewe elders in die land identies sal wees vir Letsitele of enige ander streek. Boonop, anekdotiese inligting dui aan dat daar verskille in die werking van sekere produkte in sekere streke mag wees. Gedurende dit 2015/6 seisoen is twee proewe uitgevoer: een het paringsontwrigting (PO) en lok-en-vrek (L&V) produkte vergelyk en die ander het geregistreerde spuit-produkte vergelyk. Monitering van lokvalle en vrugbesmetting is tot Maart 2016 weekliks uitgevoer en is toe gestop omdat VKM besmetting te laag was om verdere monitering te regverdig. VKM besmetting was te laag om enige betekenisvolle verskille tussen spuit behandelings en die onbehandelde kontrol te kry. Al was VKM besmetting in die PO en L&V proef ewe laag, was daar groot verskille in lokval vangste, ŉ goeie aanduiding van doeltreffendheid van die behandelings. Lokval vangste is laagste in die Isomate behandeling, gevolg deur Checkmate en Last Call, en al die behandelings is laer as die onbehandelde kontrole. Ons het beplan om die spuit proef in Maart 2017 te herhaal maar die proef is uitgestel as gevolg van lae vlakke van VKM. Die finale verslag van hierdie projek sal dus volgende jaar ingedien word.

2.2.8 PROGRESS REPORT: Assessment of the pest status of FCM on citrus in various southern African production regions

Project 1111 (Oct 2014 – Mar 2017) by Sean Moore and Sean Thackeray (CRI)

Summary

Although this project was scheduled to be conducted for only one season, it was decided to run it for a second consecutive season. In the first season (2015), a total of 29 citrus growers and citrus cooperatives/companies in seven general regions throughout South Africa and Zimbabwe were contacted to collect and supply monitoring data for FCM for the duration of the season until harvest. Additionally, Xsit agreed to supply their data from Citrusdal, the Sundays River Valley and the Gamtoos River Valley. Data from the same regions and growers must still be collected for the 2016 season. However, other research and extension commitments have held up completion of this. The data will be used to determine the current ability in each region to comply with the proposed standards of the drafted FCM Management System, of which the FCM Systems Approach is a part. A final report on this project will be submitted next year.

Opsomming

Al is hierdie projek net vir een seiseon geskeduleer, is dit besluit om dit vir ŉ tweede agtereenvolgende seisoen te laat loop. In totaal is 29 sitrus produsente en sitrus koöperatiewe/maatskapye in sewe algemene streke deur Suid-Afrika en Zimbabwe genader om VKM moniterings data deur die loop van die eerste (2015) seisoen tot oestyd te versamel en om dit aan ons te voorsien. Boonop het Xsit ingestem om hulle data van Citrusdal, die Sondagsrivier Vallei en Gamtoosrivier Vallei te voorsien. Data van dieselfde streke en produsente moet nog vir die 2016 seisoen ingesamel word. Die data sal gebruik word om te bepaal wat die huidige vermoë in elke streek is om die voorgestlede standaarde in die VKM Bestuurs-stelsel, waarvan die VKM Stelselsbenadering ŉ deel vorm, na te kom. ŉ Finale verslag op hierdie projek sal volgende jaar ingedien word.

2.2.9 PROGRESS REPORT: Identifying volatile emissions associated with false codling moth infestation of citrus fruit

Project 1090 (April 2014 – March 2019) by Wayne Kirkman, Sean Moore (CRI), Martin Hill, Rui Krause and Roman Tandlich (RU)

Summary

A Solid Phase Microextraction (SPME) probe has been shown to effectively trap as well as concentrate headspace volatile compounds surrounding intact fruit. Volatile compound detection is achieved by inserting this probe into a Gas Chromatography-Mass Spectrometry (GCMS) system. GCMS analysis was conducted on five major volatile compounds of interest previously shown to be released by FCM infested oranges. These major volatile compounds are D-limonene, 3,7-dimethyl-1,3,6-octatriene, (E)-4,8-dimethyl-1,3,7-nonatriene, caryophyllene and naphthalene. This served as training for W Kirkman.
Segments of healthy and infested fruit, as well as larvae extracted from fruit, were analysed in 250 ml containers. Oxime and methyl eugenol were found in volatiles from larvae and infested fruit, but not in healthy fruit. Whole Witkrans Navel oranges infested 14 and 21 days previously, as well as naturally infested fruit from the same orchard were analysed. D-limonene levels decreased with time after infestation, while levels of a naphthalene derivative increased. The ratio of these compounds was significantly different between healthy and infested fruit for all time periods. No differences were found in caryophyllene levels. The ability of an electronic nose to detect FCM infested fruit was investigated. Initially results were variable, due to citrus contaminants in the laboratory. Trials were then conducted under a laminar flow cabinet. Lane Late Navel oranges, infested 2, 6 and 10 days previously were analysed, and the electronic nose could detect 70, 90 and 80 percent of infested fruit respectively for the three treatments. The reaction of individual sensors in the electronic nose was examined, and a new array of sensors, comprising of the most sensitive ones, will be manufactured and will be tested in the new season. W Kirkman visited the University of California – Davis, to be trained on a Differential Mobility Spectrometry (GCDMS). During training trials were conducted to evaluate the ability of the GCDMS unit to detect phytophthora infected rhododendron plants by sampling leaf volatiles. The trials were successful, and it is intended to test the unit on FCM infested fruit in the future.

Opsomming

Soliede Fase Mikro-ekstraksie (SPME) het hoofruim vlugtigestowwe rondom vrugte effektief opgevang en gekonsentreer. Daarna is Gaskromatografie-Massaspektrometrie (GCMS) analise gedoen op die SPME naald. ŉ Vorige studie het gewys dat D-limonien, 3,7-dimetiel-1,3,6-oktatrieen, (E)-4,8-dimetiel-1,3,7-nonatrieen, kariofileen en naftaleen deur VKM-besmette vrugte afgeskei word. GCMS analise is gedoen op die vlugtigestowwe, as opleiding vir W Kirkman. Analise is in 250 ml bottels gedoen op skyfies van gesonde en besmette vrugte, sowel as larwes afkomstig van besmette vrugte. Oxime en methyl eugenol is in vlugstowwe van besmette vrugte en larwes gevind, maar nie in gesonde vrugte nie. Analise is gedoen op heel Witkrans Nawellemoene wat 14 en 21 dae vroeër besmet is, asook op natuurlik besmette vrugte uit dieselfde boord. D-limonien vlakke het afgeneem met tyd na besmetting, terwyl vlakke van naftaleen toegeneem het.

Die verhouding van die 2 vlugtigestowwe verskil beduidend tussen gesonde en besmette vrugte. Daar was geen verskille in kariofileen-vlakke nie. Proewe is gedoen om te kyk of ŉ elektroniese neus tussen besmette en gesonde vrugte kon onderskei. Voorlopige resultate was wisselvallig weens sitrus kontaminasie in die laboratorium. Proewe is toe onder ŉ Laminêrevloei kabinet uitgeoefen. Analise is gedoen op Lane Late Nawellemoene wat 2, 6 en 10 dae vroeër besmet is. Die elektroniese neus kon onderskeidelik 70, 90 en 80 persent van besmette vrugte uitken. Die reaksie van elke individuele sensor is ondersoek, en ŉ nuwe groep sensors wat bestaan uit die mees sensitiewe sensors sal vervaarig word, en in die nuwe seisoen getoets word. W Kirkman het die Universiteit van Kalifornia-Davis besoek vir opleiding op hulle Differential Mobility Spectrometry (GCDMS) eenheid. Tydens opleiding is proewe gedoen om te kyk of die eenheid phytophthora-besmette rhododendron plante kan uitken deur analise van vlugtigestowwe afkomstig van blare. Die proewe was suksesvol, en daar is beplan om te kyk of die eenheid tussen VKM-besmette en gesonde vrugte kan onderskei.

2.2.10 PROGRESS REPORT: Verification of proposed inspections standards within an FCM systems approach

Project 1085 (April-October 2014) by Sean Moore, Wayne Kirkman, Vaughan Hattingh (CRI), Mat Goddard (RU) and Sean Thackeray (CRI/RU)

Summary

The International Plant Protection Convention describes different options to attaining requisite risk mitigation for pests of phytosanitary concern in international trade. These include the use of Pest Free Areas; Pest Free Places of Production; Pest Free Production Sites; Pest Free Consignments; Post harvest Disinfestation Treatments; Non Host Status; Areas of Low Pest Prevalence; and the Systems Approach to combining partial controls into a composite system that achieves the requisite level of phytosanitary security. The Systems Approach is a concept that has a high level of flexibility inherent in choice of components that can be combined. This allows for tailoring of a Systems Approach to a particular pest by selection of treatments that are of particular relevance to the pest, the production environment and the export country's infrastructural and regulatory capacity. A risk management framework was developed for FCM in citrus fruit for export from South Africa. A component of the framework was a Systems Approach that included a series of pre- and post-harvest controls and infestation assessments. It was determined that the proportion of fruit that could be infested with FCM after application of the Systems Approach was between $P \leq 5.328 \times 10^{-6}$ and $P \leq 8.380 \times 10^{-7}$, 6 to 38 times less than the proportion associated with the probit 9 ($P \leq 3.2 \times 10^{-5}$) standard for a stand-alone cold treatment, being 3 survivors in 100,000 at the 95% confidence level. This was published in the Journal of Economic Entomology in 2016. During the 2015/6 season, a validation trial for the Systems Approach was conducted in 10 Nova orchards in the Sundays River Valley for the full duration of the season. The validation ended with a 2°C cold treatment for 18 days. One of the 10 orchards was rejected on delivery to the packhouse for further participation in the Systems Approach due to exceeding the stipulated FCM infestation threshold. Of the other nine orchards, one exceeded the stipulated FCM infestation threshold post-packing. However, after the cold treatment, all larvae were dead. Nevertheless, the one aberration in the validation trial caused us to revisit and improve the Systems Approach calculations. Currently, a statistician is being consulted in this regard, with the aim to publish the Systems Approach validation and improvement during 2017.

Opsomming

Die Internasionale Plant Beskermings Konvensie beskryf verskillende opsies om nodige risko vermindering te bereik vir plae van fitosanitêre belang vir internasionale handel. Hierdie sluit in die gebruik van Plaagvrye Areas; Plaagvrye Plekke van Produksie; Plaagvrye Produksie Persele; Plaagvrye Besendings; Na-oes Ontsmettingsbehandelings; Nie Gasheer Status; Areas van Lae Plaag Voorkoms; en die Stelselsbenadering tot samestelling van onvoldoende beheer maatreels in ŉ saamgestelde stelsel wat die nodige vlak van fitosanitêre sekuriteit behaal. Die Stelselsbenadering is ŉ konsep wat ŉ hoë mate van buigsaamheid het in die keuse van komponente wat gekombineer kan word. Hierdie maak dit moontlik om ŉ Stelselsbenadering aan te pas by ŉ spesifieke plaag deur seleksie van behandelings wat direk relevant is tot die plaag, die produksie omgewing en die uitvoerende land se infrastruktuur en regulatoriese kapasiteit. ŉ Risiko bestuursraamwerk is ontwikkel vir VKM in sitrusvrugte vir uitvoer uit Suid-Afrika. ŉ Komponent van die raamwerk was ŉ Stelselsbenadering wat ŉ reeks voor- en na-oes behandelings en besmetting analieses behels het. Dit is bepaal dat die proporsie vrugte wat na toepassing van die Stelselsbenadering met VKM besmet kon wees tussen $P \leq 5.328 \times 10^{-6}$ en $P \leq 8.380 \times 10^{-7}$, 6 tot 38 keer minder as die proporsie wat met die probit 9 ($P \leq 3.2 \times 10^{-5}$) standaard vir ŉ alleenstaande koue behandeling geassosieer is dws 3 oorlewendes in 100 000 teen ŉ 95% vlak van vertroue. Hierdie is in die Journal of Economic Entomology in 2016 gepubliseer. Gedurende die 2015/6 seisoen is ŉ validasie proef vir die Stelselbenadering in 10 Nova boorde in die Sondagsriviervallei vir die volle loop van die seisoen uitgevoer. Die validasie het geëindig met ŉ 2°C koue behandeling vir 18 dae. Een van die boorde is op aflewering by die pakhuis afgekeur vir verdere

deelname aan die Stelselsbenadering omdat die bepaalde VKM besmettings drempelwaarde oorskry is. Van die ander nege boorde het een die bepaalde VKM besmettings drempelwaarde na verpakking oorskry. Na die kouebehandeling is alle larwes egter dood. Nietemin het die een afwyking in die validasie proef veroorsaak dat ons die berekeninge van die Stelselsbenadering herbesoek het om verbeterings in te bring. Tans word ŉ statistikus in verband hiermee gekonsulteer met die doel om die Stelselsbenadering validasie en verbetering gedurende 2017 te publiseer.

2.2.11 PROGRESS REPORT: Evaluation of 7-Vinyl-Decyl Acetate for mating inhibition in FCM

Project 1063 (April 2012 – March 2015): Sean Moore, Wayne Kirkman, Claire Love (CRI), Mat Goddard (RU) and Ben Burger (SU)

Summary

Several years ago it was discovered, almost accidently, that 7-vinyldecyl acetate 1 (7-VDA) was capable of preventing adult false codling moth (FCM) males from locating virgin females. Consequently, we decided to examine this further with a view to developing a novel mating disruption, or rather a mating inhibition, technology. A novel polyethylene dispenser was developed, which allowed a consistent release rate of 7-VDA, comparable to or better than that of the female FCM pheromone from a commercial dispenser. Laboratory mating inhibition trials were conducted with virgin pairs of moths in plastic containers, with dispensers loaded 24 h before moths were introduced. Both FCM pheromone and 7-VDA showed the ability to reduce fecundity, an indication of reduced mating. Results were variable, but on average FCM pheromone appeared to be more effective. However, a subsequently tested combination of 7-VDA (5-10%) and FCM pheromone (90-95%) proved to be the most effective treatment, reducing fecundity by 69%. Trials were repeated using a modified protocol, designed to reduce variability, where females were removed from the plastic containers after 48 h and placed into petri dishes to record fecundity and fertility. Four replicates were completed, showing a similar reduction in fecundity (by 69%) with FCM pheromone, 7-VDA and 10% 7-VDA in FCM pheromone. However, the last two treatments completely eliminated fertility of eggs, whereas there was a small percentage of fertilized eggs with the FCM pheromone treatment. Results nevertheless continued to be variable. 7-VDA is currently being synthesized for field trials. The intention was to initiate these during November 2016. However, there have been difficulties in achieving synthesis and therefore field trials have been postponed to November 2017.

Opsomming

Jare gelede is dit ontdek, amper toevalig, dat 7-vinieldesielasetaat 1 (7-VDA) die vermoë het om volwasse valskodlinmot (VKM) mannetjies te verhoed om ongepaarde wyfie motte te vind. Daarom het ons besluit om hierdie verder te ondersoek met die moontlikheid van ŉ oorspronklike paringsontwrigting – of liewer paringsverhoeding – tegnologie te ontwikkel. ŉ Nuwe polietileen vrysteller is ontwikkel, wat ŉ konstante vrystellings tempo van 7-VDA toegelaat het en wat vergelykbaar of selfs beter was as die VKM wyfie feromoon in ŉ kommersiële vrysteller. Laboratorium parings inhibisie proewe is met ongepaarde mot pare in plastiek houers uitgevoer. Vrystellers is in die houers gelaai 24 ure voor die motte ingesit is. Albei VKM feromoon en 7-VDA het die vermoë getoon om fekunditeit te verminder, ŉ aanduiding van verminderde paring. Resultate is wisselvalig maar oor die algemeen het die VKM feromoon meer doeltreffend voorgekom. In proewe daarna was ŉ kombinasie van 7-VDA (5-10%) en VKM feromoon (90-95%) die mees doeltreffend en het fekunditeit met 69% verminder. Proewe is met ŉ verbeterde protokol herhaal, wat ontwikkel is om variasie te verminder. Na 48 ure is wyfies van die plastiek houers verwyder en in petribakke gesit om hulle fekunditeit en fertiliteit te meet. Vier replikate is voltooi en het ŉ vergelykbare vermindering in fekunditeit (met 69%) met die verskillende behandelings getoon: VKM feromoon, 7-VDA en 10% 7-VDA in VKM feromoon. Die laaste twee behandelings het egter fertiliteit van die eiers heeltemaal uitgeskakel, waar daar was nog ŉ klein persentasie bevrugte eiers met die VKM feromoon behandeling. Resultate was nietemin nogsteeds wisselvallig. 7-VDA word tans vir veldproewe gesintetiseer. Die doel was om in November 2016 met hierdie proewe te begin, maar probleme is met die sintese ondervind en proewe is dus tot November 2017 vertraag.

2.2.12 PROGRESS REPORT: Evaluating hot air treatments for postharvest FCM control

Project 1060 (2013/4, 2015/6-2017/8) by T G Grout, P R Stephen and K C Stoltz (CRI)

Summary

Although the USDA-APHIS has treatment schedules for vapour heat on citrus and several papers were published in the 1980s and 90s that said it could be done safely, only Thailand is risking this treatment on a commercial basis. Their treatment is at 43°C and 50-65% relative humidity (RH) and the fruit is held at 5-10°C afterwards. Previous research showed that the third instar of FCM was more tolerant to heat than younger instars or the egg stage, and that when fruit was placed in 46°C vapour heat for 6 h, 100% mortality of 681

larvae was obtained. At 44°C for 6 h, there were survivors so this temperature and period was used in 2016/7 to compare the susceptibility of third, fourth and fifth instars. Third instars were more susceptible than the other two instars, which were similar in their susceptibility, although mortalities of the latter instars ranged from 37 to 58%. Further work may require a laboratory culture to minimise variation between egg batches. In one trial, FCM fifth instars in media showed similar susceptibility to fifth instars in fruit, so this will be investigated further in order to process higher numbers of larvae per trial.

Opsomming

Alhoewel die USDA-APHIS behandelingskedules vir damphitte-behandelings op sitrus het, en verskeie artikels in die 1980's en 90's gepubliseer is wat bevestig dat die behandelings veilig uitgevoer kan word, is Thailand die enigste land wat dit waag om hierdie behandeling op kommersiële vlak te gebruik. Hulle behandeling word teen 43°C en 50-65% relatiewe humiditeit (RH) uitgevoer, waarna die vrugte teen 5-10°C gestoor word. Vorige navorsing het aangetoon dat die derde instar van valskodlingmot (VKM) meer bestand teen hitte was as jonger instars of die eierfase, en dat wanneer vrugte in 46°C damphitte vir 6 h geplaas word, 100% mortaliteit van 681 larwes verkry is. Daar was oorlewendes teen 44°C vir 6 h, gevolglik is hierdie temperatuur en periode in 2016/7 gebruik ten einde die vatbaarheid van derde, vierde en vyfde instars met mekaar te vergelyk. Derde instars was meer vatbaar as die ander twee instars, wat soortgelyk in hul vatbaarheid was, hoewel mortaliteit van laasgenoemde instars van37 tot 58% gevarieer het. Verdere werk mag 'n laboratorium teling vereis, ten einde variasie tussen verskillende eier groepe te minimaliseer. In een proef het VKM vyfde instars in media soortgelyke vatbaarheid getoon as vyfde instars in vrugte, so dit sal verder ondersoek word ten einde hoër getalle larwes per proef te verwerk.

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

Project 1039 (April 2012 – March 2015): Sean Moore, Wayne Kirkman and Vaughan Hattingh (CRI)

Summary

This project was initiated as a result of the announcement that the European and Mediterranean Plant Protection Organisation (EPPO) was conducting a Pest Risk Assessment (PRA) on FCM. This PRA was completed in September 2013 and FCM was consequently placed on the EU phytosanitary organism alert list. Subsequently, regulations have been announced for export of FCM susceptible fresh produce from Africa and Israel to the EU. However, regulations are yet to become active. Most work conducted in this project between 2012 and 2014 was aimed at demonstrating the efficacy of incomplete cold treatments for use as a step in a systems approach. Subsequently, studies were conducted to determine whether probit 9 efficacy of cold treatments against fourth and fifth instar FCM, the most cold-tolerant larval life stages, could be achieved with improved postharvest cold treatments i.e. warmer temperatures and/or shorter duration. During the research year in question, this was conducted at a temperature of 1°C. Durations from 17-24 days were tested against fourth and fifth instars, establishing that 19 days exposure were required to kill all individuals in a sample. Three replicates were then conducted at 1°C for 19 d with a total of 109 751 fourth and fifth instars. This therefore provided probit 9 efficacy (99.9968% efficacy at the 95% confidence level) of the treatment. Thereafter, work on incomplete cold treatments was resumed. A replicated trial with 4°C for 16 to 26 days was conducted, achieving average mortalities of between 90.56% and 99.83% for these periods of exposure in the first two replicates. However, results from the last replicate differed notably from the other two and necessitated a repeat of the trial. In the second trial, three replicates were again conducted, this time providing mortality ranging from 70.28% to 99.85% for the durations mentioned. Mortality for the shorter durations was thus notably lower than in the previous trial. It was speculated that this may have been a result of using larvae from a laboratory culture with a different regional origin. However, this must still be tested. Finally, two replicates to measure the effect of 7°C for periods ranging from 16 to 26 days, were conducted. Mortality of 4th and 5th instars after 26 days was only 17%.

Opsomming

Hierdie projek is aangepak as gevolg van die aankondiging dat die Europese en Meditereense Plantbeskermings Organisasie (EPPO) besig was met 'n Plaag Risiko Analise (PRA) op VKM. Hierdie PRA is in September 2013 voltooi en VKM is toe op die EU fitosanitêre organisme waarskuwingslys geplaas. Daarna is regulasies vir die uitvoer van VKM vatbare vars produkte van Afrika en Israel EU toe aangekondig. Regulasies moet egter nog in plek kom. Meeste van die werk in hierdie projek wat tussen 2012 en 2014 uitgevoer is het die doeltreffendheid van onvoledige koue behandelings as 'n stap in 'n stelselsbenadering gedemonstreer. Daarna is studies uitgevoer om te bepaal of probit 9 doeltreffendheid van koue behandelings teen vierde en vyfde instar VKM, die mees koue tolerante lewensstadiums, met verbeterde na-oes koue behandelings bereik kon word dws warmer temperature en/of korter blootstelling. Gedurende die betrokke

navorsingsjaar is hierdie teen ŉ temperatuur van 1°C uitgevoer. Tydperke van 17-24 dae is teen vierde en vyfde instars getoets en het bepaal dat 19 dae bloostelling nodig was om alle individue in ŉ monster dood te maak. Drie replikate is toe teen 1°C vir 19 dae uitgevoer met ŉ totaal van 109 751 vierde en vyfde instars. Hierdie het dus probit 9 doeltreffendheid (99.9968% effektiwiteit teen ŉ vertrouevlak van 95%) van die behandeling voorsien. Daarna is werk op onvoledige koue behandelings hervat. ŉ Herhaalde proef met 4°C vir 16 tot 26 dae is uitgevoer met mortaliteit van tussen 90.56% en 99.83% vir hierdie blootstellingstye vir die eerste twee replikate. Resultate van die laaste replikaat het egter heelwat van die ander twee verskil en dit was dus nodig om die proef te herhaal. In die tweede proef is drie replikate weer uitgevoer en het hierdie keer mortaliteit van tussen 70.28% en 99.85% vir die betrokke tydperke veroorsaak. Mortaliteit vir die korter tydperke was oplettend laer as in die vorige proef gewees. Die is gespekuleer dat hierdie moontlik gebeur het omdat larwes gebruik is van ŉ laboratorium kultuur wat van ŉ ander streek gekom het. Hierdie teorie moet egter nog getoets word. Laastens is twee replikate uitgevoer om die effek van 7°C vir tydperke van 16 tot 26 dae te toets. Mortaliteit van vierde en vyfde instars na 26 dae is net 17%.

2.2.14 PROGRESS REPORT: Entomopathogenic fungi for control of soil-borne life stages of FCM

Project 1024 (Apr 2016 – Mar 2017) by S.D. Moore (CRI), Martin P Hill (RU), J.F. Dames (RU) and C.A. Coombes (RU)

Summary

Since 2010, research focussing on the use of entomopathogenic fungi as a biological control agent against the soil-dwelling life stages of *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae) (fifth instars, pre-pupae and pupae) has been ongoing. Field trials conducted using two promising isolates, *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae) isolate G Ar 17 B3 and *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) isolate FCM Ar 23 B3, between 2013 and 2016, have been encouraging with a 34% – 82% and 28% – 63% reduction in *T. leucotreta* infestation, respectively, after fungi were applied to the soil surface at acceptable rates. These isolates were also capable of persisting for at least five months in the field and were found to be compatible with most commonly used fungicides in citrus orchards. Further research was identified to broaden our knowledge on the impact of these fungi against this pest in the field. This includes sublethal effects, the efficacy of these isolates have against *T. leucotreta* eggs and neonates in comparison to the commercially available mycopesticide, Broadband and the influence of commonly applied agrichemicals such as fertilisers and insecticides on growth parameters of both fungal isolates. This research was delayed during April 2016 to March 2017 due to an unexpected reduction in *B. bassiana* virulence recorded during soil bioassays against *T. leucotreta* fifth instars, but is now underway. This problem was suspected to be due to an old stock of dried aerial conidia and thus bioassays utilising a new, recently produced stock of dried aerial conidia were initiated. Preliminary results suggest slightly higher levels of mycosis against all five different *T. leucotreta* cultures (Addo, Citrusdal, Marble Hall, Nelspruit and Old Colony) than the old stock. However, mycosis is still much lower (< 50%) than what it should be (> 80%). *Metarhizium anisopliae* isolate FCM Ar 23 B3 continues to achieve above 80% mycosis against all *T. leucotreta* cultures. A means to genetically distinguish these two isolates from other indigenous isolates in field samples is also under investigation.

Opsomming

Sedert 2010, navorsing gefokus op die gebruik van entomopatogeniese swamme as biologiese beheeragente teen die grondgedraagde lewensstadiums van *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae) (vyfde instars, voorpapies en papies) is voortgesit. Veldproewe uitgevoer met twee belowende isolate, *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae) isolaat G Ar 17 B3 en *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) isolate FCM Ar 23 B3, tussen 2013 en 2016, het bemoedigende resultate gelewer, met 34% - 82% en 28% - 63% vermindering in *T. leucotreta* besmetting, onderskeidelik, na swamme teen geskikte dosisse op die grondoppervlak toegedien is. Hierdie isolate het ook vir ten minstens vyf maande in die veld voortgeduur en is verenigbaar met meeste swamdoders wat algemeen in sitrusboorde gebruik is. Verdere navorsingsbehoeftes is geïdentifiseer om ons kennis oor die impak van hierdie swamme teen VKM in die veld te verbeter. Dit sluit in subletale effekte, doeltreffendheid teen *T. leucotreta* eiers en pasuitgebroeide larwes in vergelyking met die kommersieel beskikbare produk, Broadband, en die invloed van algemene landbouchemikalieë, soos kunsmis en insekdoders, op die groei van beide swam isolate. Hierdie navorsing is tussen April 2016 en Maart 2017 vertraag as gevolg van 'n onverwagte verlaging in *B. bassiana* se virulensie wat tydens grond biotoetse teen *T. leucotreta* vyfde instars opgelet is, maar word tans voortgesit. Hierdie probleem is vermoedelik as gevolg van ou voorraad van gedroogde lugkonidia en dus is biotoetse met nuwe voorraad van gedroogde lugkonidia begin. Voorlopige resultate wys effens hoër vlakke van mikose as die ou voorraad teen al vyf van die verskillende *T. leucotreta* kulture (Addo, Citrusdal, Marble Hall, Nelspruit en Ou Kolonie). Mikose is egter steeds heelwat laer (< 50%) as wat dit behoort te wees (> 80%). *Metarhizium anisopliae* isolaat FCM Ar 23 B3 veroorsaak nogsteed meer as 80% mikose teen alle *T. leucotreta*

kulture. 'n Manier om hierdie twee isolate van ander inheemse isolate in die veld geneties te kan onderskei word ook ondersoek.

2.2.15 PROGRESS REPORT: Movement of false codling moth (FCM) and fruit flies (FF) in multi-crop (citrus, stone fruit, grape, pomegranate) systems

Project 1081 (2013/14 – 2017/8) by Martin Gilbert and Claire Love (CRI)

Summary

Fruit fly and false codling moth are polyphagous pests attacking many different cultivated fruit types as well as wild host plants. They are of special significance in the Western Cape because in this region many fruit crop types may be grown close together in relatively small blocks. The aim of this project is to investigate the occurrence of these pests on farms where different fruit types are cultivated in close proximity to one another. In areas west of the Cedarberg, where most citrus in the Western Cape is grown, fruit production tends to occur in relatively distinct “islands” e.g. Riebeek Kasteel, Porterville, Stellenbosch, Citrusdal, Piketberg, etc., with the intervening agricultural land being utilized for cereals. The environment of cereal lands is probably unsuitable for pests such as FF and FCM as there is little or no natural vegetation remaining in these areas. Pest presence may vary greatly between the different “pockets” of fruit production. Weekly monitoring of FCM, fruit flies, and carob moth, continued during the 2016/17 season on two farms in the Western Cape. FCM and FF were monitored on nectarines, peaches, plums, table grapes, wine grapes and citrus at the first farm situated in Riebeek Kasteel. All three pest species were monitored in pomegranates and citrus at the second farm in Porterville, as carob moth is a known pest on pomegranates and can be confused with FCM. In 2016, the highest peaks were noted in wine grapes with an average of 8.5 FCM / trap / week in April, and nectarines with an average of 7 FCM /trap /week in late May. All crops showed increases in FCM trap catches around these times, including the citrus. This is repeating the pattern of trapping from previous years which indicates that peaks of FCM adult activity as measured by pheromone traps are not associated with fruit maturity. Nevertheless, in terms of numbers caught, the results for the 2016/17 fruit-growing season are very different from those of the previous years studied. Late 2016 / early 2017 showed very low levels of FCM presence in all blocks, with trap catches increasing the most in the citrus in late April. In general, in the Western Cape, much greater attention has been focussed upon FCM incidence and control during 2016 / 17. Fruit inspections done on the tree for each fruit type for a number of weeks prior to harvest showed no evidence of FCM infestation. The collection and monitoring of fruit collected after harvest and kept in the laboratory for approximately four weeks also produced no FCM adults or larvae. Fallen fruit from under five marked data trees, which was collected in each of the two citrus blocks, also indicated a very low level of fruit infestation.
Regarding fruit fly, numbers increased as fruits neared maturity and were harvested. However, fruit fly peaks were also recorded long after fruit harvest. The highest peak for fruit flies in citrus occurred in June 2016 with a mean of 102 flies/trap/week. Fruit fly traps placed in the nectarines recorded the highest average count/trap/week in 2016 with a mean of 545 flies. In 2017, table grapes recorded the highest numbers of fruit flies. Mediterranean fruit fly was the most common species recorded. Cape fly (*Ceratitis quilicii*) was present in very low numbers and only at cooler times of the year. The collection and analysis of fruit did not yield any fruit flies. At Porterville, an area where both citrus and pomegranates are grown, FCM counts remained below 4 per trap throughout 2016, with moth numbers generally being higher in the pomegranates than the citrus. Numbers increased in March and April 2017 with greater numbers in the pomegranates than in the citrus. The same trend was found for carob moth in both crops in 2016 being nearly all caught in the pomegranates. However, from January 2017 carob moth numbers increased substantially in the pomegranates with a peak mean of 13.5 moths / trap / week being recorded in this month and numbers remaining high throughout April and May. While peaks occurred in the citrus at this time, they were much lower than in the pomegranates. Nevertheless, citrus grown close to pomegranates could well be more vulnerable to false codling moth and carob moth attack than citrus grown without this crop in close proximity. This would have implications for different production areas in the Western Cape. Mediterranean fruit fly numbers caught in pomegranate blocks were almost non-existent. Although *C. capitata* is mentioned in a previously published list of pests recorded on pomegranates, the numbers trapped here indicate that this crop is not a preferred host for fruit flies. On the nearby citrus, a peak of 63 flies / trap / week was recorded in early June. Cape fly (*C. quilicii*) numbers were very low in both citrus and pomegranates. Between June and August 2016, pomegranates remaining on the tree after harvest were sampled and dissected to determine FCM and carob moth infestation. Ten randomly chosen fruit from each of two pomegranate orchards were collected weekly. An average of 9.1% of pomegranates sampled throughout the winter over 11 weeks were infested with FCM. This sampling was continued in 2017, with pre-harvest fruit being collected from February. In this case, five fruit from each tree were randomly sampled in order to limit losses for the grower, as it was difficult to detect infestation via fruit inspection on the tree.

Opsomming

Vrugtevlieg en valskodlingmot (VKM) is plae wat verskillende vrugtesoorte sowel as wilde gasheer plante aanval. Die plae is van spesiale belang in die Wes-Kaap omdat, in hierdie streek, baie verskillende vrugtetipes naby aan mekaar gekweek kan word in relatiewe klein blokke. Die doel van die projek is om hierdie peste te ondersoek op plase waar verskillende vrugsoorte naby aan mekaar voorkom. Wes van die Sedarberg, waar die meerderheid sitrus in die Wes-Kaap gekweek word. Die produksie van vrugte neig na diskreete areas of "eilande", bv Riebeek-Kasteel, Porterville, Stellenbosch, Citrusdal, Piketberg ens., met die areas tussen in wat vir die kweek van graan gebruik word. Die omgewing van graanlande is waarskynlik nie geskik vir vrugtevlieg en VKM omdat daar geen of baie min natuurlike plantesoorte is in die areas oorbly. Plaag voorkoms kan tussen die "eilande" van vrugteproduksie wissel. Weeklikse moniteering van VKM, vrugtevliee, en karobmot, het aangehou deur die 2016/17 seisoen op twee plase in die Wes Kaap. VKM en vrugtevlieë op nektariens, perskes, pruime, tafeldruiwe en sitrus is by Riebeek-Kasteel gemoniteer. By Porterville, was al drie plae op granate en sitrus gemoniteer, omdat karobmot ŉ bekende plaag op granate is en kan met VKM verwar word. In 2016, die hoogste pieke van VKM was in wyndruiwe in April en in nektariens in laat Mei met ŉ gemiddeld van 8.5 en 7 onderskeidelik. Alle gewasse het toenames in VKM vangste getoon om en by die tye, insluitend die sitrus. Die patroon van vorige jare is herhaal waar pieke van VKM aktiwiteit, gemeet deur feromoon lokvalle, nie noodwendig saam met vrug rypheid gaan nie. Nietemin, wat VKM getalle betref, verskil die resultate vir 2016/17 seisoen weelwat met die van vorige jare. Laat 2016 / vroeë 2017 het baie lae vlakke van VKM getoon met getalle wat in sitrus eers in laat April gestuig het. Oor die algemeen in die Wes-Kaap, is baie meer aandag op VKM voorkoms en beheer gedurende 2016/17 gefokus. Vrug inspeksies op die boom vir elke vrugtipe oor ŉ paar weke voor oes het geen teken van VKM infestasie getoon nie. Die versameling en moniteering van vrugte wat na-oes gehou is in die laboratorium het ook geen VKM volwassenes of larwes opgelewer nie. Vrugte wat van die boom afgeval het is van twee sitrus blokke versamel en ŉ baie lae infestasie vlak is aangeteken. Wat vrugtevlieg aanbetref, het getalle geklom toe vrugte begin ryp word het. Nietemin, het pieke van vrugtevlieg vangste lank na oes voorgekom. Die hoogste piek in sitrus van 102 vrugtevlieg / lokval / week het in Junie 2016 gebeur. Lokvalle wat in ŉ nektarien boord uitgesit is het die hoogste gemiddelde telling in 2016 van 545 vrugtevlieë / lokval / week gewys. In 2017 het lokvalle in tafeldruiwe die meeste vlieë gevang. Meditereënse vrugtevlieg was die mees algemeen van die vlieë. Die Kaapse vlieg (*Ceratitis quilicii*) was net in baie lae getalle en net by koeler tye van die jaar teenwoordig. Die versameling en analise van vrugte het geen vrugtevlieë opgelewer nie. By Porterville, ŉ vrugte produksie area waar sitrus en granate naby aan mekaar gekweek word, het VKM getalle onder 4 per lokval reg deur 2016 gebly, met mot getalle in die algemeen hoër in granete as in sitrus. Getalle het wel in Maart en April toegeneem met dieselfde tendens van hoër getalle in granate. Dieselfde tendens is vir karobmot in albei gewasse in 2016 gevind waar amper al die motte in granate gevang is. Nietemin, vanaf Januarie 2017 het karobmot getalle in die granaat boorde noemenswaardig toegeneem met ŉ piek gemiddeld van 13.5 motte / lokval / week in dié maand and getalle wat hoog gebly het gedeurende April en Mei. Terwyl motpieke op sitrus dieselfde tyd gebeur het, was hulle baie laer as in die granate. Nietemin, sitrus wat naby aan granate gekweek word kan wel hoër VKM en karobmot besmetting ondervind as sitrus wat alleen gekweek word. Meditereënse vrugtevlieg getalle gevang in granaat blokke was feitlik naby aan nul. Alhoewel *C. capitata* in a voorafgaande gepubliseerde lys van granaat plae genome is, dui die getalle wat in dié eksperiment gevang is dat granate nie ŉ voorkeur gasheer is nie. Op die nabye geplante sitrus, is ŉ piek van 63 vlieë / lokval / week vroeg in Julie aangeteken. Vanaf Junie tot Augustus 2016, is granate wat na oes op die bome oorgebly het gemonster. Tien vrugte was van elke van twee granaat boorde ewekansig verkies. Hulle was na die laboratorium toe geneem, oop gesny, en die teenwoordegheid van enige VKM of karobmot larwas vasgestel. ŉ Gemiddeld van 9.1% van die vrugte was gedeurende die winter met VKM besmet. Monsterneming is in 2017 vanaf Februarie voortgesit. Net vyf vrugte is per week ewekansig van elk van twee bome versamel om die verlies vir die boer te beperk, omdat dit moeilik is om infestasie op die boom self te bevestig.

2.2.16 **PROGRESS REPORT: Novel approaches to mating disruption of FCM**

Project 1080 (2013/14 – 2017/18) by Martin Gilbert and Claire Love (CRI)

Summary

In 2016/17, Isomate was not available due to increased demand from stone fruit producers. It was therefore decided that Checkmate would be applied for this season. Both Isomate and Checkmate are FCM mating disruption products albeit in different forms / concentrations. Three treatments were used: a 1x treatment (110 ml per hectare per application), a 3x treatment (330 ml Checkmate per hectare) and an untreated control. Three sprays were applied: in mid-January, late February and early April. This was applied in 8000 L of water per hectare with an air-blast sprayer. The grower also incorporated Cryptogran sprays into his control programme for this season on all blocks. For the sterile moths, the trap catches in the 1x and control treatment followed a similar trend. There was no clear indication that the 1x treatment was resulting in fewer moth numbers. The 3x treatment showed far lower sterile moth trap catches throughout the season. This same trend of the 3x treatment trapping the lowest number of moths was also noted for wild FCM catches. Overall, the wild FCM catches were lower for the 1x treatment than the control treatment. This trend of progressive

reduction of moth catches with increasing dosage of Checkmate did not persist into the fruit infestation part of the experiment. Fruit were collected from December to April. Fruit infestation by FCM was assessed through weekly collection of: i) dropped fruit from the ground, and ii) prematurely colouring fruit from the tree, from five marked data trees in each treatment. The mean FCM infestation per tree per week did not show clear differences between treatments and fruit infestation was not noticeably lower in the 3x treatment.

Opsomming

In 2016/17 Isomate was, as gevolg van verhoogte gebruik deur steenvrug kwekers, nie vir dié proef beskikbaar nie. Daarom is dit besluit om Checkmate vir die proef vir dié seisoen te gebruik. Albei Isomate en Checkmate, word vir VKM paringsontwrigting gebruik, alhoewel in verskillende formulasies. Drie behandelings is in die proef ingesluit: Checkmate 1x (110 ml per hektaar per toediening); Checkmate 3x (330 ml per hektaar per toediening) en ŉ kontrole blok (geen Checkmate). Daar was drie toedienings gedeurende die seisoen: middel Januarie, laat Februarie en vroeg in April. Toedienings is met ŉ newelblaser, teen 8000 L water per hektaar gespuit. Die boer het ook Cryptogran bespuitings op die hele blok gespuit. Wat die sterile motte aanbetref, het VKM vangste in delta lokvalle in die kontrole en 1x Checkmate blokke dieselfde tendens gewys. Daar was geen duidelike aanduiding dat die 1x Checkmate toediening laer sterile mot vangste as die kontrol gewys het. Die 3x Checkmate behandeling het wel die steriele mot vangste deur die seisoen onderdruk. Wat wilde VKM aanbetref, het die 3x Checkmate behandeling ook die minste motte gevang. Hierdie tendens van progressiewe vermindering van motvangste met die verhoging in die Checkmate konsentrasie het nie na die vrug infestasie deel van die proef voortgeduur nie. Vrugte was vanaf Januarie tot Mei versamel. VKM besmetting is deur die weeklikse versameling van i) vrugte wat geval het, en ii) vrugte wat voortydig begin opkleur het, vanaf vyf gemerkde bome per behandeling. Die gemiddelde VKM besemetting per boom per week het nie duidelike verskille tussen behandelings gewys nie.

2.3 PROGRAMME: FRUIT FLY

Programme Coordinator: Aruna Manrakhan

2.3.1 Programme summary

In southern Africa, there are currently four fruit fly species: *Ceratitis capitata* (Medfly), *Ceratitis rosa* (Natal fly), *Ceratitis quilicii* (Cape fly) and *Bactrocera dorsalis* (Oriental fruit fly), which are problematic to the citrus industry. While Medfly occurs in all citrus production regions of southern Africa, Natal fly, Cape fly and Oriental fruit fly have a more restricted distribution. Natal fly and Cape fly are absent from the Northern Cape and drier parts of Western Cape. Oriental fruit fly is currently present in the north and north eastern parts of South Africa.

The main objectives of the fruit fly programme for 2016-2017 were: (1) understanding the use of citrus by fruit fly pests, (2) understanding the population ecology of Oriental fruit fly, Natal fly and Cape fly, (3) determining efficacy of fruit fly monitoring tools and (4) optimising pre-harvest control measures for fruit fly pests.

Five fruit fly colonies: Medfly, Natal fly, Cape fly, *Ceratitis cosyra* (marula fly) and Oriental fruit fly were maintained and used for CRI funded projects (2.3.8).

In studies on utilisation of citrus by fruit flies, commercial export grade Eureka lemon was not found to be infested by any fruit fly pest species at harvest (colour plates randing from 4-7; yellow green to green), this despite the presence of adult fruit flies in lemon orchards (2.3.7). Oviposition of Oriental fruit fly did not occur in the laboratory on undamaged lemon, grapefruit, Navel orange, Valencia orange and mandarin. Development of Oriental fruit fly was only recorded when eggs were placed in the pulp of some citrus types. In laboratory studies, the Natal fly and Cape fly were found to have similar developmental peaks in four citrus types tested: lemon, grapefruit, Valencia orange and mandarin.

Population ecology studies were carried out on Oriental fruit fly, Natal fly and Cape fly (2.3.4). The field dispersal capacity of the Oriental fruit fly was determined using mark release recapture methods. Males of the Oriental fruit fly could be recovered in methyl eugenol based trapping system up to approximately 1 km from the release points. A study on the relative abundance and distribution of Natal fly and Cape fly in citrus orchards in the northern regions of South Africa revealed that the Cape fly was the dominant fruit fly species in these regions. The Natal fly was absent in the high altitude areas (above 800 m above sea level).

New monitoring tools were found for some fruit fly pest species of citrus (2.3.5). The EGO lure trapping system was found to be an effective monitoring tool for Medfly and Natal fly. Efficacy of male lure (trimedlure and methyl eugenol) based trapping system was found to be influenced by trap type and lure dispenser type. As such, thresholds of fruit fly catches for Capilure and Questlure baited Sensus traps recommended in citrus would have to be adjusted when other trapping systems are utilised in orchards.

On pre-harvest fruit fly control measures, the focus was on development and application of fruit fly baits. A new paper based fruit fly bait station developed by CRI was found to be promising in control of fruit flies in citrus. The paper based fruit fly bait station at 200 units per ha was as effective as the standard M3 bait station at 300 units per ha. Finally, with increasing use of netting over citrus orchards, the deposition of aerial fruit fly baits in netted and open citrus orchards was investigated. Aerial bait spray coverage was found to be fairly similar in netted and open citrus orchards. Aerial GF-120 bait droplet sizes were found to be within recommended ranges in both netted and open orchards.

Programopsomming

Daar is tans in suidelike Afrika vier vrugtevliegspesies: *Ceratitis capitata* (Mediterreense vrugtevlieg), *Ceratitis rosa* (Natalse vlieg), *Ceratitis quilicii* (Kaapse vlieg) en *Bactrocera dorsalis* (Oosterse vrugtevlieg), wat problematies vir die sitrusbedryf is. Terwyl die Mediterreense vrugtevlieg in alle sitrus produksie-areas van suidelike Afrika voorkom, het die Natalse vlieg, Kaapse vlieg en Oosterse vrugtevlieg ŉ meer beperkte verspreiding. Die Natalse en Kaapse vlieg kom nie in die Noord-Kaap en droër dele van die Wes-Kaap voor nie. Die Oosterse vrugtevlieg kom tans in die noord en noord-oostelike dele van Suid-Afrika voor.

Die hoof doelwitte van die vrugtevliegprojek vir 2016-2017 was: (1) om die benutting van sitrus deur vrugtevliegplae te verstaan, (2) die populasie ekologie van die Oosterse vrugtevlieg, Natalse vlieg en Kaapse vlieg te verstaan, (3) effektiwiteit van vrugtevlieg moniteringshulpmiddels te bepaal en (4) om vóór-oes beheermaatreëls vir vrugtevliegplae te optimaliseer.

Vyf vrugtevliegkolonies: Mediterreense vrugtevlieg, Natalse vlieg, Kaapse vlieg, *Ceratitis cosyra* (Marula vlieg) en Oosterse vrugtevlieg is in stand gehou en vir CRI befondsde projekte gebruik (2.3.8).

In studies om die benutting van sitrus deur vrugtevlieë te ondersoek, is gevind dat kommersiële uitvoergraad Eureka suurlemoene nie deur enige vrugtevliegplaagspesies tydens oes geïnfesteer was nie (kleurplate wat van 4-7 varieer; geelgroen tot groen), dit ten spyte van die teenwoordigheid van volwasse vrugtevlieë in suurlemoenboorde (2.3.7). Oviposisie van die Oosterse vrugtevlieg het nie in die laboratorium op onbeskadigde suurlemoene, pomelo's, navel lemoene, Valencia lemoene en mandaryne voorgekom nie. Ontwikkeling van die Oosterse vrugtevlieg is slegs aangeteken wanneer eiers in die pulp van sommige sitrustipes geplaas is. In laboratoriumstudies het die Natalse en Kaapse vlieg soortgelyke ontwikkelingspieke in vier sitrustipes wat getoets is, getoon: suurlemoene, pomelo's, Valencia lemoene en mandaryne.

Populasie ekologie studies is op die Oosterse vrugtevlieg, Natalse vlieg en Kaapse vlieg uitgevoer (2.3.4). Die verspreidingsvermoë van die Oosterse vrugtevlieg in die veld is bepaal deur van merk-vrylaat-hervang metodes gebruik te maak. Mannetjies van die Oosterse vrugtevlieg kon wéér gevang word in ŉ metiel eugenol-gebaseerde lokvalsisteem tot ongeveer 1 km vanaf die vrylatingspunte. ŉ Studie oor die relatiewe volopheid en verspreiding van Natalse en Kaapse vlieg in sitrusboorde in the noordelike streke van Suid-Afrika, het aangedui dat die Kaapse vlieg die dominante vrugtevliegspesie in hierdie streke was. Die Natalse vlieg was in die hoër liggende areas (bó 800 m bo seevlak) afwesig.

Nuwe moniteringshulpmiddels is vir sommige vrugtevliegplaagspesies van sitrus gevind (2.3.5). Die EGO lokaasmiddel lokvalsisteem was effektief as moniteringshulpmiddel vir Mediterreense vrugtevlieg en Natalse vlieg. Effektiwiteit van manlike lokaasmiddels (trimedlure en metiel eugenol-gebaseerde lokvalsisteme) is deur lokvaltipe en lokaasmiddel houertipe geaffekteer. Gevolglik moet drempelwaardes van vrugtevliegvangste vir Capilure en Questlure lokaasmiddel Sensus lokvalle wat in die sitruswêreld aanbeveel word, aangepas word wanneer ander lokvalsisteme in boorde gebruik word.

Op vóór-oes vrugtevlieg beheermaatreëls, was die fokus op die ontwikkeling en toedien van vrugtevlieg lokaasmiddels. ŉ Nuwe papier-gebaseerde vrugtevlieg lokaasstasie, deur CRI ontwikkel, het belofte getoon in die beheer van vrugtevlieë in sitrus. Die papier-gebaseerde vrugtevlieg lokaasstasie teen 200 eenhede per ha was so effektief as die standaard M3 lokaasstasie teen 300 eenhede per ha. Ten slotte, met die toenemende gebruik van net oor sitrusboorde, is die neerlegging van luggedraagde vrugtevlieg lokaasmiddels in net-bedekte en oop sitrusboorde ondersoek. Luggedraagde lokaas spuitbedekking was min of meer soortgelyk in net-bedekte en oop sitrusboorde. Luggedraagde GF-120 lokaasmiddel druppelgroottes was binne die aanbevole reekse in beide net-bedekte en oop boorde.

2.3.2 FINAL REPORT: Evaluation of male annihilation treatments for control of *Bactrocera dorsalis*

Project 1093 (February 2014 – March 2016) by Aruna Manrakhan, John-Henry Daneel, Rooikie Beck, Glorious Shongwe and Tertia Grove (ARC)

Summary

The male annihilation technique (MAT) is recommended for control of the Oriental fruit fly, *Bactrocera dorsalis*. In MAT, *B. dorsalis* is targeted through deployment of stations or substrates containing a mixture of the male attractant, methyl eugenol (ME), and an insecticide. The aim of MAT is to realise high levels of male kill, thereby reducing the number of matings and the fruit fly population level. In South Africa, a number of MAT products are commercially available for control of the Oriental fruit fly. In 2014 and 2015, trials to determine the performance of these different MAT products for *B. dorsalis* control were conducted in Star Ruby grapefruit orchards in Constantia, Limpopo Province. Three types of fibre board blocks containing ME and malathion: Invader-b-lok, Dorsalure block and B.I. Toolkit, were compared with drop applications of SPLAT Spinosad ME (ME and spinosad) and Last Call B.I. (ME and permethrin). Additionally, two application rates of Invader-b-lok were evaluated: 4 blocks per ha versus 12 blocks per ha. All MAT treatments were evaluated in combination with a standard fruit fly protein bait- M3 fruit fly bait station targeting mainly females. As a control (no MAT), a treatment with only M3 fruit fly bait station was included in the field studies. In the field trials, efficacy of all treatments were evaluated using ME baited traps and traps baited with food-based attractants. An assessment of fruit was also carried out at harvest to determine fruit fly infestation.

In a separate study under this project, a new method of control using ME was explored. The efficacy of the insect growth regulator, lufenuron, in combination with ME in inducing sterility of *B. dorsalis* males was determined in laboratory studies at CRI. Ten-day old virgin *B. dorsalis* males were exposed to technical grade lufenuron (99.9% purity) mixed with ME at 6 concentrations of lufenuron: 0.0 (control), 5.0, 10.0, 30.0, 60.0 and 120.0 mg, per g of ME. Males were exposed to the different treatments for 2 hours. One day after exposure to the lufenuron and ME mixture, treated males were paired with virgin females of the same age. Flies were then provided with a mixture of sugar and yeast hydrolysate and water. An oviposition device was placed in each cage daily for egg laying. Daily fly mortality, fecundity and fertility were determined for the different treatments for a period of 2 weeks.

In field trials on the efficacy of MAT treatments, numbers of *B. dorsalis* males were generally lower in blocks treated with MAT and M3 fruit fly bait stations compared to blocks treated with only M3 fruit fly bait stations. In the first year, there were no significant differences in catches of *B. dorsalis* males between the different MAT treatments. In the second year, Static spinosad ME performed poorly whilst there were no differences in catches of *B. dorsalis* males between the other MAT treatments. A higher reduction in numbers of *B. dorsalis* males was observed in blocks treated with Invader-b-lok at 12 units per ha than those treated with Invader-b-lok at 4 units per ha. No fruit fly infestation was found on Star Ruby grapefruit on trees at harvest. However, in the second year, *B. dorsalis* flies were reared from ground collected Star Ruby grapefruit in blocks treated with only M3 bait stations and in blocks treated with Static Spinosad ME & M3 bait stations where average *B. dorsalis* male catches were above 5 flies per ME trap per week. The conclusion from these field studies is that MAT is an essential component in the control of *B. dorsalis* and the fibre board blocks, such as Invader-b-lok, would be more effective when deployed at 12 units per ha than at 4 units per ha.

In the laboratory studies on the use of lufenuron as a chemosterilant for *B. dorsalis*, partial sterility was observed when *B. dorsalis* males were exposed to methyl eugenol and the highest concentration of lufenuron (120 mg). Since male sterility was only partial at the highest concentration of lufenuron tested, no further studies on this chemosterilisation technique were pursued.

Opsomming

Die mannetjie-uitwissingstegniek (MAT) word aanbeveel vir die beheer van die Oosterse vrugtevlieg, *Bactrocera dorsalis*. *Bactrocera dorsalis* word in MAT geteiken deur ontplooiing van stasies of substrate bevattende ŉ mengsel van die mannetjie lokmiddel, metiel-eugenol (ME), en ŉ insekdoder. Die doel van MAT is om hoë getalle van die mannetjies dood te maak en daardeur die aantal parings en vrugtevlieg populasievlak te verlaag. In Suid-Afrika is ŉ aantal MAT produkte kommersieel beskikbaar vir die beheer van die Oosterse vrugtevlieg. In 2014 en 2015 is proewe in Star Ruby pomelo boorde in Constantia, Limpopo Provinsie, uitgevoer om die werking van hierdie verskillende MAT produkte vir *B. dorsalis* beheer te bepaal. Drie tipes veselbordblokke, bevattende ME en malathion: Invader-b-lok, Dorsalure blok en B.I. Toolkit, is met druppeltoedienings van SPLAT Spinosad ME (ME en spinosad) en Last Call B.I. (ME en permethrin) vergelyk. Twee toedieningstempo's van Invader-b-lok is addisioneel geëvalueer: 4 blokke per ha teenoor 12 blokke per ha. Alle MAT behandelings is in kombinasie met ŉ standaard vrugtevlieg proteïen lokaas geëvalueer (M3 vrugtevlieg lokaasstasie) wat hoofsaaklik wyfies teiken. As ŉ kontrole (geen MAT), is ŉ behandeling met slegs M3 vrugtevlieg lokaasstasies in die veldstudies ingesluit. In hierdie veldproewe is die effektiwiteit van alle behandelings geëvalueer deur gebruik te maak van ME lokvalle en valle wat met voedsel-gebaseerde lokmiddels gelaai is. Vrugtevlieg besmetting van vrugte is ook by oestyd uitgevoer.

In ʼn afsonderlike studie onder hierdie projek, is ʼn nuwe metode van beheer deur die gebruik van ME ondersoek. Die effektiwiteit van die insek groeireguleerder, lufenuron, in kombinasie met ME in die indusering van steriliteit van *B. dorsalis* mannetjies is in laboratoriumstudies by CRI ondersoek. Tien-dae-oue ongepaarde *B. dorsalis* mannetjies is blootgestel aan tegniese graad lufenuron (99.9% suiwerheid) gemeng met ME teen 6 konsentrasies van lufenuron: 0.0 (kontrole), 5.0, 10.0, 30.0, 60.0 en 120.0 mg, per g van ME. Mannetjies is vir 2 ure aan die verskillende behandelings blootgestel. Een dag ná blootstelling aan die lufenuron en ME mengsel, is behandelde mannetjies met ongepaarde wyfies van dieselfde ouderdom gepaar. ʼn Mengsel van suiker en gishidrolisaat en water is daarna aan die vlieë verskaf. ʼn Eierleggingsapparaat is daagliks in elke hok vir eierlegging geplaas. Vliegmortaliteit en vrugbaarheid is daagliks vir die verskillende behandelings vir ʼn tydperk van 2 weke bepaal.

In veldproewe rakende die effektiwiteit van MAT behandelings, was getalle van *B. dorsalis* mannetjies oor die algemeen laer in blokke wat met MAT en M3 vrugtevlieg lokaasstasies behandel is in vergelyking met blokke wat slegs met M3 vrugtevlieg lokaasstasies behandel is. In die eerste jaar was daar geen betekenisvolle verskille in vangste van *B. dorsalis* mannetjies tussen die verskillende MAT behandelings nie. In die tweede jaar het Static spinosad ME swak gevaar terwyl daar geen verskille in vangste van *B. dorsalis* mannetjies tussen die ander MAT behandelings was nie. ʼn Groter verlaging in getalle van *B. dorsalis* mannetjies is waargeneem in blokke wat met Invader-b-lok teen 12 eenhede per ha behandel is, in vergelyking met blokke wat met Invader-b-lok teen 4 eenhede per ha behandel is. Geen vrugtevlieg infestasie is op Star Ruby pomelo op bome tydens oes gevind nie. In die tweede jaar is *B. dorsalis* vlieë egter geteel vanaf Star Ruby pomelo wat vanaf die grond versamel is, in blokke wat slegs met M3 lokaasstasies behandel is en in blokke wat met Static Spinosad ME plus M3 lokaasstasies behandel is, waar gemiddelde *B. dorsalis* mannetjie vangste bó 5 vlieë per ME lokval per week was. Die gevolgtrekking vanuit hierdie veldstudies is dat MAT ʼn noodsaaklike komponent in die beheer van *B. dorsalis* is en die veselbordblokke, soos Invader-b-lok, sal meer doeltreffend wees wanneer hulle teen 12 eenhede per ha as teen 4 eenhede per ha ontplooi word.

In die laboratoriumstudies rakende die gebruik van lufenuron as ʼn chemo-steriliseerder vir *B. dorsalis*, is gedeeltelike steriliteit waargeneem wanneer *B. dorsalis* mannetjies aan metiel-eugenol en die hoogste konsentrasie lufenuron (120 mg) blootgestel is. Aangesien mannetjie steriliteit slegs gedeeltelik teen die hoogste konsentrasie van lufenuron wat getoets is, verkry is, is geen verdere studies op hierdie chemo-sterilisasie tegniek gedoen nie.

Introduction

The Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), has now been declared present in the northern and north eastern areas of South Africa (Manrakhan et al., 2015). The Oriental fruit fly can be effectively controlled by a combination of methyl-eugenol based male annihilation technique (MAT) and bait application technique (BAT) (Fay et al., 1997; Grout and Stephen, 2013; Seewooruthun et al., 2000; Vargas et al., 2010). BAT involves application of poisoned protein baits in the form of sprays or bait stations, a technique currently practiced widely for local fruit fly control in commercial fruit production areas in South Africa. In MAT, the fruit fly males are targeted using male lures and killed by an insecticide incorporated in the lure in order to reduce the number of matings and therefore viable offsprings. Methyl eugenol (ME) is the male lure used to target *B. dorsalis* (Cunningham, 1989). A number of male annihilation methods (MAT methods) such as wooden blocks impregnated with methyl eugenol and malathion (e.g Invader-b-Lok, B.i ToolKit), SPLAT technology containing methyl eugenol and spinosad such as STATIC Spinosad ME (Vargas et al., 2008) and gels containing methyl eugenol and permethrin (e.g Last Call B.I) have been registered for *B. dorsalis* control in South Africa. For some registered application methods such as wooden blocks containing ME and malathion, a wide range of densities of these “attract and kill” units are recommended from 4 (Manrakhan et al., 2012) to 16 per ha. A commonly asked question among the grower community is which density of blocks should be used for effective *B. dorsalis* control? Moreover, there is currently an uncertainty on the correct placement of male annihilation blocks. Some *B. dorsalis* affected production areas deploy the blocks along the perimeter of the orchards whilst in other areas the blocks are equally distributed within the orchards. The efficacy of these different MAT methods for control of *B. dorsalis* under South Africa field conditions is yet to be evaluated.

It was also deemed important to investigate on new methods of *B. dorsalis* control using ME. The auto sterilization or chemosterilisation of fruit flies has been previously proposed as a potential field control method (Casana-Giner et al., 1999). Insect growth regulators such as lufenuron have been shown to have sterilising effect in both females and males of *Ceratitis capitata* (Wiedemann) (Casana-Giner et al., 1999). Egg hatch has been found to be highly suppressed when female and male *C. capitata* were administered with lufenuron mixed with adult food in laboratory assays (Casana-Giner et al., 1999). In other fruit fly species such as *Anastrepha striata* Schiner, egg hatch was found to be reduced when untreated females were crossed with males treated with lufenuron (Moya et al., 2010). In Valencia in Spain, field control of *C. capitata* was achieved

using a lufenuron protein bait gel placed in traps at 24 units per ha (Navarro-Llopis et al., 2007). The chemosterilisation treatment was found to be comparable to aerial bait sprays using malathion (Navarro-Llopis et al., 2007). In recent laboratory assays, lufenuron was found to impact negatively on the fertility of *B. dorsalis* (Chang et al., 2012). Since ME is a powerful attractant to males of *B. dorsalis* (Cunningham, 1989; Shelly et al., 2010), an investigation on the effect of exposure of *B. dorsalis* males to a combination of ME and lufenuron was warranted.

In this study, the performance of different MAT methods for control of the Oriental fruit fly was determined. The combination of ME and lufenuron as a chemosterilant bait was also investigated.

Stated objectives

A. To compare efficacy of different male annihilation methods for *B. dorsalis* control in citrus and mango orchards within *B. dorsalis* affected areas in South Africa
B. To determine effect of MAT block densities on efficacy of *B. dorsalis* control
C. To determine effect of MAT block deployment strategy (perimeter versus uniform distribution) on efficacy of *B. dorsalis* control
D. To determine attractiveness of various MAT products at different ages
E. To determine effects of weathering of attractiveness of Invader-b-lok
F. Develop a new method of *B. dorsalis* control using methyl eugenol in combination with lufenuron – an insect growth regulator (chitin synthesis inhibitor)

Different MAT methods and two MAT block densities (as per objectives A & B) were evaluated in the same field trials over 2 years. Additionally, the effect of aging of MAT products on their attractiveness was also determined in field trials. The efficacy of some of the MAT methods evaluated in citrus orchards was also investigated by Tertia Grove (ARC) in mango orchards. Results of the combined citrus and mango trials in the first year were published in the proceedings of the 9th International Symposium on Fruit Flies of Economic Importance and are not detailed out in this final report.

The investigation on MAT block deployment strategy (Objective C) was however not studied since the number of treatments in the field trials over 2 years were already high (7 in total). Additional field trials planned at the beginning of 2016 could not be carried out due to lack of time.

Three further objectives D, E & F were added after the first year of the project. Investigation of lufenuron as a chemosterilant for *B. dorsalis* males (Objective F) was only carried out as a laboratory study.

Materials and methods

<u>A & B. Efficacy of male annihilation methods for *B. dorsalis* suppression in citrus orchards</u>

Study site

In 2014 and 2015, studies were conducted in Star Ruby grapefruit orchards in Van Veijren Boerdery at Constantia (S23º 38’ 45.6” E30º 42’ 30.1”), Limpopo Province, for a period of 14 weeks between February and June.

Treatments
Six male annihilation treatments were compared:
(1) Invader-b-Lok (River BioScience (Pty) Ltd) was placed at 4 units per ha evenly distributed.
(2) Invader-b-Lok (River BioScience (Pty) Ltd) was placed at 12 units per ha evenly distributed.
(3) Dorsalure/malathion blocks (River BioScience (Pty) Ltd) were placed at 12 units per ha evenly distributed.
(4) STATIC Spinosad ME (Dow Agro Sciences Southern Africa (Pty) Ltd) was applied at 300 ml per ha. The product was placed in rolled up paper cylinders which were distributed in the orchard at every 49 m. In each cylinder, 5 ml of Static Spinosad ME were placed.
(5) Last call B.I (Insect Science (Pty) Ltd) was applied at 150 g/ha. Last Call B.I. was applied as drops using an applicator, with each drop containing 0.05g of the product. Ten drops were placed per tree in the orchard.
(6) B.I. Toolkit (Insect Science (Pty) Ltd) was placed at 12 units per ha evenly distributed

All treatments included a bait application treatment- M3 fruit fly bait station. M3 fruit fly bait stations were placed on every second tree in the orchard with a resulting density of 240 stations per ha. A control treatment of only M3 fruit fly bait stations (No MAT) was also included in the trial. There were 2 replicate blocks per treatment in each orchard. Each treatment was applied to a block of about 1 ha of Star Ruby grapefruit orchard. Treatments

remained exposed within treated blocks for a period of 12 weeks. Treatments including the M3 fruit fly bait stations were removed after 12 weeks.

Fruit fly monitoring

Three attractants were used for monitoring *B. dorsalis* adult population levels: Torula Yeast (for monitoring female population), 3-component Biolure (for monitoring female population), methyl eugenol (for monitoring of male population). Torula yeast and 3-component Biolure were contained in Chempac Bucket traps. Torula yeast was prepared as 1 tablet in 300 ml of water which was then poured into the trap. In the Torula Yeast traps the capturing mechanism was by drowning. Methyl eugenol was placed in Lynfield traps. A dichlorvos strip was placed inside the ME baited Lynfield trap and the 3-component Biolure baited Chempac Bucket trap to kill attracted flies. Traps were hung about 1.5 m above ground. In a treatment block, there were 2 traps of all attractants except methyl eugenol. For methyl eugenol, only one trap was placed in a treatment block in order not to add to any control effects. The distance between each trap in a block was approximately 30 m. Adult fruit fly trapping in each block was initiated 1 week before start of treatment and was carried out on a weekly basis until 1 week after end of treatment. Attractants and insecticides were renewed after 6 weeks. With the Torula yeast traps, water was added whenever required in order to maintain the liquid level to the original level when first baited. Catches were identified to species and sex.

Fruit damage assessment

Each year, a fruit damage assessment was carried out at harvest where 500 fruit in each block were selected at random on the trees (10 fruit on each of 50 trees) and visually examined for fruit fly stings. Any suspected damaged fruit were brought to the lab, weighed and reared individually in aerated containers to determine percentage infestation and degree of infestation. In the second year, 9-10 fruit showing fruit fly damage symptoms were collected from the ground from each block. The fruit collected from the ground were weighed and incubated in aerated plastic containers over a layer of fine sand for a period of 8 weeks to determine fruit fly infestation rates.

Statistical analysis

Trapping data for each year were first summarised as *B. dorsalis males* or *B. dorsalis females* per trap per week for a particular trap type and treatment block. For *B. dorsalis* males, catches in ME baited traps were used. For *B. dorsalis* females, catches for Torula yeast and Biolure baited traps were combined. Trapping data for *B. dorsalis* males and females were categorised under 3 periods: before treatment, during treatment and after treatment. Differences in *B. dorsalis* male and females trap catches between treatments were compared using repeated measures ANOVA (Mixed Models, XLSTAT version 2016.02.27913, Addinsoft).

D. Attractiveness of various male annihilation products at different ages

In year 2 (2015) of the above field trial, the attractiveness of Invader-b-lok, Dorsalure block, B.I. Toolkit, STATIC spinosad ME and Last call B.I. was additionally compared at different ages of each product: 1 week, 4 weeks, 8 weeks and 12 weeks after exposure in the field. All MAT products tested were placed on the northern sides of the tree. In order to compare attractiveness of MAT products, a specific product of a specific age was enclosed in a Morocco bucket trap. For Invader-b-lok, Dorsalure block and B.I. Toolkit, this constituted as 1 unit per trap. For STATIC Spinosad ME and last call B.I. which are gel types, 18 g of the gel were contained inside the trap to match the volume or weight of the mixture of methyl eugenol and malathion contained in the Invader-b-lok, Dorsalure block and B.I. Toolkit. STATIC Spinosad ME was placed inside an open paper cylinder. Last call B.I. was placed on 2 paper tags (Tag 8, Buff Vanilla, Croxley). MAT products were enclosed in the Morocco trap for a period of approximately 24 H. After 24 H, MAT products were removed from the traps and left exposed on the trees for further aging. There were 4 replicate traps for each product at each age. Two replicate traps were selected per block of about 1 ha. Flies captured in each trap were collected and taken back to the CRI laboratory in Nelspruit for identification to species and sex.

Weekly catches of *B. dorsalis* males for all MAT products were summarised as flies per trap. Data did not follow a normal distribution (P=0.05, Shapiro-Wilk test). Differences in catches between MAT products were analysed using Kruskal-Wallis test. Differences in ages of each MAT product were also analysed using Kruskal-Wallis test.

E. Effects of weathering on field attraction of Invader-b-lok

Studies on weathering of Invader-b-lok were carried out in two *Citrus sinensis* orchards (cv. Valencia) in Siyalima Boerdery (S25º 40’ 44” E31º 10’ 45”) and Vergenoeg farm (S25º 20’ 39” E31º 54’ 26”) in Mpumalanga between February and May 2016 in order to coincide with peak fruit fly populations in the field.

Invader-b-lok were aged by suspending the blocks on branches of a Natal Mahogany tree in the compounds of Citrus Research International, Nelspruit, South Africa. Blocks were aged for 84 consecutive days. Every week, aged blocks were removed from the line and evaluated in each of the field sites by comparing to fresh

stations of the same type in paired tests. Stations of each age tested were placed in a Lynfield bucket trap. Each trap contained a DDVP strip to kill attracted flies.

In each orchard, there were 3 traps baited with an aged block and 3 traps baited with a fresh block to represent 3 paired replicates of each treatment in each site. Paired replicates were evaluated in different rows. Spacing between traps were kept at 90 m in each orchard.

Traps were exposed for a week in the field and were checked after one week. Flies captured were emptied in vials and brought to the laboratory for identification and counting.

Captures of *B. dorsalis* males were summarised as flies per trap for each test week. Fresh and weathered Invader-b-lok were compared using Student's paired t-test for each test week. All paired differences followed a normal distribution (P>0.05, Shapiro-Wilk test), except for the paired difference on week 11 (fresh block versus 77-day old block). For that particular week, catches were log (x+1) transformed before analysis to normalise the data.

G. Investigating lufenuron-methyl eugenol mixture as a chemosterilant bait for *B. dorsalis* males

Room conditions

Tests were carried out in a room with natural light. The temperature of the room was at 21.97°C ± 0.05°C during the tests.

Insect materials

Ten-day old virgin *B. dorsalis* males fed on sugar, yeast and water were used for all experiments. Approximately fifteen virgin males were used for each treatment tested.

Lufenuron and methyl eugenol mixture

Technical grade lufenuron (99.9% purity) provided by Syngenta was used in the tests. Lufenuron was mixed with liquid methyl eugenol (Farma Tech, U.S.A) at different concentrations: 0, 5, 10, 30, 60 and 120 mg of lufenuron per g of methyl eugenol.

Effect of a lufenuron-methyl eugenol mixture on mortality, fecundity and fertility of B. dorsalis

Exposure of *B. dorsalis* males to treatments were carried out in aerated plastic containers (30 cm x 30 cm x 14 cm). Adult males were exposed to 0.2 g of methyl eugenol or methyl eugenol and lufenuron mixture placed on a filter paper inside a 6.4 cm Petri dish. No water, sugar and yeast were provided in the cages during exposure. Adults were exposed to methyl eugenol and methyl eugenol lufenuron mixture for 2 hours. Thereafter the filter paper containing either methyl eugenol or methyl eugenol and lufenuron mixture was removed from the cage. Water and food (sugar and yeast mixture in the ratio of 3:1) were then added to the cages. A control cage was also included in the tests whereby adult males with no prior exposure to methyl eugenol or methyl eugenol lufenuron mixture were held for two hours with no food and water after which they were provided with water and food in the same way as the males exposed to either methyl eugenol or lufenuron and methyl eugenol mixtures. Twenty-four hours later, treated and untreated males were transferred to a cage containing approximately fifteen virgin untreated 10-11 day old *B. dorsalis* females. All cages were provided with water and a sugar/yeast mixture in the ratio 3:1.

Mortality of males and females and fecundity were assessed daily for 2 weeks following exposure. After 2 weeks of study, the numbers of surviving males and females were recorded to determine the exact number of males and females used in the tests. For fecundity, 1g of Granny Smith apple was placed inside an open plastic cup which was wrapped with a moist black cotton cloth. The plastic cup and black cloth were in turn wrapped with Parafilm. Twenty pin holes were made over the Parafilm layer directly on top of the open end of the plastic cup. Eggs collected daily were counted and placed inside a closed Petri dish wrapped with Parafilm to prevent drying up of eggs. The eggs were kept moist for 4 days, after which the number of unhatched eggs were counted in order to derive % hatching and therefore fertility. There were four replicates of each treatment using 2 batches of flies.

A repeated analysis of variance (ANOVA) was used to test for differences in percentage male mortality, percentage female mortality, fecundity (summarised as eggs per female per day) and fertility (percentage eggs hatched) between six different treatment groups (concentrations: 0, 5, 10, 30, 60 & 120) and a control. The data was acceptably normal distributed.

Results and discussion

Efficacy of male annihilation methods in citrus orchards

Treatments with MAT had lower catches of *B. dorsalis* males than treatments without MAT (only protein bait application) over the two years of study (Table 2.3.2.1). These results strongly support the inclusion of MAT for *B. dorsalis* control in areas affected by the pest in South Africa and concur with results of previous suppression and eradication programmes whereby MAT was used in combination with bait application for control of *B. dorsalis* (Fay et al., 1997; Manrakhan et al., 2011; Seewooruthun et al., 2000).

In 2014, there were no significant differences in *B. dorsalis* male catches between the different MAT treatments and even between the two densities of Invader-b-lok (Table 1). However in 2015, STATIC Spinosad ME had the least impact on catches of *B. dorsalis* males compared to the other MAT treatments. The STATIC Spinosad ME used in the 2015 trial was from the same batch which was provided to us by Dow Agro Sciences Southern Africa the previous year. The date of manufacture of the STATIC Spinosad ME product used was May 2013. Since STATIC Spinosad ME effectively controlled *B. dorsalis* males in 2014, it is likely that the efficacy of the product was compromised in the second year due to long storage on shelf before use (more than a year of storage after manufacture). Although there were no significant differences in efficacy of control between the two densities of Invader-b-lok tested, the percentage reduction of *B. dorsalis* males was higher for blocks treated with Invader-b-lok at 12 units per ha (97% and 86% in 2014 and 2015 respectively) than for those treated with Invader-b-lok at 4 units per ha (94% and 11% in 2014 and 2015 respectively). These results indicate that when using Invader-b-lok, the rate recommended on the label of the product (10-12 units per ha) would be able to suppress numbers of *B. dorsalis* at a faster rate than when using lower Invader-b-lok densities. In 2014, there were no significant differences in catches of *B. dorsalis* females between the different treatments (Table 2.3.2.2). In 2015, *B. dorsalis* female catches were higher in blocks treated with Dorsalure blocks at 12 units per ha (Table 2.3.2.2), although these were not significantly different to other MAT treatments except for the STATIC Spinosad ME.

In the two years of study, no fruit fly damage was recorded on grapefruit at harvest on the trees. In the second year, *B. dorsalis* flies were reared from Star Ruby grapefruit collected from the ground in blocks treated with only M3 bait stations and in blocks treated with STATIC Spinosad ME plus M3 bait stations. The numbers of *B. dorsalis* flies reared per kg of ground collected Star Ruby grapefruit in blocks treated with only M3 bait stations and in blocks treated with STATIC Spinosad ME plus M3 bait stations were 0.35 ±.35 and 0.49 ± 0.49 respectively. In the two treatments with positive fruit fly infestation, *B. dorsalis* male catches were on average above 5 flies per trap per week. The current DAFF established threshold of 3 *B. dorsalis* males per trap per week during fruiting season in ME baited traps would be conservative but is at the same time an attainable goal if MAT treatments, bait application and orchard sanitation are being implemented (see results of *B. dorsalis* male catches during treatment with MAT).

The lack of differences between different MAT products found in this study especially in the first year supports in part the results of field trials where attract and kill efficacy of combinations of ME and toxicants such as malathion, spinosad and permethin for *B. dorsalis* were determined (Vargas et al., 2003). Vargas et al. (2003) found that a combination of ME and malathion was as effective as ME and spinosad for at least 10 weeks when evaluated in traps on cotton wicks. A combination of ME and permethrin was found to be less or as effective as a combination of ME and malathion in the trials conducted in the same study (Vargas et al., 2003). Since all MAT products were found to be equally effective in this study, the choice of products for use in the field would be highly dependent on the cost, availability and registration.

Table 2.3.2.1. Catches of *B. dorsalis* males in ME baited traps in Star Ruby grapefruit orchards under different male annihilation treatments in Van Veijren Boerdery, Constantia, Limpopo Province South Africa in 2014 and 2015. *B. dorsalis* male catches were significantly affected by treatment and time, during the treated period in 2014 and 2015 (During treatment 2014: Treatment: $F_{6,150}$=2.776, P=0.01; Time: $F_{11,150}$=6.255, P<0.0001; During treatment 2015: Treatment: $F_{6,149}$=4.543, P=0.00; Time: $F_{11,149}$=4.77, P<0.0001). There were no significant differences in *B. dorsalis* male catches between blocks allocated for particular treatments before and after application of treatment (Pre-treatment, 2014: $F_{6,13}$=0.87, P=0.56; 2015: $F_{6,13}$=1.17, P=0.42; Post-treatment, 2014: $F_{6,13}$=1.00, P=0.49; 2015: $F_{4,9}$=0.50, P=0.74). Figures followed by the same letter within the same column for each year are not statistically significant from each other (P=0.05).

Year	Treatment	Mean *B. dorsalis* males per methyl eugenol trap per week at different stages of application		
		Pre	During	Post
2014	Invader-b-Lok at 4 units per ha	47.69 ± 33.69 a	3.04 ± 0.96 ab	0. 00 ± 0.00 a
	Invader-b-Lok at 12 units per ha	78.90 ± 28.73 a	2.32 ± 0.50 ab	0.00 ± 0.00 a
	Dorsalure blocks at 12 units per ha	87.31 ± 34.31 a	1.13 ± 0.34 a	0.00 ± 0.00 a
	B.I. Toolkit at 12 unit per ha	108.25 ± 55.75 a	2.31 ± 0.61 ab	0.00 ± 0.00 a
	STATIC Spinosad ME	25.00 ± 11.00 a	1.54 ± 0.42 a	0.00 ± 0.00 a
	Last call B.I	29.17 ± 8.17 a	1.18 ± 0.29 a	0.50 ± 0.50 a
	No MAT	107.48 ± 59.65 a	8.24 ± 1.89 b	0.00 ± 0.00 a
2015	Invader-b-Lok at 4 units per ha	3.50 ± 0.88 a	3.12 ± 0.56 abc	0. 00 ± 0.00 a
	Invader-b-Lok at 12 units per ha	13.13 ± 9.63 a	1.85 ± 0.45 ab	0.58 ± 0.58 a
	Dorsalure blocks at 12 units per ha	9.63 ± 8.75 a	2.03 ± 0.42 ab	na
	B.I. Toolkit at 12 unit per ha	16.19 ± 11.81 a	1.11 ± 0.24 a	0.58 ± 0.58 a
	STATIC Spinosad ME	26.69 ± 8.31 a	6.60 ± 1.27 c	na
	Last call B.I	3.06 ± 1.31 a	2.09 ± 0.39 ab	0.00 ± 0.00 a
	No MAT	10.50 ± 3.50 a	5.61 ± 0.87 bc	0.58 ± 0.58 a

Table 2.3.2.2. Catches of *B. dorsalis* females in food-baited traps in Star Ruby grapefruit orchards under different male annihilation treatments in Van Veijren Boerdery, Constantia, Limpopo Province South Africa in 2014 and 2015. In 2014, number of *B. dorsalis* females were not significantly affected by treatment and time (Treatment: $F_{6,150}$=0.99, P=0.44; Time: $F_{11,150}$=1.78, P=0.06). In 2015, *B. dorsalis* female catches were significantly affected by both treatment and time during the treated period ($F_{6,150}$=2.34, P=0.03; Time: $F_{11,150}$=6.28, P<0.0001). Figures followed by the same letter within the same column for each year are not statistically significant from each other (P=0.05).

Year	Treatment	Mean *B. dorsalis* females per food-baited* trap per week during treatment
2014	Invader-b-Lok at 4 units per ha	0.04 ± 0.03 a
	Invader-b-Lok at 12 units per ha	0.04 ± 0.02 a
	Dorsalure blocks at 12 units per ha	0.01 ± 0.01 a
	B.I Toolkit at 12 unit per ha	0.06 ± 0.03 a
	STATIC Spinosad ME	0.06 ± 0.03 a
	Last call B.I	0.00 ± 0.00 a
	No MAT	0.07 ± 0.03 a
2015	Invader-b-Lok at 4 units per ha	0.12 ± 0.02 ab
	Invader-b-Lok at 12 units per ha	0.17 ± 0.03 ab
	Dorsalure blocks at 12 units per ha	0.25 ± 0.05 b
	B.I. Toolkit at 12 unit per ha	0.11 ± 0.02 ab
	STATIC Spinosad ME	0.05 ± 0.01 a
	Last call B.I	0.09 ± 0.02 ab
	No MAT	0.12 ± 0.02 ab

*****Food baited traps include both 3-component Biolure traps and Torula yeast traps

Effect of age on attractiveness of male annihilation products

Only 11 *B. dorsalis* males were collected out of 80 trap services with each trap being exposed for a period of only 24H. It was interesting to note however that *Bactrocera dorsalis* males were still responding to Invader-b-lok, Dorsalure block, B.I. Toolkit which were aged for 8 weeks under field conditions (Table 3). Since there was no empty trap as a control trap, it cannot be ruled out that the fly catches in traps with 8 weeks old blocks was random.

There were no significant differences in *B. dorsalis* male catches between MAT products when evaluated at the different ages: 1 week, 4 weeks, 8 weeks and 12 weeks (All ages: Kruskal-Wallis test, P>0.05). For up to 12 weeks of evaluation, the age of each MAT product was not significant in influencing catches of *B. dorsalis* males (All MAT products: Kruskal-Wallis test, P>0.05).

Table 2.3.2.3. Attraction responses of wild *B. dorsalis* males to 5 MAT products: Invader-b-lok, Dorsalure block, B.I. Toolkit, STATIC spinosad ME and Last call B.I. at of different ages (1 week, 4 weeks, 8 weeks and 12 weeks).

MAT product	Age of MAT product	Mean catches of wild B. dorsalis males (± SE)
Invader-b-lok	1 week	0.50 ± 0.50
	4 weeks	0.00 ± 0.00
	8 weeks	0.25 ± 0.25
	12 weeks	0.00 ± 0.00
Dorsalure block	1 week	0.00 ± 0.00
	4 weeks	0.00 ± 0.00
	8 weeks	0.25 ± 0.25
	12 weeks	0.25 ± 0.25
B.I. Toolkit	1 week	0.25 ± 0.25
	4 weeks	0.00 ± 0.00
	8 weeks	0.25 ± 0.25
	12 weeks	0.00 ± 0.00
STATIC Spinosad ME	1 week	0.25 ± 0.25
	4 weeks	0.75 ± 0.48
	8 weeks	0.00 ± 0.00
	12 weeks	0.00 ± 0.00
Last Call B. I.	1 week	0.00 ± 0.00
	4 weeks	0.00 ± 0.00
	8 weeks	0.00 ± 0.00
	12 weeks	0.00 ± 0.00

Effect of age on attractiveness of Invader-b-lok

Studies on aging of Invader-b-lok in particular showed that 12 weeks old (84 days) Invader-b-lok were still attractive to *B. dorsalis*, with attraction which was comparable to a fresh Invader b-lok (Fig. 2.3.2.1). Catches of *B. dorsalis* in traps containing weathered Invader-b-lok were not significantly different from catches of *B. dorsalis* in traps containing fresh Invader-b-lok except in week 7 where catches in 49 day old Invader-b-lok baited traps were lower than catches in fresh Invader-b-lok baited traps. In previous studies on *B. dorsalis* in Hawaii, Vargas et al. (2000) also found that fibre board disks impregnated with methyl eugenol and malathion were effective for up to 14 weeks. Our results support a longer replacement period for Invader-b-lok compared to the current 6 weeks replacement period. This will save on costs on control of the pest.

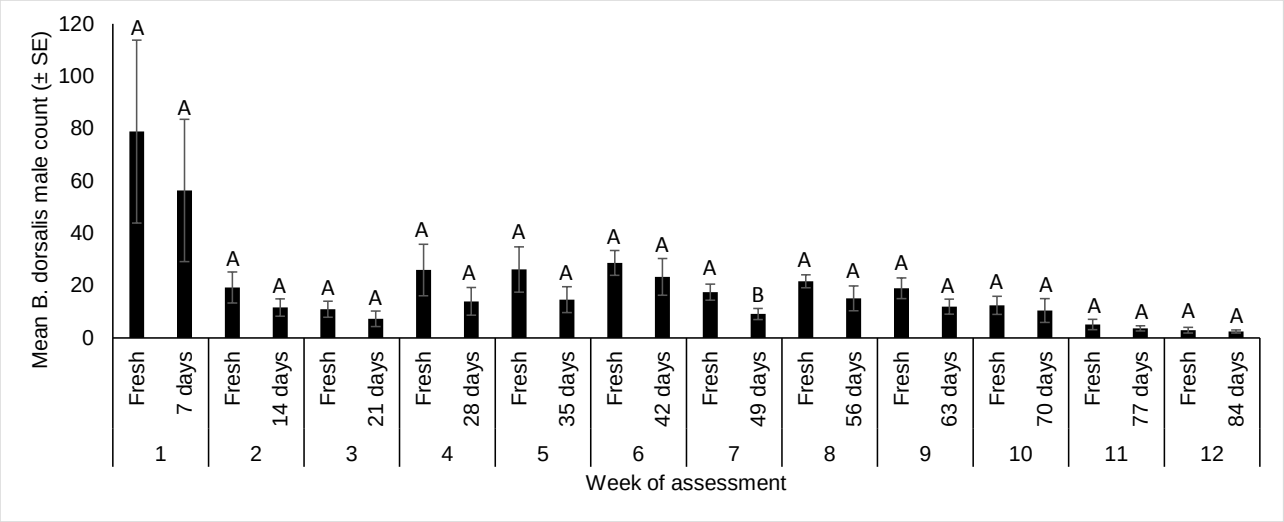

Figure 2.3.2.1. Catches of *B. dorsalis* males in Lynfield traps containing fresh and aged Invader b-lok which were placed in two citrus orchards in Mpumalanga between February and May 2016. Paired values in each week of assessment which are followed by the same letter are not significantly different (P=0.05).

Assessment of lufenuron-methyl eugenol mixture as chemosterilant

None of the lufenuron-methyl eugenol mixtures prevented complete egg hatching over the 14 days of the study. There were however significant effects of treatment on egg fertility ($F_{6,391}$=10.99, P<0.001), with the lowest egg fertility recorded for treatments containing males exposed to lufenuron-methyl eugenol mixture at 120 mg of lufenuron per g of methyl eugenol (Figure 2). This implies that lufenuron in a lufenuron and methyl eugenol mixture conferred partial sterility to *B. dorsalis* males and not full sterility. Fecundity ($F_{6,391}$=3.41, P=0.0029) was also influenced by treatment with lower fecundity recorded for treatments containing males exposed to methyl eugenol only and those containing males exposed to lufenuron-methyl eugenol mixture at 120 mg of lufenuron per g of methyl eugenol. Male mortality and female mortality were both not affected by treatment (Male mortality: $F_{6,391}$=2, P=0.05; Female mortality: $F_{6,391}$=0.83, P=0.5437). In previous studies on *C. capitata*, exposure of males to lufenuron at 5000 ppm (24 times lower than the highest concentration in this study) was found to confer full sterility to females for at least 25 days, provided that the treated males mated with the unexposed females within a day after exposure (Casana-Giner et al., 1999). When lufenuron treated *C. capitata* males mated with females 2 days after treatment, duration of sterilization was reduced (Casana-Giner et al., 1999).
Based on the results from this study, there is no prospect for the use of lufenuron as a chemosterilant for *B. dorsalis* males.

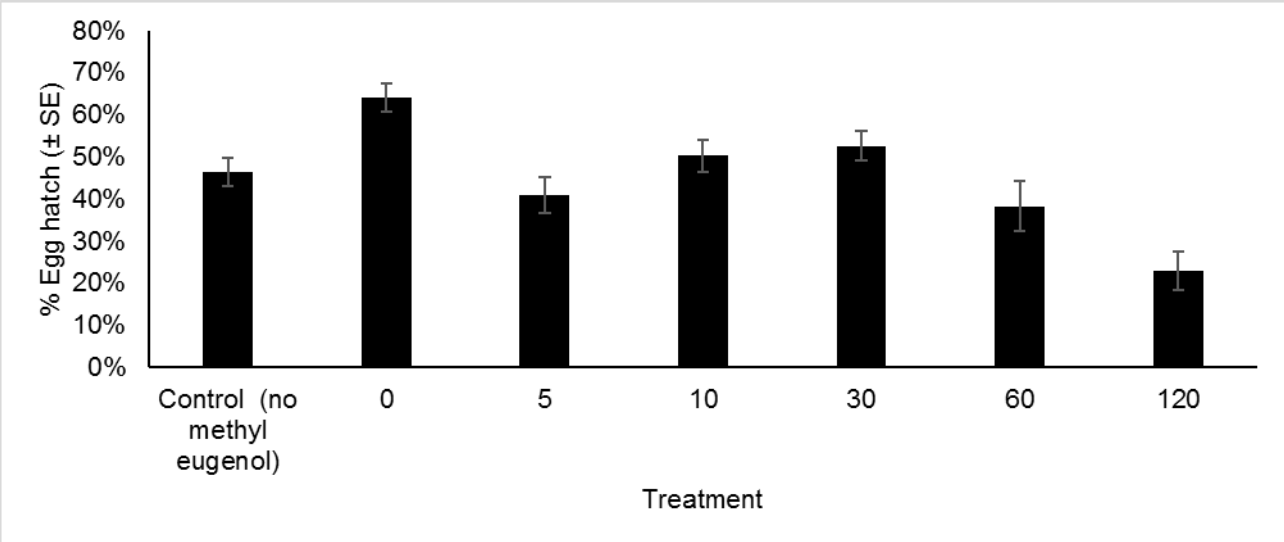

Figure 2.3.2.2. Egg fertility (percentage of eggs hatched over total eggs laid) following mating of 10-11 day old virgin *B. dorsalis* females with 10 day old *B. dorsalis* males exposed to treatments consisting of mixtures of lufenuron and methyl eugenol (figures on x-axis presented as mg of lufenuron per g of methyl eugenol). The control treatment consisted of males which had no prior exposure to neither methyl eugenol nor lufenuron.

Conclusions

In areas where *B. dorsalis* is present, the male annihilation technique targeting *B. dorsalis* should be included in fruit fly control programmes implemented during the fruiting season. This will help mitigate risk of fruit infestation by this pest. Invader-b-lok would be more effective when placed at 12 units per ha than at 4 units per ha. Invader-b-lok would however still be attractive and most likely effective after 12 weeks. The results from this study support a longer replacement time of Invader-b-lok than what is currently on the label of this product (6 weeks). Finally, results in this project have shown no prospect of a new male sterilisation technique for *B. dorsalis* using the insect growth regulator- lufenuron

Future research

The non-response of *B. dorsalis* males to methyl eugenol should be investigated to determine potential development of resistance of *B. dorsalis* to the methyl eugenol based male annihilation technique. Although *B. dorsalis* resistance to methyl eugenol based male annihilation technique has not been reported in areas where the pest is present and the technique is utilised, it may be worthwhile to determine any possibility of resistance.

Technology transfer

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2.3.3 FINAL REPORT: Invasion and expansion of *Bactrocera dorsalis* in South Africa: a genetic analysis

Project 1105 (April 2014 – March 2017) by Minette Karsten (SU), Pia Addison (SU), Aruna Manrakhan (CRI), Caroline Knox (RU), Melissa Llyod (RU), Vaughan Hattingh (CRI) and Jan Hendrik Venter (DAFF)

Summary

Bactrocera dorsalis (Hendel) (Diptera: Tephritidae), the Oriental fruit fly, is a polyphagous agricultural pest of Asian origin that has become invasive in many countries worldwide. *Bactrocera dorsalis* was first detected in South Africa in 2010 after which an eradication effort was launched, which was declared successful. Subsequently *B. dorsalis* was redetected and it is currently restricted to the northern parts of South Africa. Despite the associated economic risk that this fly poses to the fruit industry in South Africa, the population genetics of the invasion process has not yet been explored. Here we make use of previously described microsatellites to investigate the population structure and invasion pathways of *B. dorsalis* with a focus on southern Africa. *Bactrocera dorsalis* individuals were collected from 26 geo-referenced localities worldwide and 10 polymorphic microsatellite markers were used to genotype all individuals. We calculated genetic diversity and investigated the current macrogeographic population structure. Furthermore, in a Bayesian framework using Approximate Bayesian computations (ABC) we reconstructed and tested invasion pathway hypotheses of *B. dorsalis* in Southern Africa. Our results show clear evidence of human influence on the genetic diversity and population differentiation of *B. dorsalis* on the African continent as well as within South Africa. The invasion route shows possible multiple introductions of *B. dorsalis* into Africa from Asia, but it seems plausible that spread on the African continent was by natural dispersal as well as human-mediated dispersal. A lack of population differentiation in South Africa suggests high levels of gene flow between sampled locations and therefore has significant implication for the scale at which pest management practices are employed.

Opsomming

Die Oosterse vrugtevlieg, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), is ʼn polyfage landboupes endemies aan Asië en word tans beskou as ʼn indringer in verskeie lande wêreldwyd. In 2010, is *B. dorsalis* vir die eerste keer in Suid-Afrika onderskep en ʼn uitwissingsprogram is dadelik op die been gebring wat daarin geslaag het om *B. dorsalis* in die area uit te wis. Ten spyte hiervan is *B. dorsalis* weer in lokvalle gevind, word tans beperk tot die noordelike dele van Suid-Afrika. Ten spyte van die feit dat *B. dorsalis* ʼn groot ekonomiese risiko inhou vir die vrugtebedryf in Suid-Afrika, is die bevolkingsgenetika van die indringings proses in Suid-Afrika nog nie ondersoek nie. In hierdie studie maak ons gebruik van voorheen ontwikkelde mikrosatelliet merkers om die bevolkingsstruktuur en indringingspad van *B. dorsalis* te ondersoek, met ʼn fokus op suidelike Afrika. *Bactrocera dorsalis* individue is versamle vanaf 26 lokaliteite wêreldwyd en gegenotipeer vir 10 polimorfiese mikrosatelliet merkers. Ons het die genetiese diversiteit en makrogeografiese bevolkingsstruktuur van die verskillende lokaliteite ondersoek. Deur gebruik te maak van Bayesian raamwerk (Approximate Bayesian computations (ABC)) het ons verskeie hipoteses getoets om die indringingsspad van *B. dorsalis* te rekonstrueer. Ons resultate toon duidelike bewyse van die mens se invloed op die genetiese diversiteit sowel as die bevolkings differensiasie van *B. dorsalis* op die Afrika-kontinent en in Suid-Afrika. Die indringingsroete toon moontlike veelvuldige besettings vanaf Asië na Afrika was, maar dat die verspreiding van *B. dorsalis* op die Afrika kontinent waarskynlik is as gevolg van natuurlike verspereiding sowel as verspreiding met menslike invloed. Daar is ook geen bevolkingsstruktuur in Suid-Afrika nie, wat daarop dui dat daar hoë vlakke van geenvloei is tussen bevolkings en het dus verreikende implikasies vir die skaal waarop pes beheer praktyke toe gepas word.

Introduction

Citrus production in South Africa is influenced by a number of fruit fly pests that not only cause important yield losses but also impact on trade. Increasing trade and travel to and within the African and Indian Ocean region has led to the introduction and spread of a number of exotic insect pests which directly affect citrus in South Africa (see Grout & Moore, 2015). One such exotic pest is the invasive fruit fly of Asian origin, *Bactrocera dorsalis,* previously described as *B. invadens* (Drew *et al.* 2005, Schutze *et al.* 2014). *Bactrocera dorsalis* was first detected on the African continent in Kenya in 2003 (Lux *et al.* 2003) and has since been reported in several African countries (De Meyer *et al.* 2010). Direct damage caused by *B. dorsalis* on some commercial fruit was estimated to range from 20% to 80%.

In 2008, specimens of *B. dorsalis* were reported from Southern Africa for the first time (De Meyer *et al.* 2010). Between 2007 and 2008, 2 isolated single specimens of the pest were found in the northern parts of South Africa (Manrakhan *et al.* 2015). In 2010 and 2011, a number of specimens of *B. dorsalis* were found in separate areas in the northern parts of the country and the pest was declared eradicated from these areas when no further flies were found in the affected areas for a period of at least 12 weeks after eradication actions had stopped. Eradication actions were carried out using a combination of male annihilation technique with methyl eugenol baited blocks and bait application technique (Manrakhan et al. 2011). In 2012, detections of *B. dorsalis* were widespread in the northern areas of South Africa and despite implementation of eradication actions in all areas where the pest was detected, *B. dorsalis* specimens continued to be detected in those areas (Manrakhan *et al.* 2015). *Bactrocera dorsalis* has subsequently been declared present in specified areas in the northern and north-eastern parts of South Africa (Manrakhan *et al.* 2015).

The invasion process of *B. dorsalis* in South Africa has raised a number of questions with regards to the pathways of introduction into South Africa. Questions were also raised on the pathways of spread within South Africa. An understanding of the invasion and expansion patterns in South Africa will provide insights on the dispersal patterns of this new pest from outside of the country and within the country. This project aims to understand the *B. dorsalis* invasion patterns observed in Africa, focusing on Southern Africa. Information gained can be used to develop a targeted fruit fly detection system within the region. In addition, such information may also give an indirect measure of insect dispersal, integrated over many generations, since increased levels of gene flow among populations are associated with improved dispersal ability (Bohonak *et al.* 2002). Such studies may reveal the distance over which a species is able to disperse and establish and, as such, determine the scale at which pest control measures should be implemented (for example Timm *et al.* 2010, Zepeda-Paulo *et al.* 2010, Buckley *et al.* 2012, Silva-Brandão *et al.* 2012, Gaither *et al.* 2013). An understanding of gene flow at various spatial levels is therefore essential, particularly for control measures that are maintained for long periods of time (Dorn *et al.* 1999).

The population genetic structure of *B. dorsalis* has been investigated previously to establish the dispersal patterns of the species in Asia and Pacific Area (Aketarawong *et al.* 2007, Shi *et al.* 2009, Li *et al.* 2011, Wan *et al.* 2011, Shi *et al.* 2012, Isasawin *et al.* 2012). These studies relied on the use of microsatellite analysis (Aketarawong *et al.* 2006, 2007, Aketarawong 2010, Shi *et al.* 2012) as well as sequence analysis of the cytochrome oxidase I gene (Li *et al.* 2011, Shi *et al.* 2012) and additional mitochondrial genes (Wan *et al.* 2011). These studies found that the most likely route of dispersal for the species was from China to the west, and traced the most likely invasion pattern of the species throughout Asia. In addition, since dispersal was investigated over local scales, the results were applicable to the design of an SIT eradication program against the fly (Aketarawong 2010, Isasawin *et al.* 2012). The population genetic structure of *B. dorsalis* has also been previously characterised in Africa, to trace the rapid invasion pattern of the species on the continent, using microsatellite markers (Khamis *et al.* 2008, Khamis *et al.* 2009). However, this study was conducted before the presence of the pest in southern Africa (including northern areas of South Africa) and before the synonymy of *B. invadens* with *B. dorsalis* was established. As such in the population genetic structure studies of *B. dorsalis* in Africa, Khamis *et al.* 2009 only considered one site outside of Africa where *B. invadens* was considered present (Sri Lanka).

Objectives

1. To determine the population genetic structure of B*. dorsalis* in Africa, focusing on southern Africa and South Africa.
2. To determine the patterns of spread of *B. dorsalis* within South Africa.

Materials and Methods

Sampling and microsatellite genotyping

Bactrocera dorsalis individuals (N= 351) were collected from 11 African countries and two Asian countries with sampling focused on sub-Saharan Africa, specifically South Africa (Table 2.3.3.1). Flies were predominantly

collected using Methyl eugenol traps, but also reared from infested fruit. In the rest of this study we will refer to individuals from the same sampling location as a population. A DNeasy® tissue kit (Qiagen Inc.) was used to extract DNA from all of the collected individuals. Individuals were subsequently genotyped at ten microsatellite markers following Khamis *et al.* (2008, 2009). To check for inconsistencies and errors in the runs a positive and negative control was included in each plate. All genotyping was done on an ABI 3130 Automated Sequencer (Applied Biosystems, Foster City, California, USA).

Microsatellite analysis

GENEMAPPER v3.7 (Applied Biosystems, Foster City, California) was used to score alleles and all markers were tested for deviations from Hardy-Weinberg equilibrium (HWE) and Linkage disequilibrium using 10 000 in GENEPOP v4.01 (Raymond & Rousset 1995; Rousset 2008). Basic statistics for all populations was calculated and included: expected heterozygosity (H_E), observed heterozygosity (H_O), number of alleles (N_A), number of private alleles (N_P), as well as the inbreeding coefficient (F_{IS}) (GENETIX v4.05.2, Belkhir *et al.* 2004; GenAlEx v6.5, Peakall & Smouse 2006, 2012). We also calculated the frequency of null alleles (A_n, FREENA v1.0, Chapuis & Estoup 2007) within our dataset.

The degree of differentiation between populations was investigated using a number of different techniques. First we calculated the pairwise F_{ST} between genetic clusters identified in the STRUCTURE analysis (*see Results and Discussion* section) as well as overall F_{ST} in FREENA v1.0 running 10 000 replications and compared including (INA) and excluding (ENA) null alleles (Chapuis & Estoup 2007). Moreover, we assessed the relationships between populations using a Principal Coordinate Analysis (PCoA) based on allele frequencies (GenAlEx v6.5, Peakall & Smouse 2006, 2012).

To further assess the population structure, STRUCTURE v2.3.4 (Pritchard *et al.* 2000; Falush *et al.* 2003) was used to assign individuals to populations without including any *a priori* information. We ran both the admixture and no-admixture models with correlated allele frequencies (frequencies can be similar due to continued migration or shared ancestry). We ran 1 000 000 MCMC permutations of which 100 000 was discarded as part of the burn-in for each possible cluster (*K*) between one and 26 performing 10 independent runs. We determined the most likely value for *K* by implementing two strategies. The log-probabilities of the different clusters were inspected for high values with low variance (Pritchard *et al.* 2000) in combination with the second order rate of change Ln P(X/Y) (according to Evanno *et al.* 2005) utilizing in the online resource STRUCTURE HARVESTER (Earl & Von Holdt 2012). After inspection, two additional analyses were performed investigating the hierarchical structuring within each cluster. We used the same parameters as for the first run, except *K* was bound by the number of populations within each cluster. Thereafter the STRUCTURE runs for optimal *K* value was averaged in CLUMPP v1.2.2 (Jakobsson & Rosenberg 2007) and visualized in DISTRUCT v1.1 (Rosenberg 2004).

Using multilocus genotypes we tested 28 scenarios in seven separate tests with regards to the invasion pathway of *B. dorsalis* on the African continent; we were specifically interested in the pathway in southern Africa (Table 2.3.3.3). This was done by making use of Approximate Bayesian Computations in DIYABC v2.03 (Cornuet *et al.* 2014). The hypotheses considered were based on results from this study as well as information from literature. Based on DIYABC results from Aketarawong *et al.* (2014) which showed movement between China and Thailand, we started our invasion pathway with this information, but also included an unsampled population that represents the actual native range. Using results from the Bayesian cluster analyses dates of colonization and the number of generations that *B. dorsalis* can support in a year, we subsequently investigated the spread of *B. dorsalis* across the African continent (Table 2.3.3.3). The following prior distributions of parameters were used to test the different scenarios: 100<Unsampled population<10 000; 10<N1,N2,N3,N4,N5,N6,N7,N8,N9,N10,N11, N12<1000; 1<*db*<10; 2<*Nib*<500; 10<*t*<500 where *N* is the effective population size, *Nib* is the number of founders in each colonization event, *db* is the duration of the bottleneck event (number of generations) and *t* the timing of an event (generations back in time). Prior distributions of parameters for the microsatellite mutation model represented the Generalized Stepwise Mutation model and we included all summary statistics. For each test we simulated 2 000 000 to 3 000 000 computations and computed a posterior probability and 95% confidence intervals (CI) using a logistic regression approach (Cornuet *et al.* 2014). We used the highest posterior probability to identify the most likely scenario in each test.

Results and discussion

Analyses of the ten microsatellite markers in *B. dorsalis* showed that some locations, although not all (Tanzania, Botswana, Mazowe (Zimbabwe), South Africa: Baltimore, Beitbridge, Deerpark, Komatipoort, Musina, Nelspruit, Weipe, Zeerust) deviated from HWE. In those populations where deviations from HWE was observed moderate levels of inbreeding (F_{IS}) was also observed (Table 2.3.3.1) This deviation could further be explained by the Wahlund effect and/or the presence of null alleles. The frequency of null alleles in all

populations was low to intermediate and ranged between 0.000 and 0.151 (Table 2.3.3.1), which are common in species with large effective population sizes (Chapuis & Estoup 2007). Despite the presence of null alleles there was no difference between F_{ST} values with and without correction for null alleles and we therefore assume that null alleles also do not influence other analyses in this study. No linkage disequilibrium was observed between any of the microsatellite loci included in this study. Population genetic diversity measured as expected heterozygosity (H_E) and the number of alleles (*N*A) indicated moderate levels of genetic diversity and ranged between 0.343 (Deerpark, South Africa) to 0.549 (Inhambane, Mozambique) and 2.4 (Chimoio, Mozambique) to 4.7 (Thailand) respectively (Table 1). Thailand had the highest number of private alleles (N_P) 0.8, with only Fôret des Monts Kouffé (Benin), Kenya, Inhambane Cidade (Mozambique) and Weipe (South Africa) containing private alleles out of the rest of the sampled locations (Table 2.3.3.1). The genetic diversity estimates in this study are similar to those in other *B. dorsalis* studies for African populations (Khamis *et al.* 2009), but lower than those from studies investigating locations in the native range (Aketarawong *et al.* 2014). Lower levels of genetic diversity estimates are not unexpected as during the invasion process populations are often founded with only a small number of founders which results in low levels of genetic diversity.

We assessed genetic differentiation by examining F_{ST} overall and pairwise values between populations. The overall F_{ST} values with (F_{ST}=0.175) and without (F_{ST}=0.186) null allele correction indicated that there were genetic differentiation between the populations sampled. Pairwise F_{ST} values (Table 2) between the different clusters indicated clear differentiation supporting the clusters identified in the STRUCTURE analysis. In the Principal Coordinate Analysis (Fig. 2.3.3.1) the first three axes explained 63.90% of the genetic variation. The first axis (23.45%) separated China from all other populations included, whereas the second axis (22.00%) separated Kenya from all other populations included. The detailed population genetic structure from the Bayesian clustering in STRUCTURE supported the presence of population differentiation in the dataset. The results from Evanno *et al.* (2005) and the log-probabilities indicated that there were two clusters (*K*=2). The first cluster included China, Thailand and Kenya (Cluster 1) and the second included all other sampling locations in Africa (Cluster 2) (Fig. 2.3.3.2 (A)). The two clusters identified in the first STRUCTURE analyses were further investigated by running each cluster separately (Fig. 2.3.3.2 (B), (C)). Sub-structuring in Cluster 1 (Fig. 2.3.3.2 (B)) identified three separate clusters corresponding to China, Thailand and Kenya. Within Cluster 2 (Fig. (C)) two further clusters were identified, however no obvious geographic pattern could be observed. This lack of differentiation across large distances on the African continent and within South Africa indicates high levels of movement either by natural dispersal or aided by the trade of goods (formal or informal) as well as human travel. This pattern of macrogeographic population differentiation (between Africa, China and Thailand) and the lack of similar patterns across regional (across Africa) and local (within South Africa) scales have also been shown in other *Bactrocera* species including *Bactrocera cucurbi*tae (Virgilio *et al.* 2010) and *Bactrocera oleae* (Nardi *et al.* 2005).

To further investigate the invasion pathway of *B. dorsalis* we tested 28 scenarios in seven tests using Approximate Bayesian Computations. Based on posterior probabilities the combined route of invasion shows an initial introduction into both China and Thailand from a location unsampled in our study after which flies were introduced into Kenya from Thailand. The second wave of introductions seems to have happened from China into Tanzania from which flies were introduced to Burundi, from Burundi to the DR Congo, from DR Congo to Benin and then from Benin to Senegal. Introductions in southern Africa seem to have moved from Tanzania to Mozambique, from Mozambique to Botswana and from Botswana to Zimbabwe. Introductions into South Africa seems to have come from Botswana, however it is important to note that we were unable to separate Mozambique, Zimbabwe and Botswana in our STRUCTURE analysis and it is probable that any of these countries may have also contributed flies to South Africa. From these analyses we conclude that there were multiple introductions from Asia into Africa. After introduction to the African continent flies spread across the continent likely by natural dispersal as well as assisted by the trade of goods and human travel between countries.

Conclusion

These results suggest that area-wide pest management practises should be implemented on larger scales than is currently being done. Potentially the whole of South Africa can be considered a management unit. This notion however, seems unfeasible and raises multiple practical challenges especially for current control practices. The small-scale control efforts currently employed may therefore fail to contain *B. dorsalis* and therefore eliminating or at least lessening the number of dispersal pathways is critical. This study provides baseline information to be used as part of an integrated pest management strategy.

Future research

This study focused on the current distribution of *B. dorsalis* in South Africa. However, despite current control practices *B. dorsalis* continues to spread. Future work should focus on including these samples in population

genetic studies and re-evaluating the movement patterns within South Africa. This data could be used to create friction maps to further investigate connectivity across the landscape.

Technology transfer

1. The invasion of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) on the African continent: Population structure and invasion routes. Minette Karsten, Pia Addison, Aruna Manrakhan. The Third International Symposium of TEAM (Tephritid Workers of Europe, Africa and the Middle East), Stellenbosch, 2016.

2. The invasion of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae): Population structure and invasion routes for Southern Africa. Minette Karsten, Pia Addison, Aruna Manrakhan. XXV International Congress of Entomology, Orlando, Florida, 2016.

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Table 2.3.3.1. Sampling sites of *Bactrocera dorsalis* as well as sample size (*N*), number of alleles (*N*A), number of private alleles (*N*P), expected (*H*E) and observed (*H*O) heterozygosity (±standard error), the inbreeding coefficient (*F*IS) and the mean null allele frequency (*A*n, Dempster *et al.* 1977; SD in parentheses).

	Country	Location	ID	GPS coordinates		N	N_A	N_P	H_E	H_O	F_{IS}	A_n
				Latitude	Longitude							
Africa	Benin	Fôret des Monts Kouffé	BEN	2.064	8.742	15	3.100	0.100	0.494±0.230	0.449±0.269	0.127	0.054 (0.069)
	Botswana		BOT	25.469	-24.534	12	3.100	0.000	0.493±0.243	0.446±0.279	0.139	0.052 (0.066)
	Burundi	Ngagagara	BUR	29.359	-3.356	23	3.500	0.000	0.489±0.252	0.367±0.261	0.273	0.088 (0.093)
	DR Congo	Kisangani	CDR	25.202	0.520	17	3.200	0.000	0.500±0.261	0.476±0.305	0.079	0.039 (0.071)
	Kenya		KEN	-1.290	36.830	23	3.700	0.200	0.402±0.292	0.345±0.292	0.162	0.048 (0.062)
	Mozambique	Chimoio	MOZ	33.735	-19.000	9	2.400	0.000	0.432±0.234	0.283±0.262	0.41	0.115 (0.131)
		Inhambane Cidade	INH	35.380	-23.870	18	3.300	0.300	0.549±0.183	0.424±0.307	0.261	0.113 (0.118)
	Senegal	Dakar	SEN	-17.379	14.768	25	3.100	0.000	0.504±0.234	0.396±0.301	0.239	0.084 (0.139)
	South Africa	Baltimore		-23.197	28.479	5	2.500	0.000	0.416±0.253	0.517±0.331	-0.056	0.002 (0.005)
		Beitbridge		-22.191	29.872	8	2.800	0.000	0.511±0.240	0.451±0.317	0.191	0.072 (0.100)
		Deerpark		-23.701	30.286	6	2.300	0.000	0.343±0.272	0.330±0.315	0.135	0.032 (0.075)
		Komatipoort		-25.356	31.899	5	2.800	0.000	0.446±0.253	0.548±0.338	-0.108	0.000 (0.000)
		Levubu		-23.117	30.351	7	2.600	0.000	0.442±0.222	0.220±0.144	0.577	0.151 (0.121)
		Louis Trichardt		-23.071	30.050	5	2.600	0.000	0.436±0.223	0.280±0.169	0.451	0.112 (0.098)
		Musina		-22.493	30.000	6	2.500	0.000	0.391±0.211	0.328±0.288	0.258	0.089 (0.117)
		Nelspruit		-25.435	30.820	6	2.900	0.000	0.466±0.229	0.420±0.268	0.194	0.068 (0.093)
		Pontdrift		-22.237	29.037	7	3.100	0.000	0.469±0.283	0.317±0.311	0.402	0.108 (0.146)
		Tshidzini		-22.803	30.748	7	2.600	0.000	0.424±0.220	0.202±0.180	0.578	0.151 (0.110)
		Weipe		-22.181	29.858	8	2.800	0.100	0.456±0.236	0.351±0.293	0.300	0.093 (0.110)
		Zeerust		-25.525	26.074	7	2.800	0.000	0.481±0.242	0.438±0.250	0.170	0.053 (0.066)
	Tanzania		TAN	35.569	-2.803	26	3.000	0.000	0.471±0.246	0.404±0.225	0.162	0.050 (0.065)
	Zanzibar		ZAN	39.205	-6.167	19	3.100	0.000	0.456±0.231	0.388±0.267	0.176	0.065 (0.077)
	Zimbabwe	Mazowe	MAZ	30.976	-17.508	23	3.000	0.000	0.466±0.258	0.418±0.274	0.126	0.045 (0.060)
		Victoria Falls	VIC	25.830	-17.935	22	3.300	0.000	0.513±0.243	0.438±0.243	0.170	0.053 (0.054)
Asia	Thailand		THA	99.869	12.620	20	4.700	0.800	0.505±0.332	0.357±0.377	0.323	0.104 (0.143)
	China		CHI	119.335	26.090	22	2.700	0.000	0.373±0.246	0.270±0.278	0.302	0.075 (0.130)

Table 2.3.3.2. Pairwise *F*ST values calculated in FREENA v1.0 (Chapuis & Estoup 2007) with null allele correction between the four genetic clusters China, Thailand, Kenya, Africa (all other African locations) inferred from Bayesian Clustering in STRUCTURE for *Bactrocera dorsalis.*

	China	Thailand	Kenya	Africa
China	0.000			
Thailand	0.284			
Kenya	0.385	0.230		
Africa	0.215	0.115	0.182	0.000

Table 2.3.3.3. Results from seven tests with 28 different scenarios tested in DIYABC v2.03 (Cornuet *et al*. 2014) with Posterior Probabilities with 95% confidence intervals for all simulated data sets.

Test		Scenario	Posterior probabilities (95% CI)
1	1	Unsampled→China→Thailand	0.169 (0.160,0.177)
	2	Unsampled→Thailand→China	0.162 (0.155,0.169)
	3	Unsampled→Thailand; Unsampled→China	0.130 (0.124,0.136)
	4	Unsampled→China; Unsampled→Thailand	**0.539 (0.529,0.549)**
2	1	Unsampled→China; Unsampled→Thailand; China→Kenya	0.020 (0.010,0.029)
	2	Unsampled→China; Unsampled→Thailand; Thailand→Kenya	**0.531 (0.520,0.543)**
	3	Unsampled→China; Unsampled→Thailand; Unsampled→ Kenya	0.449 (0.438,0.461)
3	1	Zimbabwe→Botswana→Mozambique	0.019 (0.006,0.033)
	2	Zimbabwe→Botswana; Zimbabwe→Mozambique	0.024 (0.010,0.038)
	3	Zimbabwe→Mozambique→Botswana	0.091 (0.074,0.107)
	4	Botswana→Mozambique→Zimbabwe	0.029 (0.016,0.043)
	5	Botswana→Zimbabwe→Mozambique	0.020 (0.007,0.034)
	6	Botswana→Zimbabwe; Botswana→Mozambique	0.206 (0.179,0.234)
	7	Mozambique→Botswana→Zimbabwe	**0.376 (0.341,0.411)**
	8	Mozambique→Zimbabwe→Botswana	0.203 (0.175,0.230)
	9	Mozambique→Zimbabwe; Mozambique→Botswana	0.032 (0.018,0.045)
4	1	Mozambique→Botswana→Zimbabwe; Mozambique→South Africa	0.117 (0.068,0.165)
	2	Mozambique→Botswana→Zimbabwe; Botswana→South Africa	**0.523 (0.478,0.568)**
	3	Mozambique→Botswana→Zimbabwe; Zimbabwe→South Africa	0.361 (0.301,0.421)
5	1	Tanzania→Burundi→Congo→Benin→Senegal	**0.930 (0.895,0.964)**
	2	Tanzania→Senegal→Benin→Congo→Burundi	0.002 (0.000,0.004)
	3	Senegal→Benin→Congo→Burundi→Tanzania	0.069 (0.034,0.103)
6	1	Unsampled→China; Unsampled→Thailand; Thailand→Kenya→ Tanzania→Burundi→Congo→Benin→Senegal	0.00 (0.000,0.456)
	2	Unsampled→China; Unsampled→Thailand; Thailand→Kenya; Thailand→Tanzania→Burundi→Congo→Benin→Senegal	0.012 (0.006,0.017)
	3	Unsampled→China; Unsampled→Thailand; Thailand→Kenya; China→ Tanzania→Burundi→Congo→Benin→Senegal	**0.980 (0.971,0.989)**
7	1	Unsampled→China; Unsampled→Thailand; Thailand→Kenya; China→Tanzania→Burundi→Congo→Benin→Senegal→ Mozambique→Botswana→Zimbabwe; Botswana→South Africa	0.020 (0.000,1.000)
	2	Unsampled→China; Unsampled→Thailand; Thailand→Kenya; China→ Tanzania→Burundi→Congo→Benin→Senegal; Kenya→Mozambique→ Botswana→Zimbabwe; Botswana→South Africa	0.000 (0.000,1.000)
	3	Unsampled→China; Unsampled→Thailand; Thailand→Kenya; China→Tanzania→Burundi→Congo→Benin→Senegal; Tanzania→Mozambique→Botswana→Zimbabwe; Botswana→South Africa	**0.980 (0.951,1.000)**

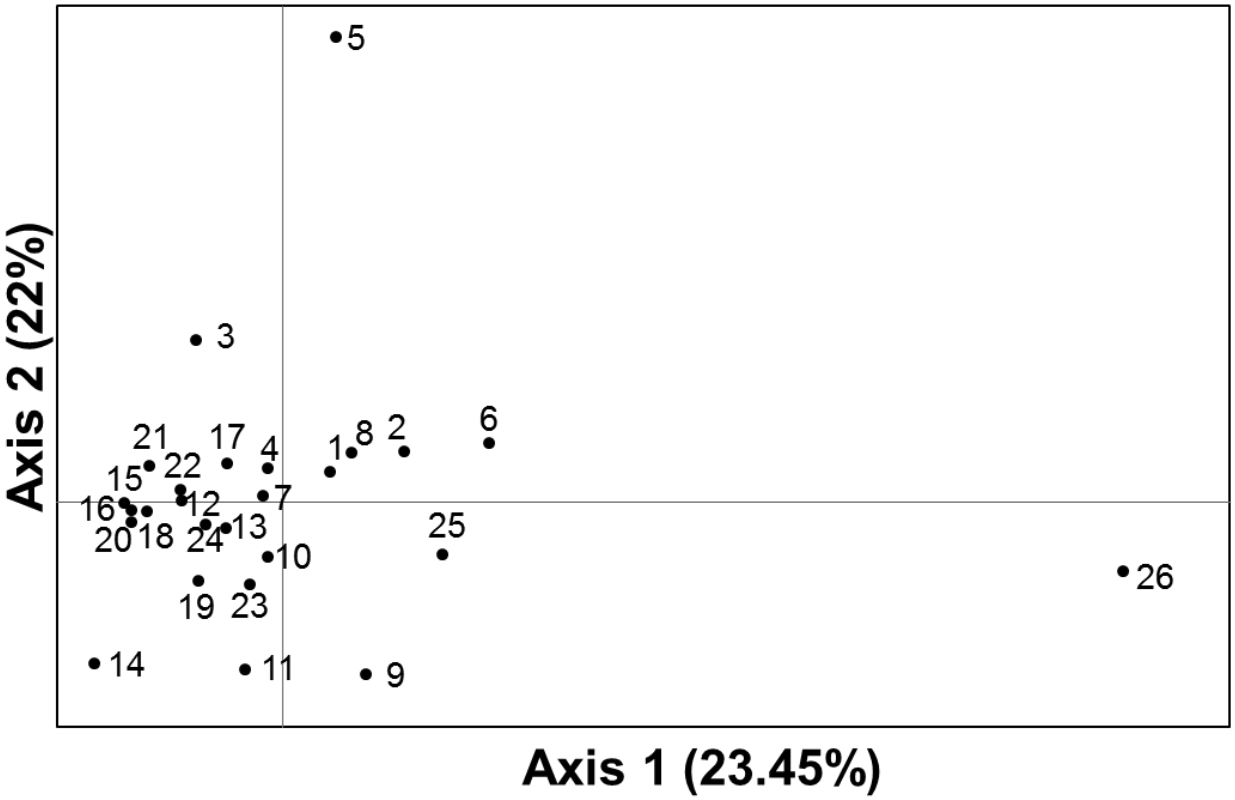


Figure 2.3.3.1. The Principal Coordinate Analysis (PCoA) for 26 *Bactrocera dorsalis* populations. 1-Benin, 2-Botswana, 3-Burundi, 4- Democratic Republic of the Congo, 5- Kenya, 6- Chimoio (Mozambique), 7- Inhambane Cidade (Mozambique), 8- Senegal, 9- Baltimore (South Africa),10- Beitbridge (South Africa), 11- Deerpark (South Africa), 12- Komatipoort (South Africa), 13- Levubu (South Africa), 14- Louis Trichardt (South Africa), 15-Musina (South Africa), 16- Nelspruit (South Africa), 17- Pontdrift (South Africa), 18- Tshidzini (South Africa), 19-Weipe (South Africa), 20- Zeerust (South Africa), 21- Tanzania, 22- Zanzibar, 23- Mozowe (Zimbabwe), 24-Victoria Falls (Zimbabwe), 25 Thailand, 26- China.

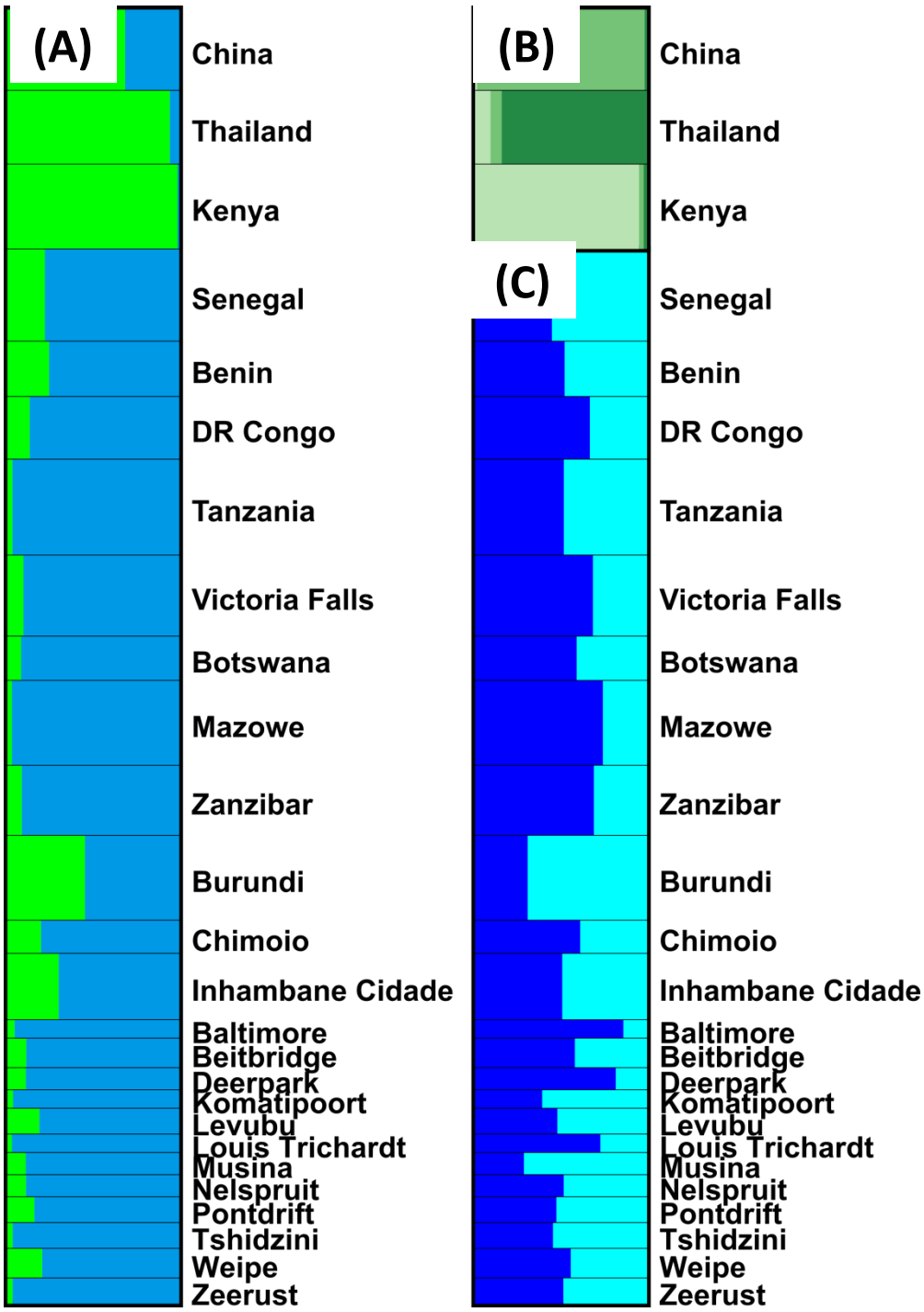


Figure 2.3.3.2. Assignment results from STRUCTURE for (A) all locations included as well as (B) hierarchical population genetic structure for the two clusters identified in (A). One cluster includes China, Thailand and Kenya and the other all other sampled locations.

2.3.4 PROGRESS REPORT: Dispersal capacity of *Bactrocera dorsalis*

Project number 1075 (2013/14 – 2016/17) by C W Weldon (UP), R Anguelov (UP) and A Manrakhan (CRI)

Summary

The key outcome of this project is to establish the dispersal capacity of the Oriental fruit fly, *Bactrocera dorsalis*, with regard to environmental and physiological variables. The PhD student assigned to the project, Mrs Louisa Makumbe, performed mark-release-recapture experiments using sterile *B. dorsalis* to determine the effects of fly maturity, sex and availability of host fruit on dispersal. All releases of sterile *B. dorsalis* have been completed for the dispersal study at three study sites near Louis Trichardt in Limpopo province. Release of female *B.*

dorsalis among host plants reduced their dispersal, whereas younger *B. dorsalis* males travel further than older males. The placement of traps in host trees had no effect on female recaptures, but did affect male recaptures. For both females and males, recaptures increased significantly as average minimum temperature increased. Rainfall positively affected female recaptures in BioLure traps, and negatively affected male receptures in methyl eugenol traps. To determine how temperature may affect population growth, Mrs Makumbe has recorded mating behaviour of *B. dorsalis* over a range of temperatures. She found no male calling at temperatures lower than 20°C or above 32°C. Male calling was greatest at 24 and 28°C. Copulation was not observed during the experiment. Mrs Makumbe is now focused on writing her PhD thesis, which will be ready for examination by September 2017.

Opsomming

Die hoofdoel van die projek is om die verspreidingsvermoë van die Oosterse Vrugtevlieg, *Bactrocera dorsalis*, te bepaal met betrekking tot omgewings en fisiologiese veranderlikes. Me Louisa Makumbe, die Doktorale student aan wie die projek toegeken is, het merk-vrylaat-vang eksperimente van steriele *B. dorsalis* onderneem, waar die uitwerking van volwassenheid, geslag en vrug beskikbaarheid op verspreiding bepaal is. Alle vrylatings is reeds voltooi by die drie studie areas naby Louis Trichardt in Limpopo. Die vrylating van vroulike *B. dorsalis* tussen vrug-draënde bome het verspreiding verminder, alhoewel jonger manlike *B. dorsalis* vêrder versprei as ouer mannetjies. Die gekose plasing van lokvalle het nie vroulike vangsgetalle beïnvloed nie, maar wel die van manlike *B. dorsalis.* Vangsgetalle van beide geslagte het ingrypend gestyg soos die gemiddelde minimum temperatuur toegeneem het. Reënval het ook ’n positiewe uitwerking op vroulike vangsgetalle met BioLure lokvalle gehad, en ’n negatiewe uitwerking op manlike vangsgetalle met methyl eugenol lokvalle. Me Makumbe het die invloed van temperatuur op bevolkingsgroei ondersoek deur die paargedrag van *B. dorsalis* oor ’n reeks temperature te ondersoek. Daar is geen roep deur manlike *B. dorsalis* teen temperature laer as 20˚C of hoër as 32˚C opgemerk nie. Die meeste roep aktiwiteit is tussen 24˚C en 28˚C opgemerk. Paring is nie tydens die eksperiment opgemerk nie. Me Makumbe is tans toegespits op die skryf van haar PhD proefskrif, wat teen September 2017 voltooi sal wees.

2.3.5 PROGRESS REPORT: Detection methods for fruit flies of economic significance to fruit and vegetable production in Africa and Indian Ocean islands

Project ERAfrica (project funded by DST: Jan 2014- Jan 2017) by Aruna Manrakhan, John-Henry Daneel, Martin Gilbert, Claire Love, Rooikie Beck, Glorious Shongwe (CRI), Christopher Weldon (UP), Louisa Makumbe (UP), Caroline Knox (RU), Marc De Meyer (Royal Museum for Central Africa), François Hala N’Klo (Centre National de Recherche Agronomique), Helene Delatte (CIRAD), Pierre Francois Duyck (CIRAD).

Summary

The aim of the ERAfrica Fruit fly project was to develop effective and accurate detection methods for fruit fly pests in the Afrotropical region. The specific objectives of this project were to (1) determine the efficacy and sensitivity of different trapping systems for monitoring fruit fly pests, (2) analyse the population genetic structure of key indigenous and exotic fruit fly pests in the Afrotropical region, (3) develop identification tools for fruit flies nd (4) set up a standardised fruit fly detection system in Africa and the Indian Ocean region. Activities carried out by CRI were under specific objective 1.

The efficacy of different combinations of traps and attractants for detection of adult populations of fruit fly pests was evaluated in natural and commercial fruit orchards in Mpumalanga and Limpopo. Responses of a number of fruit fly species including nine important pest species in Africa to food-based attractants and male lures were quantified. New associations of fruit fly pests and male lures were discovered. The sensitivity of the novel EGO lure based trapping system for detection of Medfly, Natal fly and Marula fly, was evaluated in commercial fruit orchards in Mpumalanga using a mark-release-recapture method. With a trapping grid consisting of 5 EGO lure based traps per 2.59 km^2, a detection probability of over 95% was estimated for pest populations of Medfly, Natal fly and Marula fly each containing 1000 adult males. Effects of trap types, lure dispensers and trapping systems (combined traps and lure dispensers) on trapping efficacy of two important fruit fly pests: Medfly and Oriental fruit fly were also determined in commercial orchards. Both traps and lure dispensers had significant effects on catches of both Medfly and Oriental fruit fly in trimedlure and methyl eugenol based trapping systems respectively.

Opsomming

Die doel van die ERAfrica Vrugtevliegprojek was om effektiewe en akkurate opsporingsmetodes vir vrugtevliegplae in die Afrotropiese streek te ontwikkel. Die spesifieke doelwitte van hierdie projek was om (1) die effektiwiteit en sensitiwiteit van verskillende lokval sisteme vir monitering van vrugtevliegplae te bepaal, (2)

die populasie genetiese struktuur van die hoof inheemse en uitheemse vrugtevliegplae in die Afrotropiese streek te analiseer, (3) identifikasie hulpmiddels vir vrugtevlieë te onwikkel en (4) om ʼn gestandaardiseerde vrugtevlieg opsporingssisteem in Afrika en die Indiese Oseaan streek op te stel. Aktiwiteite wat deur CRI uitgevoer is, was onder spesifieke doelwit 1.

Die effektiwiteit van verskillende kombinasies van lokvalle en lokaasmiddels vir die opsporing van volwasse populasies van vrugtevliegplae is in natuurlike en kommersiële vrugteboorde in Mpumalanga en Limpopo geëvalueer. Reaksies van ʼn aantal vrugtevliegspesies, insluitende nege belangrike plaagspesies in Afrika, vir voedingsgebaseerde lokaasmiddels en manlike lokaasmiddels is gekwantifiseer. Nuwe assosiasies van vrugtevliegplae en manlike lokaasmiddels is ontdek. Die sensitiwiteit van die nuwe EGO lokaas-gebaseerde lokvalsisteem vir die opspoor van Mediterreense vrugtevlieg, Natalse vlieg en Marula vlieg, is in kommersiële vrugteboorde in Mpumalanga geëvalueer deur gebruik te maak van ʼn merk-vrylaat-hervang metode. Met ʼn lokvalrooster bestaande uit 5 EGO lokaas-gebaseerde lokvalle per 2.59 km^2, is ʼn opsporingswaarskynlikheid van meer as 95% vir plaagpopulasies van Mediterreense vrugtevlieg, Natalse vlieg en Marula vlieg, elk bevattende 1000 volwasse mannetjies, beraam. Effek van lokval tipes, lokaashouers en lokval sisteme (gekombineerde lokvalle en lokaashouers) op lokval effektiwiteit van twee belangrike vrugtevliegplae: Mediterreense vrugtevlieg en Oosterse vrugtevlieg, is ook in kommersiële boorde bepaal. Beide lokvalle en lokaashouers het betekenisvolle effekte op vangste van beide Mediterreense vrugtevlieg en Oosterse vrugtevlieg in onderskeidelik trimedlure en metiel eugenol-gebaseerde lokval sisteme gehad.

2.3.6 PROGRESS REPORT: Utilisation of citrus and other fruit grown in South Africa by *B. dorsalis*

Project 1107 (April 2014 – March 2018) by C W Weldon (UP) and A Manrakhan (CRI)

Summary

The key outcomes of this project are to determine the fruit species and varieties used by the Oriental fruit fly, *Bactrocera dorsalis*, with a particular emphasis on citrus, and the properties of citrus varieties that make them more or less susceptible to attack. The PhD student assigned to the project, Ms Charmaine Theron, has published the results of her first study in *Journal of Applied Entomology*. She reports that *B. dorsalis* was reared from seven plant species: two from commercial orchards (*Mangifera indica* cv. [Tommy Atkins, Sensation], *Citrus sinensis* cv. [Valencia]), and five from other plant species (*Psidium guajava*, *Anacardium occidentale*, *Solanum mauritianum*, *Xylotheca kraussiana*, *Vangueria infausta*). Fruit utilised by *B. dorsalis* was also infested or damaged by other species, which may indicate opportunism by the pest. Charmaine also attended the International Congress of Entomology in Florida, USA, where she presented her results on oviposition likelihood and development of *B. dorsalis* on citrus varieties. No oviposition was found on undamaged Star Ruby, Glen Ora Late, Delta Valencia or Nadorcott. Oviposition was only detected when Star Ruby, Glen Ora Late and Nadorcott were damaged. These results contrast with oviposition occuring on both undamaged and damaged Tommy Atkins mangoes. When eggs were artificially deposited into the flavedo and albedo of Star Ruby, Eureka, Glen Ora Late, Delta Valencia or Nadorcott, no pupae were recovered. Larval development occurred when eggs were placed in the pulp of the five citrus varieties. Larval development time in the pulp increased with higher pupal recovery, which provides evidence for resource competition. Overall, these results suggest that citrus varieties are not preferred hosts for *B. dorsalis*, with oviposition and development occurring only when the pulp is exposed by other pests. This highlights the importance of integrating the management of *B. dorsalis* with other citrus pests.

Opsomming

Die hoof doel van die projek is om die vrugte spesies en variëteite te bepaal wat deur die Oosterse vrugtevlieg, *Bactrocera dorsalis*, benut word, met spesifieke klem op sitrus en die eienskappe van sitrus variëteite wat die vrugte meer of minder vatbaar maak vir aanvalle. Die PhD student aan wie die projek toegeken is, Me Charmaine Theron, het die resultate van haar eerste studie in die '*Journal of Applied Entomology*' gepubliseer. Sy berig dat *B. dorsalis* op sewe plant soorte grootgemaak is: twee vanuit kommersiële boorde (*Mangifera indica* cv. [Tommy Atkins, Sensation], *Citrus sinensis* cv. [Valencia]) en vyf ander plant spesies (*Psidium guajava*, *Anacardium occidentale*, *Solanum mauritianum*, *Xylotheca kraussiana*, *Vangueria infausta*). Vrugte wat deur *B. dorsalis* benut word was ook deur ander spesies besmet of beskadig, wat opportunisme kan aandui deur die pes. Charmaine het die Internasionale Kongres van Entomologie in Florida, VSR, bygewoon, waar sy resultate van eierlegging waarskynlikheid en ontwikkeling van *B. dorsalis* op sitrus variëteite aangebied het. Geen eierlegging was teenwoordig op onbeskadigde 'Star Ruby', 'Glen Ora Late', 'Delta Valencia' of 'Nadorcott' nie, maar was wel teenwoordig op beskadigde 'Star Ruby', 'Glen Ora Late' en 'Nadorcott'. Die resultate verskil van eierlegging wat op beskadigde en onbeskadigde Tommy Atkins veselperskes aangetref word. Geen papies was verhaal na kunsmatige plasing van eiers in die 'flavedo' en 'albedo' van 'Star Ruby', 'Eureka', 'Glen Ora Late', 'Delta Valencia' of 'Nadorcott' nie. Ontwikkeling van larwes het plaasgevind wanneer eiers binne die

pulp van die vyf variëteite geplaas is. Die larwale ontwikkeling tydperk in die pulp het toegeneem met hoër papie verhaling, wat dui op hulpbron kompetisie. Die resultate dui aan dat sitrus variëteite nie die gekose gasheer vir *B. dorsalis* is nie. 'Oviposition' en ontwikkeling het slegs voorgekom waar die pulp deur ander peste ontbloot is. Dit lig die belangrikheid daarvan uit om die bestuur van *B. dorsalis* te integreer met ander sitrus peste.

2.3.7 PROGRESS REPORT: Determination of non-host status of lemon to Natal fly, Medfly and Oriental fruit fly

Project 1146 (February 2016 - March 2018) by Aruna Manrakhan, John-Henry Daneel, Rooikie Beck, Sean Moore and Vaughan Hattingh (CRI)

Summary

To date, there has not been any interception of fruit flies on Eureka lemon exported from South Africa. The aim of this project was to demonstrate the non-host status of export grade Eureka lemon for fruit fly pests of citrus in South Africa: Mediterranean fruit fly (*C. capitata*), Natal fly (*C. rosa*) and Oriental fruit fly (*B. dorsalis*). A total of 18106 Eureka lemon fruit obtained at harvest from 10 farms in Limpopo and Mpumalanga between March and June 2016 were peeled and dissected to determine the presence of fruit fly eggs and larvae. There were no live fruit fly eggs and larvae in all the fruit examined. Most of the lemon fruit examined were between colour plates 7-4 (green to yellowish green). Traps baited with capilure, EGO Pherolure, methyl eugenol, Biolure and Questlure showed the presence of *B. dorsalis*, *C. capitata* and *C. rosa* sensu lato in all lemon orchards. Populations of *B. dorsalis* and *C. capitata* peaked in February and June respectively in the lemon orchards.

Opsomming

Tot op hede was daar nog geen onderskepping van vrugtevlieë op uitvoer Eureka suurlemoene vanaf Suid-Afrika nie. Die doel van hierdie projek was om die nie-gasheer status van uitvoergraad Eureka suurlemoene vir vrugtevliegplae van sitrus in Suid-Afrika te demonstreer: Mediterreense vrugtevlieg (*C. capitata*), Natalse vlieg (*C. rosa*) en Oosterse vrugtevlieg (*B. dorsalis*). ʼn Totaal van 18106 Eureka suurlemoene wat tydens oes vanaf 10 plase in Limpopo en Mpumalanga tussen Maart en Junie 2016 verkry is, is geskil en gedissekteer ten einde die teenwoordigheid van vrugtevlieg-eiers en -larwes te bepaal. Daar was geen lewendige vrugtevlieg-eiers en -larwes in al die vrugte wat ondersoek is nie. Die meeste van die suurlemoenvrugte wat ondersoek is, was tussen kleurplate 7-4 (groen tot gelerig-groen). Lokvalle met lokaasmiddels capilure, EGO Pherolure, metiel eugenol, Biolure en Questlure, het die teenwoordigheid van *B. dorsalis*, *C. capitata* en *C. rosa sensu lato* in alle suurlemoenboorde aangedui. Populasies van *B. dorsalis* en *C. capitata* het onderskeidelik in Februarie en Junie in die suurlemoenboorde ʼn piek bereik.

2.3.8 PROGRESS REPORT: Fruit fly rearing

Project 407 (April 1999 – March 2018) by Aruna Manrakhan, John-Henry Daneel, Glorious Shongwe and Rooikie Beck (CRI)

Summary

Five fruit fly species: *Ceratitis capitata* (Mediterranean fruit fly or Medfly), *Ceratitis rosa* (Natal fly), *Ceratitis quilicii* (Cape Fly), *Ceratitis cosyra* (Marula fly) and *Bactrocera dorsalis* (Oriental fruit fly) were reared. Fruit fly materials were used in experiments under CRI funded Projects 915, 1075, 1107 and 1170. Colonies of Medfly, Natal fly and Cape fly were refreshed between August 2016 and March 2017 by fruit sampling and addition of wild males to the colonies.

Opsomming

Vyf vrugtevliegspesies: *Ceratitis capitata* (Mediterreense vrugtevlieg of “Medfly”), *Ceratitis rosa* (Natalse vlieg), *Ceratitis quilicii* (Kaapse vlieg), *Ceratitis cosyra* (Marula vlieg) en *Bactrocera dorsalis* (Oosterse vrugtevlieg) is geteel. Vrugtevliegmateriaal is in proewe onder CRI befondsde Projekte 915, 1075, 1107 en 1170 gebruik. Kolonies van die Mediterreense vrugtevlieg, Natalse vlieg en Kaapse vlieg is tussen Augustus 2016 en Maart 2017 versterk deur vrug-monsterneming en die byvoeg van wilde mannetjies tot die kolonies.

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

Project 915 (April 2008 – March 2018) by A Manrakhan, John-Henry Daneel, Rooikie Beck and Glorious Shongwe (CRI)

Summary

The field efficacy of the paper based station: Tephri cone containing malathion was evaluated in Midknight Valencia orchards in two farms in Mpumalanga and one farm in each of Eastern Cape and Western Cape Provinces over 8 weeks between May and July 2016. The Tephri cone was compared with standard fruit fly bait treatments. Efficacy of control was determined by weekly trapping and fruit sampling. In all provinces, trapping results showed that Tephri cones at 200 units per ha were as effective as M3 bait stations at 300 units per ha in controlling Medfly populations.

Laboratory experiments were carried out to determine the efficacy of existing fruit fly bait used as sprays: HymLure and GF-120 on *B. dorsalis*, *C. capitata* and *C. rosa*. Effect of bait concentration on attractiveness and mortality was investigated. HymLure was evaluated at 0.4%, 0.8% and 6.0%. GF-120 was evaluated at 3.3%, 5.0%, 10% and 20%. There were no significant effects of concentration of the two baits in the attractiveness and efficacy of kill for all fly species tested. *C. capitata* had the highest response to all fruit fly baits compared to the other species. Fly mortality rates were also higher for *C. capitata* compared to the other species for all baits tested.

The effect of shade netting on aerial fruit fly bait spray deposition in citrus orchards was determined in Navel orange orchards on a farm near Nelspruit, Mpumalanga Province. An aerial application of the commercially available fruit fly bait GF-120 was carried out in January 2017 in two orchards which were under a 20% white shade net and in two orchards which were uncovered. Coverage of aerial GF-120 spray deposits were fairly similar in orchards under net and in uncovered orchards. Mean droplet sizes of GF-120 were within the recommended ranges in all orchards.

Opsomming

Die veld-effektiwiteit van die papier-gebaseerde stasie, Tephri keël, bevattende malathion, is in Midknight Valencia boorde in twee plase in Mpumalanga en een plaas in elk van die Oos-Kaapprovinsie en Wes-Kaapprovinsie oor 8 weke tussen Mei en Julie 2016 geëvalueer. Die Tephri keël is met standaard vrugtevlieg lokaasbehandelings vergelyk. Effektiwiteit van beheer is deur weeklikse lokval en vrug-monsterneming bepaal. In al die provinsies het lokval-resultate getoon dat Tephri keëls teen 200 eenhede per ha so effektief soos M3 lokaasstasies teen 300 eenhede per ha in die beheer van Mediterreense vrugtevliegpopulasies was.

Laboratorium-eksperimente is uitgevoer ten einde die effektiwiteit van bestaande vrugtevlieg lokaasmiddels wat as spuite gebruik word, te bepaal: HymLure en GF-120 op *B. dorsalis*, *C. capitata* en *C. rosa*. Effek van lokaasmiddelkonsentrasie op aantrekkingskrag en mortaliteit is ondersoek. HymLure is teen 0.4%, 0.8% en 6.0% geëvalueer. GF-120 is teen 3.3%, 5.0%, 10% en 20% geëvalueer. Daar was geen betekenisvolle effekte van konsentrasie van die twee lokaasmiddels in die aantrekkingskrag en effektiwiteit van doodmaak vir alle vliegspesies wat getoets is nie. *C. capitata* het, in vergelyking met die ander spesies, die hoogste reaksie op alle vrugtevlieg lokaasmiddels getoon. Vliegmortaliteitstempo's was ook hoër vir *C. capitata* in vergelyking met die ander spesies vir alle lokaasmiddels wat getoets is.

Die effek van skadunet bedekking op luggedraagde vrugtevlieg lokaasmiddel spuitneerlegging in sitrusboorde, is in Navel lemoenboorde op 'n plaas naby Nelspruit, Mpumalanga-provinsie bepaal. 'n Luggedraagde toediening van die kommersieel beskikbare vrugtevlieg lokaasmiddel, GF-120, is in Januarie 2017 in twee boorde, wat onder 'n 20% wit skadunet was, en in twee boorde wat onbedek was, uitgevoer. Bedekking van luggedraagde GF-120 spuitneerleggings was min of meer dieselfde in boorde onder net en in onbedekte boorde. Gemiddelde druppelgroottes van GF-120 was binne die aanbevole reekse in alle boorde.

2.3.10 PROGRESS REPORT: Determining phytotoxicity of fruit fly baits on citrus fruit with previous exposure to copper sprays

Project 1147 (April 2016- March 2018) by Aruna Manrakhan, John-Henry Daneel, Charl Kotze and Rooikie Beck (CRI)

Summary

Fruit flies and Citrus Black Spot (CBS) are among the key phytosanitary pests in the citrus industry. Protein-based bait sprays are commonly used for control of fruit flies. For control of CBS, copper sprays are increasingly being used due to lower tolerances of other fungicides previously used for control of CBS. There have been previous claims of phytotoxicity on citrus fruit when fruit fly baits and copper sprays are combined. In this study, incompatibility between applications of copper sprays and fruit fly baits was investigated on Valencia fruit in an orchard at Crocodile Valley Estates, Nelspruit, Mpumalanga. Three copper products were evaluated: cuprous oxide (Nordox at 90 g/100L), copper hydroxide (Kocide at 150 g/100 L) and copper oxychloride (Cuprous super at 200g/100L) at three application rates: no application, single application and double application over two consecutive months. Two types of fruit fly baits: GF-120 and a mixture of HymLure and malathion were applied as a 2ml cover spray on fruit exposed with different copper products and

application rates. Phytotoxicity due to copper and baits were determined on immature green fruit picked one week after bait application. The fruit were thereafter dipped in an Ethephon solution to induce a colour change and were left to dry for ~3 weeks before being re-examined to determine phytotoxicity. Following the ethephon dip, visible damage (discolouration and rind collapse) were found on all fruit irrespective of copper product, copper application rate (including no application) and fruit fly bait. Fruit exposed to the copper products and protein baits were also left on the tree to mature naturally and will be examined for phytotoxicity closer to harvest.

Opsomming

Vrugtevlieë en Sitrus Swartvlek (CBS) is van die hoof fitosanitêre plae in die sitrusbedryf. Proteïen-gebaseerde lokaasmiddelspuite word algemeen vir die beheer van vrugtevlieë gebruik. Koper spuite word toenemend vir beheer van CBS gebruik weens laer toleransies vir ander fungisiedes wat voorheen vir die beheer van CBS gebruik is. Daar was al voorheen bewerings van fitotoksisiteit op sitrusvrugte wanneer vrugtevlieg lokaasmiddels met koper spuite gekombineer is. In hierdie studie is onverenigbaarheid tussen toedienings van koper spuite en vrugtevlieg lokaasmiddels op Valencia vrugte in ŉ boord by Crocodile Valley Estates, Nelspruit, Mpumalanga, ondersoek. Drie koper produkte is geëvalueer: koper-I-oksied (Nordox teen 90 g/100L), koperhidroksied (Kocide teen 150 g/100 L) en koperoksichloried (Cuprous super teen 200g/100L) teen drie toedieningstempo's: geen toediening, enkel toediening en dubbel toediening oor twee opeenvolgende maande. Twee tipes vrugtevlieg lokaasmiddels: GF-120 en ŉ mengsel van HymLure en malathion is as ŉ 2ml bedekkingspuit op vrugte toegedien wat aan verskillende koper produkte en toedieningstempo's blootgestel is. Fitotoksisiteit weens koper en lokaasmiddels is op groen vrugte, wat een week ná die toedien van die lokaasmiddel gepluk is, bepaal. Die vrugte is daarna in ŉ Ethephon oplossing gedoop om ŉ kleurverandering te induseer, en is gelos om vir ~3 weke te droog voordat dit weer ondersoek is om fitotoksisiteit te bepaal. Ná die ethephon doop is sigbare skade (verkleuring en skilverval) op alle vrugte gevind, ongeag koper produk, koper toedieningstempo (insluitend geen toediening) en vrugtevlieg lokaasmiddel. Vrugte wat aan die koper produkte en proteïen lokaasmiddels blootgestel is, is ook op die boom gelaat om natuurlik ryp te word, en sal nader aan oes vir fitotoksisiteit ondersoek word.

2.3.11 PROGRESS REPORT: Biology and ecology of *Ceratitis rosa* and *Ceratitis quilicii* (Diptera: Tephritidae) in citrus

Project 1070 (April 2016 – March 2018) by J Daneel and A Manrakhan (CRI)

Summary

The Natal fly, *Ceratitis rosa*, was recently split in two species: *C. rosa* and *Ceratitis quilicii*. The broad aim of the project was to quantify similarities and differences between *C. rosa* and *C. quilicii* in citrus. Activities carried out in 2016-2017 were to determine: (1) relative abundance of the two species in citrus orchards and (2) development of the two species in citrus. In the relative abundance studies, nine farms were selected at three altitudinal ranges: high (899 – 1086 masl), medium (426 – 550 masl) and low (162 – 278 masl) in Limpopo and Mpumalanga provinces. Population levels of *C. rosa* and *C. quilicii* in citrus orchards were determined by attractant based traps. Three attractants were used: EGO Pherolure, capilure and Biolure. Specimens were collected from the traps every month and were identified to species and sex. Fruit were also collected from each orchard every month to determine fruit infestation by the two fruit fly species. Laboratory reared *C. rosa* and *C. quilicii* were used for studying their development in four citrus types (sweet orange, soft citrus, grapefruit and lemon). The developmental time of each immature stage of each species in each citrus type was determined by inoculating eggs of each species separately and concurrently in each citrus type and dissecting fruit daily thereafter for 15 days to determine the larval developmental stages. *Ceratitis quilicii* was more dominant than *C. rosa* in citrus orchards at all three altitudes. Only one *Ceratitis rosa* male was caught at the high altitude and Cool-inland region. Fruit fly development was more optimal in the oranges compared to the other citrus types. The peaks in development of each larval instar were the same for both species. As such, each instar for both species can be exposed to a cold treatment on the same day to determine the least susceptible instar.

Opsomming

Die Natal vrugtevlieg, *Ceratitis rosa* was onlangs onderverdeel in twee spesies: *C. rosa* en *Ceratitis quilicii*. In breë trekke, is die doel van die projek was om ooreenkomste en verskille tussen *C. rosa* en *C. quilicii* in sitrus te kwantifiseer. Die aktiwiteite wat in 2016-2017 uitgevoer was, was om te bepaal: (1) die relatiewe rykdom van die twee spesies in sitrusboorde en (2) die ontwikkeling van die twee spesies in sitrus. In die relatiewe rykdom studies was nege plase op drie hoogtes bo seevlak in die Limpopo en Mpumalanga provinsies geselekteer: hoog (899 – 1086 m), medium (426 – 550 m) en laag (162 – 278 m). Die populasievlakke van *C. rosa* en *C. quilicii* in die sitrusboorde was bepaal met lokaas gebaseerde lokvalle. Drie lokase was gebruik: EGO Pherolure, capilure en Biolure. Monsters verkry vanuit die lokvalle is elke maand versamel en

geidentifiseer tot op spesievlak en geslag. Vrugte was ook versamel in elke boord elke maand om die vrugbesmetting van die twee vrugtevlieë te bepaal. Laboratorium kulture van *C. rosa* en *C. quilicii* is gebruik om hul ontwikkeling in vier sitrussoorte (soet lemoene, sagte sitrus, pomelos en suurlemoene) te bestudeer. Die ontwikkelingstyd van elke onvolwasse stadium van elke spesie in elke sitrustipe is bepaal deur eiers van elke spesie afsonderlik, maar gelyktydig in elke sitrustipe te inokkuleer en daarna vrugte daagliks vir 15 dae te dissekteer om sodoende die larwes se ontwikkelingsfases te bepaal. *Ceratitis quilicii* was meer dominant as *C. rosa* in die sitrusboorde op al drie hoogtes. Slegs een *Ceratitis rosa* mannetjie was gevang op die hoë hoogtes en Koel-binnelandse streek. Vrugtevliegontwikkeling was meer optimaal in lemoene as in vergelyking met die ander sitrustipes. Die ontwikkelingspieke van elke larwe instar was dieselfde vir albei spesies. As sodanig kan elke instar vir beide spesies op dieselfde dag aan 'n koue behandeling blootgestel word om die minste vatbare instar te bepaal.

2.4 PROGRAMME: MEALYBUG AND OTHER MARKET ACCESS PESTS

Programme coordinator: Sean D Moore (CRI)

2.4.1 Programme summary

Although the two main phytosanitary pests for citrus in southern Africa are considered to be FCM and fruit flies, certain of the mealybug species known to occur on citrus also have phytosanitary status for certain export markets. One of these species is *Delotococcus aberiae*, of which conspicuous outbreaks were reported in Letsitele a few years ago. A project was initiated to survey for parasitoids attacking this species (2.4.6). However, *D. aberiae* infestation has been a lot lower during the the last couple of seasons and consequently no parasitoids were found and it was not possible to establish a laboratory culture. Another insect which is considered to be a phytosanitary organism by China is carob moth. A comprehensive study was conducted on this pest (2.4.3), establishing a monitoring technique, quantifying its association with mealybug and determing the efficacy of various products for its control. A new parasitoid species was also identified from carob moth larvae. Another project on carob moth investigated the efficacy of a cold sterilisation treatment (2.4.2). It was demonstrated that at a subzero temperature, carob moth was more cold susceptible than FCM, opening the door for existing cold-sterilisation protocols for FCM to also include carob moth. Another project in the programme evaluated GRAS fumigants for post-harvest disinfestation of fruit of a series of phytosanitary pests, with the main focus during the past year on FCM (2.4.4). CO_2 (60%) fumigation followed by cold (2°C for 6 d) resulted in 100% mortality of third instars and 98.3% mortality of fifth instars. The final project in the programme, which aimed to investigate trunk sprays for Fuller's rose beetle, had to be terminated, as pest levels were negligible (2.4.5).

Programopsomming

Alhoewel dit beskou word dat die twee hoof fitosanitê plae op sitrus in suidelike Afrika VKM en vrugtevlieë is, sekere witluis spesies wat op sitrus voorkom het ook fitosanitêre status vir sekere markte. Een van hierdie spesies is *Delotococcus aberiae*, waarvan opvallende uitbrake te Letsitele 'n paar jaar gelede opgelet is. 'n Projek is begin om 'n opname te doen vir parasiete wat hierdie spesie aanval (2.4.6). *Delotococcus aberiae* besmetting is egeter in die laaste twee seisoene heelwat laer en gevolglik is geen parasiete gekry nie en dit was ook nie moontlik om 'n laboratorium kultuur te stig nie. Nog 'n insek wat as 'n fitosanitêre organisme deur China beskou word is karobmot. 'n Omvattende studie is op hierdie plaag uitgevoer (2.4.3). 'n Moniterings tegniek is ontwikkel, die verhouding tussen karobmot en witluis is gekwantifiseer en die werking van verskeie produkte vir karobmot beheer is bepaal. 'n Nuwe parasiet spesie is ook van karobmot larwes geïdentifiseer. Nog 'n projek op karobmot het die werking van 'n kouesterilisasie behandeling ondersoek (2.4.2). Dit is teen 'n temperatuur onder vriespunt gedemonstreer dat karobmot meer koue gevoelig as VKM was, wat die deur oopmaak vir bestaande koue sterilisasie protokolle vir VKM om karobmot ook in te sluit. Nog 'n projek in die program het GRAS berokingsmiddels ontleed vir na-oes ontsmetting van vrugte vir 'n reeks fitosanitêre plae, met die hoof fokus gedurende die laaste jaar op VKM (2.4.4). CO_2 (60%) beroking gevolg deur koue (2°C vir 6 d) het gelei tot 100% mortaliteit van derde instars en 98.3% mortaliteit van vyfde instars. Die finale projek in die program, wat stambehandelings vir Fuller se rooskewer sou ondersoek, moes geskraap word as gevolg van weglaatbare lae plaagvlakke (2.4.5).

2.4.2 FINAL REPORT: Development of a cold sterilisation treatment for carob moth

Project 1151 (Apr 2016 – Aug 2018) by Sean Moore, Sean Thackeray, Mat Goddard and Wayne Kirkman (CRI & RU)

Summary

Carob moth is regulated as a phytosanitary pest by China. There are also indications that the pest status of carob moth on citrus may be higher than originally believed, particularly in certain regions and seasons. It was

therefore necessary to demonstrate that carob moth is at least as cold susceptible as FCM, so that any cold treatment protocols which are accepted for FCM, can also be acceptable for carob moth. Firstly, head capsule sizes for carob moth larval instars were determined, in order to be able to differentiate between instars. Carob moth fifth instars were determined to be the most cold tolerant larval life stage. Carob moth larval mortality was compared with that of FCM larvae at -0.55°C. This was conducted in infested citrus, pecan nuts and artificial diet, removing samples at various intervals from 3-18 days and assessing mortality. Carob moth showed higher cold susceptibility in all instars and this was significantly so for second and third instars. At -0.55ºC for 18 days, mortality of fifth instar carob moth was 94.6% while mortality of fourth and fifth instar FCM was 87.8%. A probit analysis, comparing these most cold-tolerant instars of carob moth and FCM, showed that regression lines for empirical probits plotted against log time were significantly different. LC50 and LC90 values for carob moth were 8.2 and 16.6 days respectively, while FCM values were 10.5 and 22.1. Empirical probits for carob moth and FCM at 18 days were 6.6 and 6.1 respectively. This study therefore showed that carob moth larvae are more cold susceptible than FCM larvae, indicating that post-harvest cold disinfestation treatments for FCM will be as, or more, effective against carob moth. Due to this finding this project will be terminated and results will be published.

Opsomming

Karobmot word deur China as ŉ fitosanitêre plaag gereguleer. Daar is ook tekens dat die plaagstatus van karobmot op sitrus dalk hoër kan wees as wat oorspronklik geglo is, veral in sekere streke en seisoene. Daarom is dit as noodsaaklik beskou om te demonstreer dat karobmot minstens so koue-gevoelig as VKM is, dat enige koue-behandelings protokolle wat vir VKM aanvaar word, ook vir karobmot aanvaarbaar kan wees. Eerstens is kopkapsule grotes vir die karobmot instars bepaal, om tussen instars te kan onderskei. Dit is bepaal dat karobmot vyfde instars die mees koue-tolerante lewensstadium is. Larwe mortaliteit is tussen karobmot en VKM teen -0.55°C vergelyk. Hierdie is in besmette sitrus, pekanneute en kunsmatige dieët uitgevoer. Monsters is op verskeie intervalle tussen 3 en 18 dae verwyder en vir mortaliteit ontleed. Alle instars van karobmot was meer koue-gevoelig en hierdie verskil was betekenisvol vir tweede en derde instars. Teen -0.55ºC vir 18 dae was mortaliteit van karobmot vyfde instars 94.6%, terwyl mortaliteit van VKM vierde en vyfde instars net 87.8% was. ŉ Probit analiese wat hierdie mees koue-tolerante instars van karobmot en VKM vergelyk het, het gewys dat regressie lyne vir empiriese probits teen log-tyd betekenisvol verskil het. LC50 en LC90 waardes vir karobmot was 8.2 en 16.6 dae onderskeidelik, terwyl VKM waardes 10.5 en 22.1 was. Empiriese probits vir karobmot en VKM teen 18 dae was 6.6 en 6.1 onderskeidelik. Daarom het hierdie studie gewys dat karobmot larwes meer koue-gevoelig as VKM larwes is wat aandui dat na-oes koue-ontsmettings behandelings vir VKM net so doeltreffend, as nie meer doeltreffend, teen karobmot sal wees. As gevolg van hierdie bevinding sal hierdie projek beëindig word en die resultate sal gepubliseer word.

Introduction

Carob moth, Ectomyelois ceratoniae, has always been considered as no more than a nominal and extremely rare pest on citrus (Honiball and Catling, 1998). It has also been considered to be a secondary infester, in response to infestation of fruit by mealybug (or other sucking insects) and the honey dew and sooty mould that ensues (Grout and Moore, 2015). However, China recently opened up as a new export market for South Africa and amongst other pests, regulated carob moth as a phytosanitary organism. Additionally, Moore et al. (2015) recently discovered that levels of carob moth infestation may be a lot higher in citrus in certain cases than originally believed. For example, from one farm in the Groblersdal region, 60% of larvae found infesting Navel oranges were carob moth. The remaining 40% were FCM larvae.

The protocol for phytosanitary requirements for the export of citrus from South Africa to China, signed between the governments of the two countries, states that the mere presence of carob moth in an orchard, packhouse or during phytosanitary inspections, will lead to the expulsion of the relevant orchard from the China export programme for the duration of the season. It goes on to say that if any carob moth infestation of fruit is recorded on inspection in China, then the consignment will be returned or destroyed and the relevant orchard and packhouse suspended. This is only the case for FCM and fruit flies, if the larva found is alive. However, as there are no data to show the effect of the mandatory cold sterilisation protocol (aimed at FCM) on carob moth, China cannot assume that this cold treatment is adequate for carob moth, and consequently the finding of a larva, whether alive or dead, is simply interpreted as a sign of the presence of the pest in the consignment and thus provides reason for rejection.

Fortunately there have as yet been no findings of carob moth in citrus fruit exported from South Africa to China. However, we need to be proactive and generate the necessary cold sterilisation data in order to avert such an occurrence and in order to facilitate a change in the export protocol, meaning acceptance of the efficacy of the FCM cold sterilisation protocol for carob moth. Preliminary data generated by Moore et al. (2015) has provided

a strong indication that carob moth is substantially more cold susceptible than FCM and that the FCM cold sterilisation protocol will indeed be adequate for carob moth.

Objectives

- To demonstrate that carob moth is at least as cold susceptible as FCM and therefore that cold sterilisation protocols accepted for FCM should also be accepted for carob moth.

Materials and Methods

Head capsule size categories for carob moth larval instars

Carob moth larvae were collected from a laboratory culture (refer to the chapter in which you describe the rearing) and field samples over a 12-month period. Head capsule widths were measured using Dewinter Caliper Pro 4.6 (Dewinter Optical Inc. New Delhi, India) and these measurements were plotted according to Dyar (1890).

Cold treatment

Source of Insects
Pecan nuts and Navel oranges infested with carob moth and FCM were collected between 11 and 15 June 2016 from Vaalharts (27°52'40.54"S 24°49'37.47"E) in the Northern Cape of South Africa. Pecans nuts were collected from four different farms at sorting tables and Navel oranges from two different orchards. All collected material was kept separate in order to ensure that each collection was an independent sample and therefore a true replicate. False codling moth larvae in artificial diet were obtained from River Bioscience (Hermitage, Eastern Cape Province, South Africa), where FCM had been reared for numerous generations (Moore et al. 2014). Sixty jars were obtained when larvae were in the intended life stage, in this case fourth and fifth instar, which are the most cold-tolerant (Moore et al., 2016a). These were from two separate batches, thus two true replicates.

Cold room
A 5 m by 4 m by 2.5 m polyurethane cold room with a galvanised floor was used for the experiment at Citrus Research International in Port Elizabeth, South Africa. Temperatures were monitored with a Brainchild VR18 temperature logger with 16 PT100 probes (WIKA Technologies, Port Elizabeth, South Africa). Probes were calibrated before the experiment using the freezing point method where the probes were immersed in melting ice and the temperature recorded when they reached equilibrium. A certified calibrated thermometer (SANAS Calibration Laboratory, Pretoria, South Africa) immersed in the melting ice was used to confirm the temperature (Grout et al. 2011a, Moore et al. 2016a). The cold room was set at -0.8oC in order for product temperature to be as close to -0.55oC as possible. This temperature was used as it is the current standard cold treatment temperature when exporting citrus from South Africa to China (SA-DAFF 2016). Probes were inserted into pecan nuts (seven probes), oranges (four probes) and artificial diet (four probes) in order to monitor product temperature. Individual probes also measured air temperature at the inflow and outflow of the cold room. Temperatures were recorded at 10 minute intervals for the full duration of the study; hourly mean, maxima and minima were calculated.

Mortality induced through cold treatment
After each product had reached a mean temperature of -0.5oC, the cold treatment was initiated. Cartons of citrus, pecans and artificial diet were removed from the cold room at 3, 6, 9, 12, 15 and 18 days. These were then kept at 25 ± 2oC, 30% RH, and a photoperiod of 16:8 (L: D) for 24 h to enable any surviving larvae to become active (Moore et al. 2016a, 2016b). Immediately thereafter, pecans, fruit and artificial diet were dissected and numbers of live and dead larvae (and instar) were recorded for each species. Larvae were identified according to Rentel et al. (2012). Larvae were considered alive if colouration was normal and moved after prodding (Moore et al. 2016a). Samples (at least 25 individuals per species) of each life stage were kept in 70% ethanol, and estimated life stage was verified and numbers of larvae corrected by measurement of head-capsule size (Table 2.4.2.1).

Table 2.4.2.1. Head capsule width for carob moth and false codling moth.

Species	**Instar and head capsule size (mm)**				
	1	**2**	**3**	**4**	**5**
Carob moth[1]	0.0-0.34	0.35-0.64	0.65-0.94	0.95-0.14	1.15-wider
FCM[2]	0.0-0.28	0.29-0.46	0.47-0.77	0.78-1.16	1.17-wider

[1] Thackeray, 2017
[2] Hofmeyer et al. 2016

Larvae surviving cold treatment
To determine the fate of carob moth larvae that survive the cold treatment, all surviving individuals found after the 18-day treatment were placed onto artificial diet individually in 30 ml capacity Poly Top glass vials (Bonpak, Johannesburg, South Africa) and kept at 25 ±2oC, 30% RH, and a photoperiod of 16:8 (L: D). These larvae were monitored for survival and ability to reach the adult life stage. This was compared to a control survival where 80 carob moth larvae (20 from each replicate) where dissected from pecans and placed onto artificial diet and monitored until adult eclosion or the larva died.

Statistical analyses
All statistical analyses were conducted with Statistica 13.0 (Statsoft, Tulsa, United states) unless stated otherwise. To determine whether the mortality of FCM and/or carob moth was comparable in the different products (pecan nuts, oranges and artificial diet), comparisons of mean hourly temperature over the 18-day treatment period were made with an Analysis of Variance (ANOVA). Tukey's post-hoc analysis was used to determine where significant differences occurred.

Percentage mortality data were corrected for control mortality (Abbot 1925) and data underwent arcsine transformation. Carob moth mean survival of each instar in pecans and oranges were compared separately for each time period with a factorial ANOVA to determine instars with the highest levels of cold tolerance. Tukey's post-hoc analysis was used to determine where significant differences occurred.

A comparison of mean mortality of the least cold susceptible carob moth instar against the combined mortality of FCM fourth and fifth instars at all time periods was conducted with a General Linear Model (GLM) factorial ANOVA and Tukey's post hoc analysis was used to determine where significant differences occurred. Regression analysis was used to determine the functional relationship between log time period of cold treatment and probit of mortality of fifth instar carob moth along with fourth and fifth instar FCM larvae using PROBAN at a test level of $P < 0.05$ (Van Ark 1995).

Results

Head capsule size categories for carob moth larval instars

Five distinct size classes were visible when plotting head capsule measurements of carob moth larvae in 0.5 mm categories (Fig. 2.4.2.1). Head capsule measurements for carob moth larval instars were determined as follows: first instar (0.0-0.34 mm), second instar (0.35-0.64 mm), third instar (0.65-0.94 mm), fourth instar (0.95-1.14 mm) and fifth instar (0.15 mm and wider).

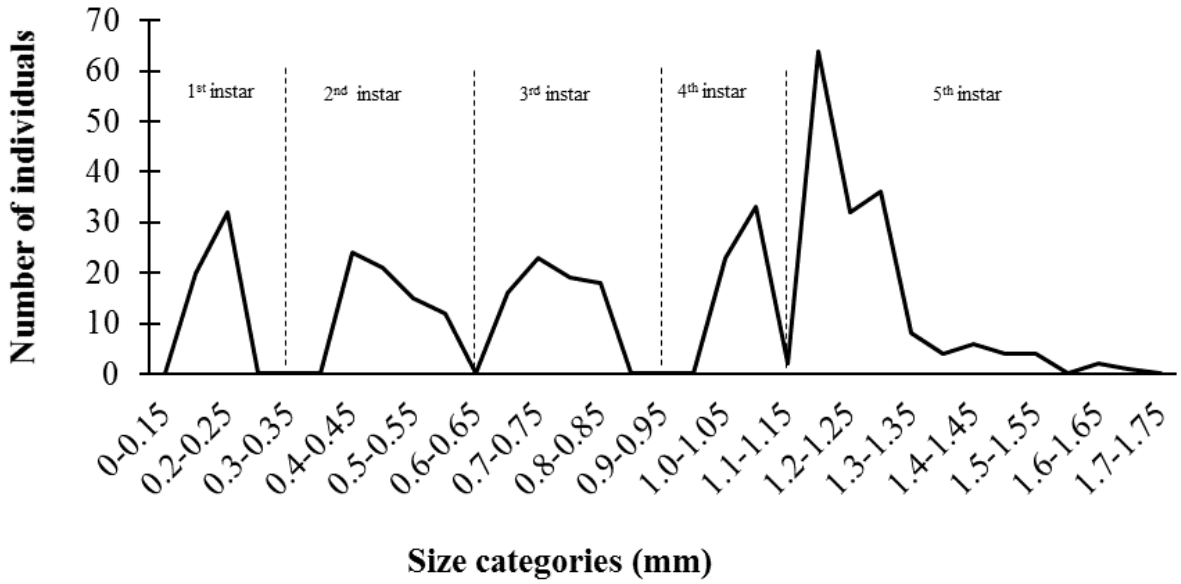


Fig. 2.4.2.1. Frequency of head capsule sizes falling into categories of 0.5 mm to determine the number of instars and size categories of instars for carob moth

Cold treatment

Temperatures within products were monitored over the duration of the study (Fig. 2) and comparison of hourly means showed that there was a significant difference in product temperature over the 18-day period ($F_{1, 2}$ = 23.26, P = 0.00) (Table 2.4.2.2). Therefore, direct comparisons could not be made on mortality of FCM and carob moth between oranges, pecans and artificial diet.

Table 2.4.2.2. Mean hourly internal product temperature over 18 days. Different letters denote significant difference between means established through Tukey's post hoc analysis (P < 0.05).

Product	Mean temp (ºC)	Standard error
Pecan nuts	-0.36[a]	± 0.0021
Navel oranges	-0.53[b]	± 0.0028
Artificial diet	-0.20[c]	± 0.0022

Carob moth mortality in pecan nuts over the duration of the cold treatment was significantly different between larval instars ($F_{1, 3}$=998.5, P = 0.000028). Post hoc analysis showed that carob moth fifth instar was the least cold susceptible with 94.6% mortality after 18 days which was significantly lower than second instar (100%, P = 0.00016), third instar (98.4%, P = 0.049) and fourth instar (99.1%, P = 0.00182) (Table 2.4.2.3). In Navel oranges, 100% mortality was observed for all instars over the 18-day treatment. There were no significant differences between cold susceptibility of instars for the shorter durations ($F_{1, 3}$ = 1.65, P = 0.924), due to the low number of treated individuals.

Table 2.4.2.3. Corrected mean (± SE) percentage mortality of carob moth second to fifth instars for four replicates at -0.36ºC for seven different time treatments in pecan nuts.

Treatment (days)	Mean n	Larval instar corrected mortality (%)			
		2	3	4	5
Control	113.25 ±21.0	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]
3	280.0 ± 128.7	19.4 ± 12.4[a]	25.7 ± 11.4[a]	20.4 ± 2.7[a]	8.9 ± 1.7[a]
6	296.0 ± 106.1	37.9 ± 14.6[a]	32.2 ± 4.81[a]	29.65 ± 5.3[a]	20.9 ± 1.7[b]
9	206.5 ± 69.4	91.2 ± 5.9[a]	56.9 ± 4.5[b]	72.4 ± 5.6[b]	51.8 ± 4.8[b]
12	232.5 ± 93.4	93.8 ± 7.2[a]	84.3 ± 7.7[a]	89.4 ± 3.7[a]	78.7 ± 2.3[b]
15	152.8 ± 60.5	100.0 ± 0.0[a]	95.2 ± 4.0[a, b]	98.3 ± 1.9[a]	90.6 ± 3.6[b]
18	258.0 ± 73.9	100.0 ± 0.0[a]	98.4 ± 1.8[a]	99.1 ± 0.6[a]	94.6 ± 1.8[b]

Table 2.4.2.4. Corrected mean (± SE) percentage mortality of carob moth second to fifth instars at -0.53ºC for seven different time treatments in Navel oranges.

Treatment (days)	Mean n	Larval instar corrected mortality (%)			
		2	3	4	5
Control	2.5 ±0.7	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
3	3.0 ±0.0	-	-	0.0 ±0.0	0.0 ±0.0
6	7.0 ±2.5	75.0 ±35.4	87.5 ±17.8	75.0 ±35.4	33.3 ±47.2
9	4.5 ±2.1	50.0 ±0.0	100.0 ±0.0	50.0 ±0.0	50.0 ±0.0
12	5.0 ±1.4	100.0 ±0.0	100.0 ±0.0	50.0 ±0.0	50.0 ±0.0
15	5.0 ±1.5	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0
18	4.5 ±0.7	-	100.0 ±0.0	100.0 ±0.0	100.0 ±0.0

A total 35 carob moth larvae survived the 18-day treatment, however, none of these larvae were able to pupate, compared to the control, where 78.8% (± 2.71) were able to reach adulthood. Due to the small number of carob moth larvae recovered in citrus, only corrected mortality data generated from pecans were used to compare species cold susceptibility. Combined mean mortality of FCM fourth and fifth instars (Table 2.4.2.5) was significantly different to carob moth fifth instar ($F_{1,1}$=1645.4, P = 0.000). There was a significant interaction between species and cold treatment duration ($F_{1, 6}$ = 243.5, P = 0.000068). For all time periods, carob moth mortality was higher than FCM. However, post hoc analysis showed that this was only significantly so at 9 (P

= 0.0000142) and 12 days (P = 0.000138). After 18 days, carob moth and FCM mortality was 95.4% and 88.7% respectively, but this was not significantly different (P = 0.947) (Fig. 2.4.2.4).

Table 2.4.2.5. Combined fourth and fifth instar false codling moth corrected mortality in different products. Different letters in each column indicate significant differences (P < 0.05) determined with Tukey's post hoc analysis.

Treatment	Corrected mortality (%)								
	Pecan nuts			Navel oranges			Artificial diet		
	n	Mean	SE	n	Mean	SE	n	Mean	SE
Control	85	0.00[a]	±0.00	10	0.00[a]	±0.00	3143	0.00[a]	±0.00
3	176	5.51[b]	±2.26	15	0.00[a]	±0.00	2066	6.27[b]	±1.35
6	183	19.19[c]	±5.89	9	25.00[ab]	±25.00	1332	30.28[c]	±7.39
9	240	24.30[c]	±5.30	13	48.75[b]	±15.10	1333	95.78[d]	±1.98
12	197	54.00[d]	±4.40	13	100.00[c]	±0.00	2124	100.00[e]	±0.00
15	228	79.28[e]	±3.74	18	100.00[c]	±0.00	1560	100.00[e]	±0.00
18	279	87.83[e]	±9.94	20	100.00[c]	±0.00	1892	100.00[e]	±0.00

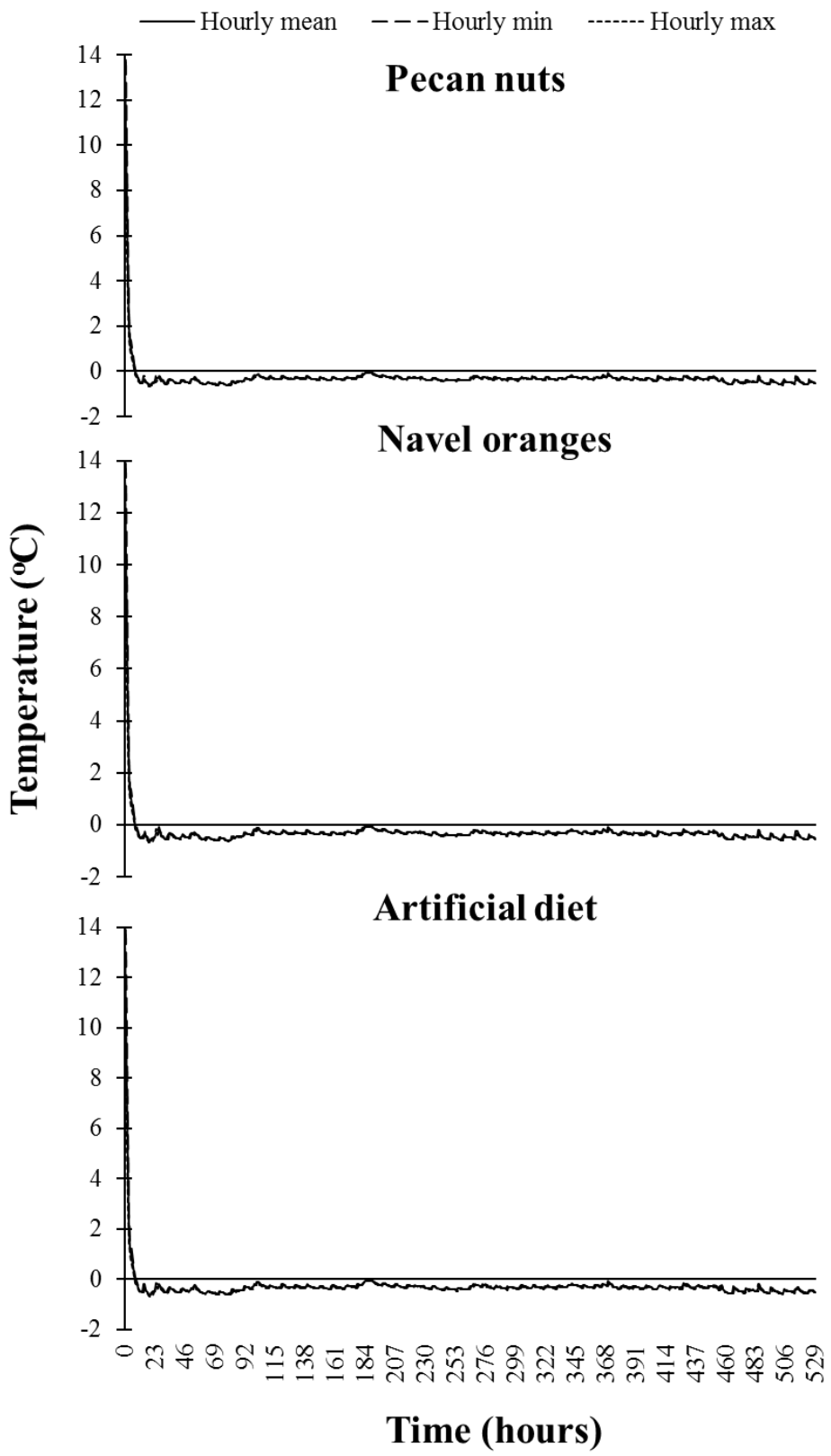
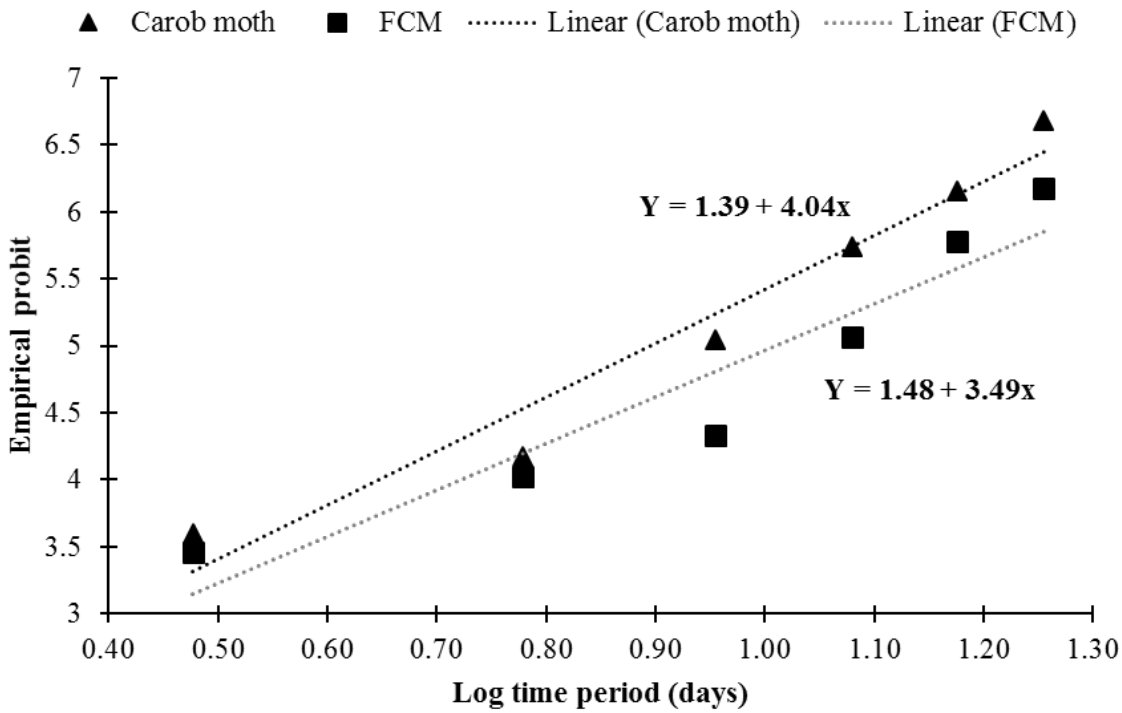


Fig. 2.4.2.2. Hourly mean temperature for products over the full 18 day treatment period including cooling down.



Time period (days)	Log time	Number of individuals exposed	
		Carob moth	FCM
3	0.48	625	176
6	0.78	666	183
9	0.95	580	240
12	1.08	617	197
15	1.18	397	228
18	1.26	669	279

Fig. 2.4.2.3. Regression of empirical probit values against the log time period in days for carob moth and false codling moth in pecan nuts with the number of individuals exposed to each time period.

Regression analysis of empirical probit values was conducted and residual variances were homogenous ($F_{4, 4} = 1.213$, $P < 0.01$), lines were parallel ($X2 = 0.567$, $P < 0.05$), and the comparison of elevations of adjusted means was found to be significantly different ($F_{1, 9} = 6.027$, $P < 0.05$) (Fig. 3). Empirical probit values at log 18 days was 6.7 for carob moth and 6.1 for FCM. The expected time period to reach LD50 was 10.5 days for FCM and 8.5 days for carob moth; and LD90 value for carob moth was 16.5 days and 22 days for FCM. The LD90 value for FCM may be unreliable due to the range between fidicular limits (UF = 66.72, LF = 15.91).

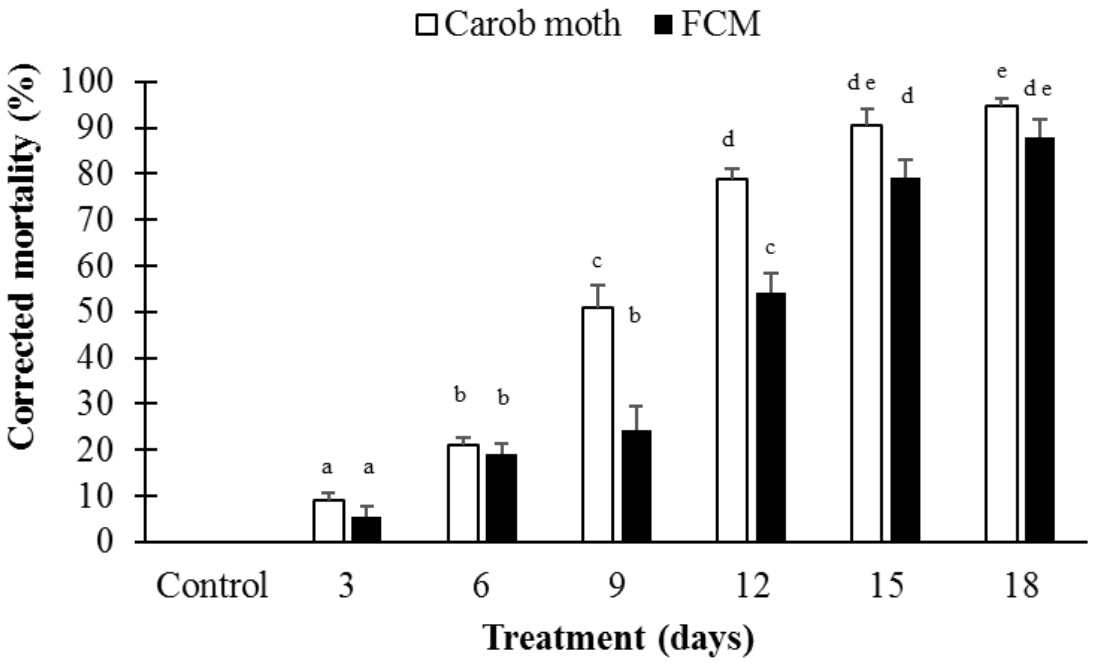


Fig. 2.4.2.4. Mean corrected survival of carob moth and false codling moth in pecan nuts at -0.34^{o}C. Letters above bars indicate significant differences between means determined with Tukey's post hoc analyses ($P < 0.05$).

Discussion

Head capsule sizes indicated five larval instars with a prepupal period. This is congruent with Gothilf (1969) who observed five head capsule moults, but went on to define instar size categories in larval length. Mediouni and Dhouibi (2007) conducted experiments to establish the development of carob moth larvae in a mass rearing facility for the sterile insect technique, however, they failed to mention how instars were separated. To the best of the authors knowledge, head capsule size categories of the carob moths' larval instars have not been previously established. In two instances there is a gap between instars (first-second and third-fourth). In a similar study for the false codling moth, Daiber (1979) also recorded gaps, which resulted in confusion when head capsule measurements did not fall within a specific instar category. Hofmeyr et al. (2016) went on to amend these categories by removing gaps to avoid further confusion. Therefore, the same approach was taken in this study and gaps between instars were avoided by extending the relevant instar size categories to a midpoint between instars.

Moore et al. (2016a) showed that FCM larvae reared in artificial diet were suitable for demonstration of cold tolerance for post-harvest treatments due to their cold susceptibility being comparable to that in oranges. In this study, internal temperatures of products were significantly different and therefore not comparable. These differences in temperatures may have been a result of probe placement and the positioning of products within the cold room or internal quality of the products.

In lepidopteran larvae, the most cold-tolerant instars have been found to be either the final or the final two instars (Daiber 1979, Neven 2004, Moore et al. 2016a). The most cold tolerant larval stage of carob moth was the fifth instar. The corrected mortality of carob moth (oranges) and FCM (oranges and artificial diet) reached 100% after 12 days. Although there is no literature on the cold treatment of carob moth, Moore et al. (2016b) recorded 100% mortality of FCM in artificial diet after 15 days at -0.5oC. The observed reduction in time to reach 100% mortality in this study was most likely due to the small sample size.

The reduced efficacy of the 18-day cold treatment on corrected mortality of both species in pecans compared to citrus or artificial diet can be attributed to multiple variables. Acclimatization is the modification of an organism's physiology in response to natural environmental change (Follet and Neven 2006). In the field, thermal fluctuations are common and both insect and host commodity modify their physiology in response to these fluctuations (Follet and Nevan 2006). Infested pecans used in this study often only consisted of the outer shell due to larval feeding within the nut, and thus oranges provided a higher level of insulation. This may have altered the range of temperatures field collected larvae experienced prior to the cold treatment. Night time

temperatures in Vaalharts often fall below freezing, allowing larvae in a less insulated environment (pecans) to potentially acclimatize to sub-zero temperatures, increasing cold-hardiness.

The cold-hardiness of insects can be estimated through supercooling points (SCP) (Baust and Rojas 1985, Khani and Moharramipour 2010). The SCP is established through the measuring of the point at which body fluids undergo a phase change from liquid to solid as represented by the onset of the latent heat of fusion (Baust and Rojas 1985). The SCP of individual species can vary depending on whether the larva is overwintering or in diapause (Baust and Rojas 1985). Salt (1936, 1963) determined that surface moisture can affect SCP capacity dramatically, while water consumption can decrease SCP capacity (Cannon et al. 1985). Super-cooling points of both FCM (Boardman et al. 2012) and carob moth (Heydari and Izadi 2014) have been established. In both species it was found that with an increase of water content, a subsequent increase in SCP was recorded and larvae were less cold-tolerant than their counterparts with low water content. The water content of pecans is negligible and the tissue which larvae consume is 60-80% oil (Beuchat 1978), while water content of Navel oranges at the growth stage used in this study was between 70-80% (Pers comm Z. Zondi, Citrus research International Cultivar Evaluator). The low water content with the proposed acclimatization of larvae infesting pecans are both likely to contribute to lower SCPs in carob moth and FCM.

Conclusion

Carob moth head capsules size categories were established for larval instars. These were used to establish that the fifth instar is the least cold susceptible in this species. Results showed that the cold tolerance of FCM was significantly higher than carob moth. Therefore, all cold treatments effective against FCM will be as effective, if not more effective, against carob moth.

Technology transfer to date

Results were included in a presentation made at the Citrus Symposium in August.

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2.4.3 FINAL REPORT: Establishment of a monitoring system and control practices for carob moth on citrus

Project 1110 (Sep 2014 – Dec 2016) by Sean Thackeray, Martin Hill (RU), Sean Moore, Wayne Kirkman, Martin Gilbert and Peter Stephen (CRI)

Summary

The aim of this study was to evaluate the pest status of carob moth in citrus and establish a species specific IPM programme by determining the autecology of carob moth in citrus. Reliable methods for monitoring carob moth in citrus orchards both for producers and for research purposes were developed. A user-friendly monitoring method for determining weekly carob moth infestation through dropped fruit was suitable for producers. A timed scouting method was also developed; although the accuracy of this method varied with the experience of the scout. The pest status of carob moth was highest in the Loskop Valley, Nelspruit and the Vaalharts production areas and economic injury to growers ranged from R512.35 to R3 719.80 per hectare as a direct result of infestation. No infestation was recorded in the Sundays River Valley and Citrusdal production areas over both the 2014-15 and 2015-16 growing seasons. A laboratory study showed the survival of carob moth larvae infesting citrus is less than 10% in the absence of mealybug. However, this increases to almost 40% in the presence of mealybug residues and sooty mould. There was a significant relationship between carob moth infestation at harvest and mealybug infestation in the middle of the growing season. It is proposed that an orchard with a history of carob moth infestation and a high mealybug infestation in the previous season should be subjected to an early season preventative application of a registered control product. Also, if mealybug infestation in December is higher than a 5% of fruit per tree, then a corrective application of a registered product is recommended. The application of 2,4-D at petal drop reduced the size of the navel-end opening, decreasing the proportion of mealybug found in the navel-end, subsequently reducing carob moth infestation. Products registered for the control of false codling moth (FCM), *Thaumatotibia leucotreta* Meyrick, were effective in reducing carob moth infestation. In a spray trial conducted over two seasons, Delegate® and Runner® reduced infestation significantly in the 2014-15 season (over 80%), while only Delegate® was effective in the 2015-16 season (over 80%). If a late season corrective chemical application is targeted at both FCM and carob moth, this application should take place between 6-7 weeks prior to harvest. The mating disruption product, SPLAT® EC, reduced carob moth infestation by 70% compared to the untreated control. A laboratory culture was established and head-capsule size categories were determined for all five carob moth instars. A parasitoid survey indicated that parasitism of carob moth larvae is generally less than 5% in citrus

orchards and a new species of Braconidae was described as *Phanterotoma carobivora* van Achterberg and Thackeray. Cold sterilisation trials with carob moth are reported in a separate project (1151).

This full report is available in the form of an MSc thesis by Sean Thackeray, entitled “The pest status and integrated management programme of carob moth, *Ectomyelois ceratoniae* Zeller, attacking citrus in South Africa”, available through Rhodes University.

Opsomming

Die hoofdoel van hierdie studie was om die plaagstatus van karobmot op sitrus te evalueer en om ŉ spesie-spesifieke IPM program te ontwikkel wat gebasseer is op die ekologie van karobmot op sitrus. Betroubare metodes vir monitering van karobmot in sitrusboorde, albei vir produsente en navorsers, is ontwikkel. ŉ Verbruikervriendelike moniterings metode deur weeklikse opname van gevalde vrugte is geskik vir produsente. ŉ Tyd-gebasseerde verkennings metode is ook ontwikkel maar die akuraatheid van die metode het gewissel met die ondervinding van die verkenner. Die plaagstatus van karobmot is die hoogste in die Loskopvallei, Nelspruit en Vaalharts produksie streke. Ekonomiese skade aan produsente as ŉ direkte gevolg van besmetting het gestrek van R512.35 tot R3 719.80 per hektaar. Geen besmetting is in die Sondagsriviervallei en Citrusdal produksie streke gedurende albei die 2014-15 en 2015-16 produksie seisoene gekry nie. ŉ Laboratorium studie het gewys dat die oorlewing van karobmot in sitrus, sonder die teenwoordigheid van witluis, minder as 10% was. Hierdie syfer het tot amper 40% gegroei in die teenwoordigheid van witluis residue en roetskimmel. Daar was ŉ betekenisvolle verhouding tussen karobmot besmetting teen oestyd en witluis besmetting in die middel van die groei-seioen. Dit word aanbeveel dat ŉ boord met ŉ geskiedenis van hoë karobmot besmetting en hoë witluis besmetting in die vorige seisoen vroeg in die seisoen voorkomend met ŉ geregistreerde middel gespuit moet word. Daarbenewens as witluis besmetting in Desember hoër as 5% van die vrugte per boom is, word ŉ korrektiewe bespuiting aanbeveel. Die toediening van 2,4-D teen blomblaarval het die grote van die nawelent verminder, wat die proporsie witluis in die nawelent verminder het, wat toe karobmot besmetting verminder het. Produkte wat geregisteer is vir beheer van valskodlingmot (VKM), *Thaumatotibia leucotreta* Meyrick, het karobmot besmetting doeltreffend verminder. In ŉ spuitproef wat oor twee seisoene geloop het, het Delegate® en Runner® besmetting betekenisvol verminder in die 2014-15 seisoen (> 80%), maar net Delegate® was doeltreffend in die 2015-16 seisoen (> 80%). As ŉ laat seisoen korrektiewe chemiese bespuiting teen albei VKM en karobmot gemik word, moet die toediening tussen 6-7 weke voor oes plaasvind. Die paringsontwrigting produk, SPLAT® EC, het karobmot besmetting met 70% verminder in vergelyking met die onbehandelde kontrole. ŉ Laboratorium kultuur is gevestig en kopkapsule grote kategorië is vir al vyf karobmot instars bepaal. ŉ Parasiet opname het aangedui dat parasitisme van karobmot larwes in sitrusboorde oor die algemeen minder as 5% was. ŉ Nuwe spesie van Braconidae is as *Phanterotoma carobivora* van Achterberg en Thackeray beskryf. Koue-sterilisasie proewe met karobmot word in ŉ verskillende projek (1151) gerapporteer.

Hierdie volle verslag is beskikbaar in die vorm van ŉ MSc tesis deur Sean Thackeray, genaamd “The pest status and integrated management programme of carob moth, *Ectomyelois ceratoniae* Zeller, attacking citrus in South Africa”, beskikbaar deur Rhodes Universiteit.

Introduction

The carob moth, *Ectomyelois ceratoniae* Zeller, is a pest of agricultural commodities and stored products around the world. Carob moth is known to infest citrus in the Mediterranean region and in southern Africa. In grapefruit cultivars, carob moth infestations are associated with high levels of mealybug. However, although this relationship has been observed in other citrus types such as Navel oranges, this has never been quantified. A recent survey of infested fruit from various production areas in South Africa indicated that the pest status of carob moth on Navel oranges may have been underestimated. As a result of the incidental pest status of carob moth on citrus in South Africa in the past, a species specific integrated pest management (IPM) programme does not exist. Therefore, the overriding aim of this study was to evaluate the pest status of carob moth in citrus and establish a species specific IPM programme by determining the autecology of carob moth in citrus.

Objectives

- To develop a reliable monitoring system for carob moth
- To determine a relationship between trap catches and fruit infestation
- To establish life-cycle and behavioural patterns of carob moth in the field
- To determine the pest status of carob moth on citrus throughout the season and throughout the country
- To search for alternative hosts for carob moth in close proximity to citrus orchards
- To test a novel mating disruption product against carob moth

- To test various spray options for control of carob moth
- To conduct a survey for parasitoids of carob moth
- Establish a laboratory culture of carob moth
- Establish the relationship between the presence of mealybug and carob moth infestation in orchards

Materials and methods

Refer to Thackeray 2017 (Msc Thesis)

Results and discussion

Reliable methods for monitoring carob moth in citrus orchards both for producers and for research purposes were developed. A user-friendly monitoring method for determining weekly carob moth infestation through dropped fruit was suitable for producers. A timed scouting method was also developed; although the accuracy of this method varied with the experience of the scout. The pest status of carob moth was highest in the Loskop Valley, Nelspruit and the Vaalharts production areas and economic injury to growers ranged from an estimated R512.35 to R3 719.80 per hectare as a direct result of infestation. No infestation was recorded in the Sundays River Valley and Citrusdal production areas over both the 2014-15 and 2015-16 growing seasons.

A laboratory study showed the survival of carob moth larvae infesting citrus is less than 10% in the absence of mealybug. However, this increases to almost 40% in the presence of mealybug residues and sooty mould (Fig. 2.4.3.1). There was a significant relationship between carob infestation at harvest and mealybug infestation in the middle months of the growing season. The relationship between carob moth and mealybug indicates that current production guidelines for the management of mealybug in citrus may need to be amended (Table 2.4.3.1). Consequently, it is proposed that an orchard with a history of carob moth infestation and a high mealybug infestation in the previous season should be subjected to an early season preventative application of a registered control product. Also, if mealybug infestation in December is higher than a 5% of fruit per tree, then a corrective application of a registered product is recommended. The application of 2,4-D at petal drop reduced the size of the navel-end opening, decreasing the proportion of mealybug found in the navel-end, subsequently reducing carob moth infestation, resulting in a direct benefit for producers.

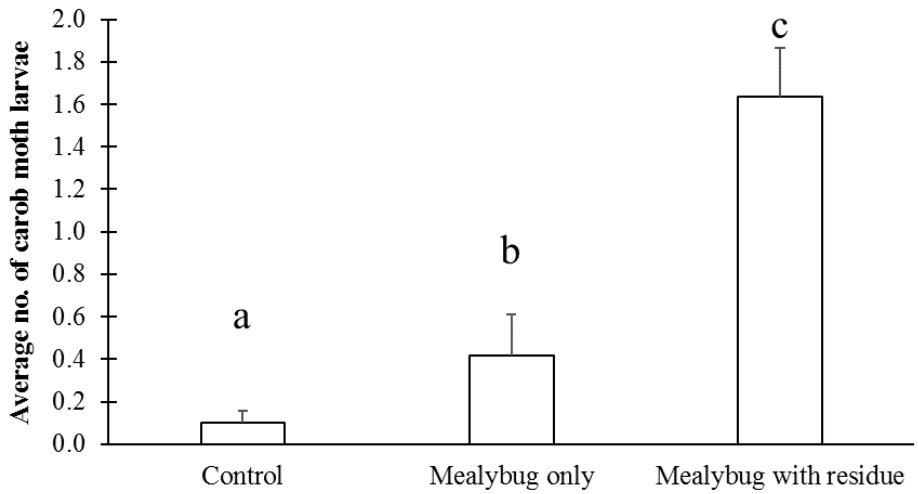

Fig. 2.4.3.1 Mean number of carob moth larvae recovered after three weeks on Washington Navel oranges with three different levels of mealybug infestation, a mealybug free control, mealybug only and mealybug with residue such as sooty mould and honeydew. Different letters indicate significant differences (P > 0.05) (Fisher's LSD).

Table 2.4.3.1. Results for regression analyses for mealybug and carob moth infestation in Navel and Valencia orange orchards.

Citrus Type	Retrospective regression	R^2	Degrees of Freedom	F value	P value
Navel	Week 6 of 2016 carob moth vs Week 50 of 2015 mealybug	0.157	1, 23	4.29	0.0498*

	Week 18 of 2016 carob moth vs Week 6 of 2016 mealybug	0.710	1, 23	56.43	0.0023*
	Week 18 of 2016 carob moth vs Week 50 of 2015 mealybug	0.232	1, 23	6.95	0.0147*
Valencia	Week 6 of 2016 carob moth vs Week 50 of 2015 mealybug	0.219	1, 13	3.65	0.0784
	Week 18 2016 carob moth vs Week 6 2016 mealybug	0.524	1, 13	14.30	0.0023*
	Week 18 of 2016 carob moth vs Week 50 of 2015 mealybug	0.011	1, 13	0.15	0.7080

* Indicates a P < 0.05 i.e. a significant relationship

Products registered for the control of false codling moth (FCM), *Thaumatotibia leucotreta* Meyrick, were effective in reducing carob moth infestation. In a spray trial conducted over two seasons, Delegate® and Runner® reduced infestation significantly in the 2014-15 season (over 80%), while only Delegate® was effective in the 2015-16 season (over 80%) (Fig 2). If a late season corrective chemical application is targeted at both FCM and carob moth, this application should take place between 6-7 weeks prior to harvest. The mating disruption product, SPLAT® EC, reduced carob moth infestation by 70% compared to the untreated control (Fig. 3).

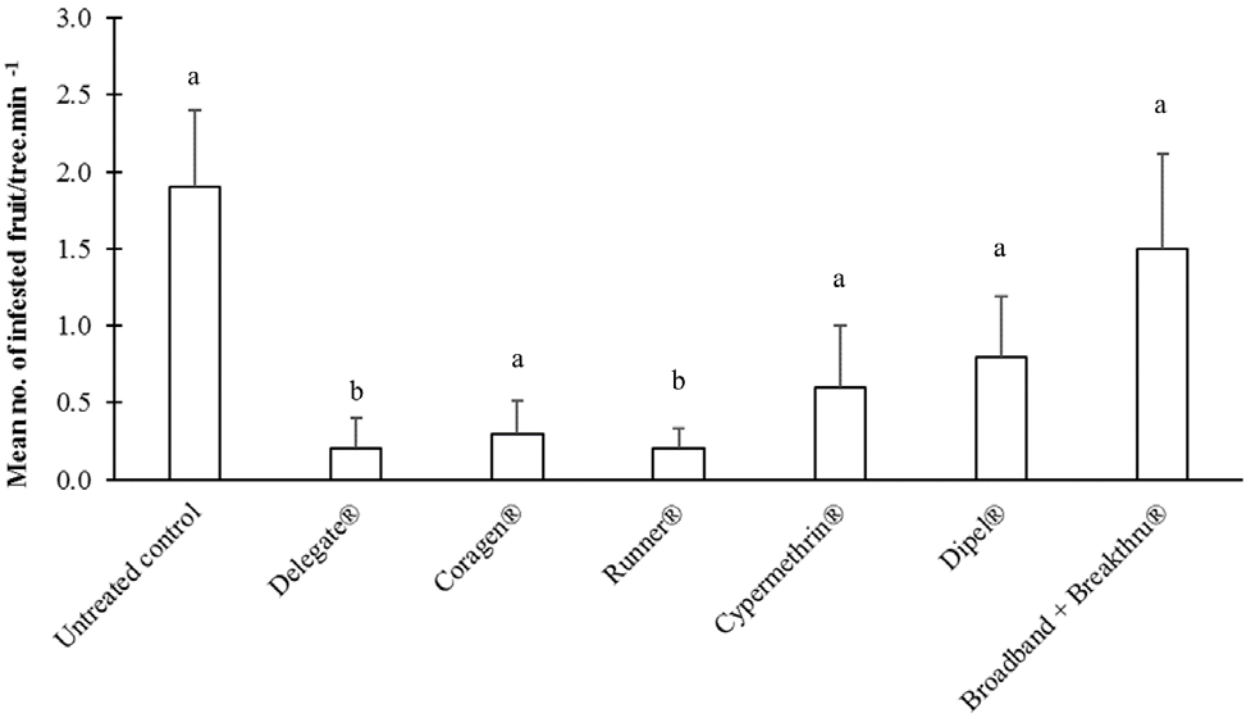

Fig. 2.4.3.2 Mean carob moth infestation per tree recorded in one minute at 11 weeks after treatment application in the 2014-15 growing season. Error bars show standard error from the mean. Different letters indicate significant differences (P < 0.05) (Tukey's post-hoc test).

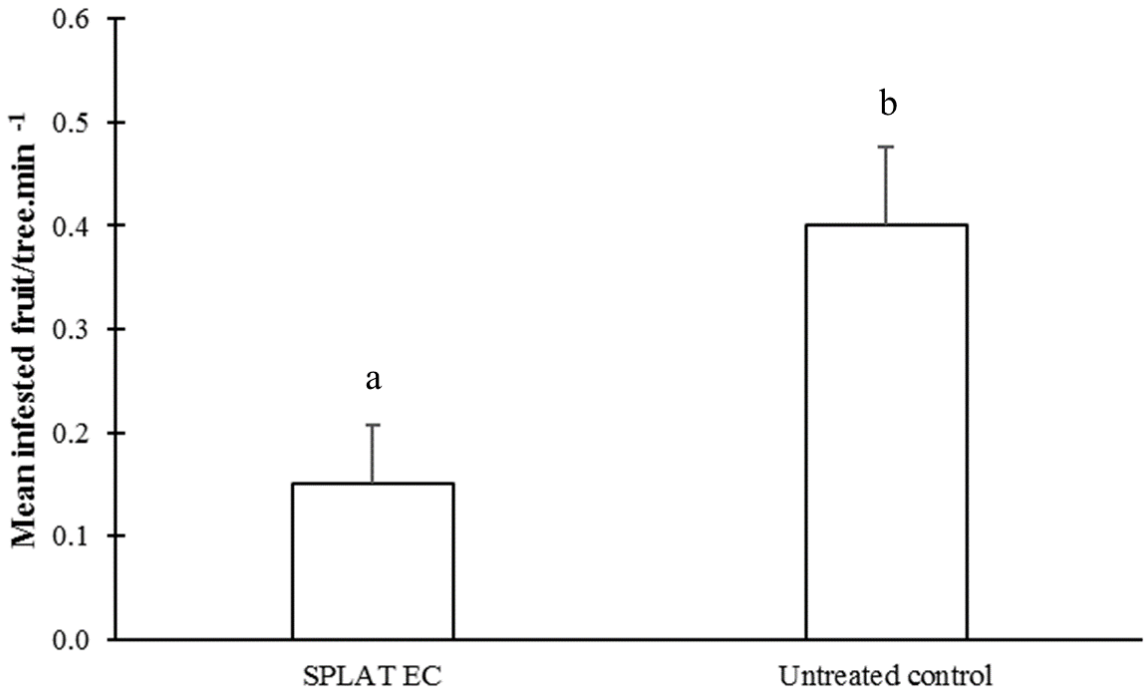

Fig. 2.4.3.3 Mean number of carob moth infested fruit in the SPLAT® EC and untreated control treatments in week 10 of 2016. Error bars show standard error from the mean. Different letters indicate significant differences ($P < 0.05$) (Tukey's post-hoc test).

A parasitoid survey indicated that parasitism of carob moth larvae is generally less than 5% in citrus orchards and a new species of Braconidae was described as *Phanterotoma carobivora* van Achterberg and Thackeray.

Conclusion

Refer to Thackeray 2017 (Msc thesis)

Future research

Future research should include how citrus cultivation under netting will influence the carob moth mealybug relationship as well as continued bioprospecting for a microbial control agent now that a methodology exists for laboratory rearing of carob moth.

Technology transfer

Peer-reviewed publications

Van Achterberg, C., Thackeray, S. R., Moore, S. D. & Hill, M. P. 2016. A new species of Phanerotoma Wesmal (Hymenoptera: Braconidae: Cheloninae) reared from carob moth in South Africa. Zootaxa, In press.

Thackeray, S.R., Kirkman, W. K., Moore, S. D. & Hill. M. P. 2017. Biology and rearing of Ectomyeolis ceratoniae Zeller (Lepidoptera: Pyralidae), carob moth, a pest of multiple crops in South Africa. African Entomology, In press.

Articles in popular press

Thackeray, S. R. 2015. Carob moth on citrus: where do we stand? Rostrum, December issue.

Scientific conference outputs

Thackeray, S. R., Kirkman, W., Moore, S. D. & Hill, M. P. The development of an IPM programme for carob moth on citrus in southern Africa.

Presented at the 9th Citrus Research Symposium 2016 (Drakensburg, South Africa) and the International Congress of Entomology 2016 (Orlando, USA).

Grower workshops

Thackeray, S., Kirkman, W. & Moore, S. IPM of carob moth on citrus.

Presented at the Citrus Research International Spring Pest Complex Growers Workshops (2015) in Hoedspruit, Groblersdal, Nelspruit, Jefferies Bay and Citrusdal.

2.4.4 PROGRESS REPORT: Evaluating GRAS post-harvest fumigants for phytosanitary pests

Project 913 (2011/2 – 2016/7) by T G Grout, P R Stephen and K C Stoltz (CRI)

Summary

Earlier research showing that Vapormate at 250 g/m^3 for 4 h at 15°C completely eliminated grain chinch bug adults and controlled the oribatulid mite *Siculobata sicula,* was published in Journal of Economic Entomology. Research during 2016/7 included eight trials with second instar Medfly and eight trials with fifth instar false codling moth in different citrus types using CO_2 fumigation at 60% followed by a short cold treatment at 2°C. The length of the cold treatment started at 9 days but one FCM larva was found to move in each of the trials with Satsuma and Nova mandarins. Even though these larvae died when placed on fresh fruit pulp and had no energy to penetrate, the cold period was extended to 11 days. All fifth instar FCM were killed with this treatment in Star Ruby, Clementine and Nadorcott mandarin but again one larva out of 329 showed movement in navel oranges, although it died within 24 h on fruit pulp. The cold period was again extended to 13 days for further trials in Turkey Valencia and Valencia with no moving larvae out of 438. Trials with Medfly were run in parallel to those with FCM and one moving Medfly larva was found in Nova mandarin when a 9 day cold treatment was used but no survivors were found in any other fruit types after 11 or 13 days. Previously, a comparison between third and fifth instar FCM following CO_2 fumigation at 60% and 6 days at 2°C, gave a mean mortality of 96% for the third instars and 82% for fifth instars. A further six trials were conducted to validate these results but two trials had to be scrapped due to fungal decay and high control mortality. Mortality levels were higher than found before with 100% mortality of 2 911 third instars. Forty-five fifth instars survived out of a total of 2 688 (98.3% mortality). Some trials will now be conducted to compare fumigation doses of 45 and 60% CO_2 before conducting verification trials of CO_2 fumigation followed by 13 days at 2°C.

Opsomming

Vroeëre navorsing wat aangetoon het dat Vapormate teen 250 g/m^3 vir 4 h teen 15°C graanstinkbesie volwassenes volledig uitgewis het en die oribatulid myt, *Siculobata sicula*, beheer het, is in die *Journal of Economic Entomology* gepubliseer. Navorsing gedurende 2016/7 het ag proewe met tweede instar Mediterreense vrugtevlieg en ag proewe met vyfde instar valskodlingmot (VKM) in verskillende sitrustipes ingesluit, deur gebruik te maak van CO_2 beroking teen 60%, gevolg deur ʼn kort koue-behandeling teen 2°C. Die lengte van die koue-behandeling het by 9 dae begin, maar een VKM larwe het nog in elk van die proewe met Satsuma en Nova mandaryn beweeg. Selfs al het hierdie larwes gedood toe hulle op vars vrugtepulp geplaas is, en hulle geen energie gehad het om te penetreer nie, is die koue-periode na 11 dae verleng. Alle vyfde instar VKM is met hierdie behandeling in Star Ruby, Clementine en Nadorcott mandaryn gedood, maar een larwe uit 329 het weereens beweging in navel lemoene getoon, hoewel dit binne 24 h op vrugtepulp gedood het. Die koue-periode is vir verdere proewe in Turkey Valencia en Valencia weereens na 13 dae verleng, met geen bewegende larwe uit 438. Proewe met Mediterreense vrugtevlieg is parallel aan die proewe met VKM geloop, en een bewegende Mediterreense vrugtevlieglarwe is in Nova mandaryn gevind toe ʼn 9-dae koue-behandeling gebruik is, maar geen oorlewendes is in enige ander vrugtetipe ná 11 of 13 dae gevind nie. Voorheen het ʼn vergelyking tussen derde en vyfde instar VKM, volgende op CO_2 beroking teen 60% en 6 dae teen 2°C, ʼn gemiddelde mortaliteit van 96% vir die derde instars en 82% vir die vyfde instars gelewer. ʼn Verdere ses proewe is uitgevoer ten einde hierdie resultate te verifieer, maar twee proewe moes geskrap word weens swambederf en hoë kontrole mortaliteit. Mortaliteitsvlakke was hoër as voorheen, met 100% mortaliteit van 2 911 derde instars. Vyf-en-veertig vyfde instars het uit ʼn totaal van 2 688 oorleef (98.3% mortaliteit). Proewe gaan nou uitgevoer word ten einde berokingsdosisse van 45 en 60% CO_2 te vergelyk voordat verifikasieproewe van CO_2 beroking, gevolg deur 13 dae teen 2°C, uitgevoer word.

2.4.5 PROGRESS REPORT: Trunk sprays for Fuller's rose beetle control

Project 1145 by Tim Grout and Peter Stephen (CRI)

Summary

This project has been terminated. Infestations of Fullers rose beetle appear to have declined markedly and it has been impossible to find trial sites. Perhaps Delegate, which is used for thrips control, may be reducing numbers of this weevil in the orchard because it is also registered for the control of weevils in other crops. Time assigned to this research project is being spent on other projects.

Opsomming

Hierdie projek is gestaak. Infestasies van Fullers rooskewer het klaarblyklik aansienlik gedaal en dit was onmoontlik om proefpersele te vind. Delegate, wat vir blaaspootjiebeheer gebruik word, kan moontlik getalle van hierdie kalander in die boord verminder aangesien dit ook vir die beheer van kalanders in ander gewasse geregistreer is. Tyd wat vir hierdie navorsingsprojek geoormerk was, is aan ander projekte spandeer.

2.4.6 PROGRESS REPORT: The natural enemies and biological control of *Delottococcus aberiae*

Project 1150 (Apr 2015 – Mar 2017) by Sean Moore and Wayne Kirkman (CRI)

Summary

Delottococcus aberiae, previously incorrectly identified as *D. elizabethae*, one of the seven mealybug species recorded to infest citrus in South Africa, has been considered extremely rare and very difficult to find, with no more than nominal pest status. However, since around 2012 fairly dramatic outbreaks of this species have been recorded on citrus in the Letsitele region, with an apparent gradual spread of the pest in the area. Due to the limited knowledge of the pest and its biological control, we proposed to regularly collect samples from infested orchards in Letsitele with the objective of monitoring the species of parasitoids and the levels of parasitism. As was the case during the previous season, infestation levels of *D. aberiae* on citrus in the Letsitele region were again very low during the 2016/7 season. Two collections were conducted but no parasitoids emerged from either of these collections. Contact has been made with a Spanish postgraduate student, who is also working on parasitism of *D. aberiae*, including in the Letsitele region, and results from this study will become available to us in time.

Opsomming

Delottococcus aberiae, voorheen bekend as *D. elizabethae*, een van die sewe witluis spesies wat op sitrus in Suid-Afrika aangeteken is, is as baie skaars en moeilik om te vind beskou, en het daarom net nominale plaagstatus. Van omtrent 2012 is redelike dramatiese uitbrake van die spesie op sitrus in die Letsitele streek egeter plaasgevind, met ŉ oënskynlike geleidelike verspreiding van die plaag in die omgewing. As gevolg van die beperkte kennis van die plaag en sy biologiese beheer, ons het voorgestel om gereeld monsters van besmette boorde in Letsitele te versamel met die doel om parasiet spesies en vlakke van parasitisme to monitor. Soos wat die geval gedurende die vorige seisoen was, was besmettings vlakke van *D. aberiae* op sitrus in die Letsitele streek weer gedurende die 2016/7 seisoen baie laag. Monsters is op twee verskillende geleenthede versamel maar geen parasiete is gekry nie. Kontak is met ŉ Spaanse nagraadse student gemaak, wat ook op parasitisme van *D. aberiae* werk, insluitend in die Letsitele streek, en resultate van hierdie studie sal in die toekoms vir ons beskikbaar wees.

2.4.7. PROGRESS REPORT: Suitability of entomopathogenic fungal isolates for microbial control of citrus pests: biological traits and effects of formulation

Project 1143 (Apr 2016 – Dec 2018) by Mavis Acheampong, Martin Hill, Candice Coombes (RU) and Sean Moore (CRI)

Summary

Temperature tolerance, determined by radial growth of seven selected fungal isolates and two commercial mycopesticides (Broadband® a.i. *B. bassiana* PPRI 5339 and Real IPM a.i. *M. anisopliae* ICIPE 69) at various temperatures (8-40°C), was studied in the laboratory. All fungal isolates generally grew at 16-27°C and optimally at 20 and 27°C. At the optimal temperatures, the two *M. anisopliae* isolates (FCM Ar 23 B3 and G 11 3 L6) recommended for further studies recorded significantly higher radial growth (2.04-3.04 mm/day) than the *B. bassiana* isolates (0.58-1.88 mm/day) and the two commercial mycopesticides (1.36-2.44 mm /day). At 8°C, growth was marginal for all *B. bassiana* isolates, including the commercial product (0.11 -0.20 mm/day), and completely absent for all *M. anisopliae* isolates, including the commercial product. The thermal threshold for all isolates was 35°C. *Beauveria bassiana* isolate, G Ar 17 B3 (which has also been recommended for further studies) recorded the least radial growth, 0.34, 0.58 and 0.66 mm/day at 16, 20 and 27°C, respectively. Moisture requirements and endophytic potential of these isolates is currently being tested.

Opsomming

Temperatuur verdraagsaamheid, bepaal deur radialegroei van sewe geselekteerde swam isolate en twee kommersiële swam produkte (Broadband® a.i. *B. bassiana* PPRI 5339 en Real IPM a.i. *M. anisopliae* ICIPE 69) teen verskeie temperature (8-40°C), is in die laboratorium ondersoek. Alle swam isolate het oor die algemeen teen 16-27°C gegroei en teen 20 en 27°C optimaal gegroei. Teen die optimale temperature het die twee *M. anisopliae* isolate (VKM Ar 23 B3 en G 11 3 L6) wat vir verdere studies voorgestel is, aansienlik hoër radialegroei (2,04-3,04 mm / dag) aangeteken in vergelyking met die *B. bassiana* isolate (0,58-1,88 mm / dag)

en die twee kommersiële produkte (1,36-2,44 mm / dag). Teen 8°C was daar min groei gewees vir alle *B. bassiana* isolate, insluitend die kommersiële produk (0.11 -0,20 mm / dag), en was heeltemal afwesig vir alle *M. anisopliae* isolate, insluitend die kommersiële produk. Die termiese drempelwaarde vir alle isolate was 35°C. *Beauveria bassiana* isolaat, G Ar 17 B3 (wat ook voorgestel is vir verdere studies) het die minste radialegroei aangeteken, 0.34, 0.58 en 0.66 mm / dag teen 16, 20 en 27°C, onderskeidelik. Vog vereistes en endofitiese potensiaal van hierdie isolate word tans ondersoek.

2.5 PROGRAMME: NON-PHYTOSANITARY KEY PESTS

Programme Coordinator: Tim G Grout (CRI)

2.5.1 Programme summary

Research in this programme is currently focused on the control of various pests shortly before harvest and developing techniques to control the Asian citrus psyllid *Diaphorina citri* before it arrives in South Africa. The drought, or natural enemies have reduced populations of woolly whitefly in the northern regions and we have been unable to re-establish a culture, so progress on late season plant protection products has been slow (2.5.2). The screening of prospective new systemic insecticides against aphids has shown that some new products may be effective as soil drenches or stem treatments for the control of *D. citri* (2.5.3). Attempts to control both *D. citri* and *Trioza erytreae* in Mauritius using products available in South Africa have had a degree of success but having to rely on traps for treatment differences has not been ideal (2.5.4). All these projects will continue in 2017/8.

Programopsomming

Navorsing in hierdie program fokus tans op die beheer van verskeie plae net voor oes, en die ontwikkeling van tegnieke om die Asiatiese sitrus bladvlooi, *Diaphorina citri*, te beheer voordat dit Suid-Afrika binnekom. Die droogte of natuurlike vyande het populasies van wollerige witvlieg in die noordelike dele verminder, en ons was onsuksesvol om ŉ kultuur te hervestig, so vordering op láát seisoen plantbeskermingsmiddels was stadig (2.5.2). Die keuring van moontlike nuwe sistemiese insekdoders teen plantluise het aangedui dat sommige nuwe middels effektief as gronddoopmiddels of stambehandelings vir die beheer van *D. citri* kan wees (2.5.3). Pogings om beide *D. citri* en *Trioza erytreae* in Mauritius te beheer, deur gebruik te maak van middels wat in Suid-Afrika beskikbaar is, het ŉ mate van sukses getoon, maar deur op lokvalle vir behandelingsverskille staat te maak, was nie ideaal nie (2.5.4). Al hierdie projekte sal in 2017/8 voortgaan.

2.5.2 PROGRESS REPORT: Short residual treatments for thrips, psylla, leafhoppers and woolly whitefly for late season usage

Project 1061 (2013/4-2017/8) by Tim G Grout and Peter R Stephen (CRI)

Summary

There is a shortage of registered control options that can be used for late season control of thrips, citrus psylla, leafhoppers and woolly whitefly. The objective of this research is to evaluate unregistered products, and products that have been recently registered on citrus against other pests that are likely to have short preharvest intervals. Unfortunately, the drought reduced populations of woolly whitefly in the field and we were unable to restart a culture. A possible site for a psylla trial was evaluated but the population was not evenly distributed and the numbers too low. The registrar was encouraged to make a call for products that could be used under an emergency registration for leafhoppers and towards the end of the report period plans were made for field trials against leafhoppers with some of the prospective products. This research will continue whenever suitable trial sites become available.

Opsomming

Daar is ŉ tekort aan geregistreerde beheermaatreëls om blaaspootjies, sitrusbladvlooi, bladspringers en wollerige witvlieg (WWV) láát in die seisoen te beheer. Die doel van hierdie navorsing is om ongeregistreerde middels en middels wat onlangs teen ander sitrusplae geregistreer is, met moontlike kort vóóroes intervalle, te evalueer. Ongelukkig het die droogte populasies van wollerige witvlieg in die veld verminder en ons was onsuksesvol om ŉ kultuur te hervestig. ŉ Moontlike area vir ŉ sitrusbladvlooiproef is geëvalueer, maar die populasie was nie eweredig versprei nie en die getalle te laag. Die registrateur is aangemoedig om te vra na middels wat in ŉ noodregistrasie vir bladspringers gebruik kan word, en teen die einde van die verslagperiode is planne vir veldproewe met sommige van die moontlike middels teen bladspringers gemaak. Hierdie navorsing sal voortgaan soos wat geskikte proef-areas beskikbaar kom.

2.5.3 PROGRESS REPORT: New systemic insecticides for citrus

Project 1148 (2016/7-2017/8) by Tim G Grout and Peter R Stephen (CRI)

Summary

In order to prepare for the arrival of *Diaphorina citri* in South Africa, we need to find more systemic insecticides that can be used frequently in nurseries and for non-bearing trees. The brown citrus aphid *Toxoptera citricida* was used as an indicator pest for screening systemic insecticides on potted lemon trees. Due to the current demand for lemons it took a long time to acquire the trees and then infest them with aphids but towards the end of the report period we were able to screen a few chemicals. The registered imidacloprid drench and recently registered acephate (Spectra Stem) stem treatment were used as standards and resulted in all aphids dropping off the leaves within 7 days. Two dosages of sulfoxaflor as a drench gave the same result after 7 days, although the mortality rate was slower. Results from an unregistered product, used in some countries as a soil drench for vegetables, were disappointing. Further trials on potted plants with these and other chemicals will be conducted, followed by field trials against aphids on non-bearing trees. We will then be in a position to evaluate the better treatments against African citrus psylla if trial sites become available.

Opsomming

Ten einde vir die binnekoms van *Diaphorina citri* in Suid-Afrika voor te berei, moet ons meer sistemiese insekdoders vind wat gereeld in kwekerye en vir nie-draende bome gebruik kan word. Die bruin sitrus plantluis, *Toxoptera citricida*, is as indikatorplaag gebruik vir die keuring van sistemiese insekdoders op suurlemoenbome in potte. Weens die huidige aanvraag vir suurlemoene, het dit ŉ lang tyd geneem om die bome te bekom en dan met plantluise te infesteer, maar teen die einde van die verslagperiode was ons in staat om ŉ paar chemikalieë te keur. Die registreerde imidacloprid doopmiddel en onlangs geregistreerde acephate (Spectra Stem) stambehandeling, is as standaarde gebruik, en het daartoe gelei dat alle plantluise binne 7 dae van die blare afgeval het. Twee dosisse van sulfoxaflor as ŉ doop het dieselfde resultaat ná 7 dae gelewer, hoewel die mortaliteitstempo stadiger was. Resultate van ŉ ongeregistreerde middel, wat in sommige lande as ŉ gronddoop vir groente gebruik word, was teleurstellend. Verdere proewe op plante in potte met hierdie en ander chemikalieë sal uitgevoer word, gevolg deur veldproewe teen plantluise op nie-draende bome. Ons sal dan in ŉ posisie wees om die beter behandelings teen die Afrika sitrus bladvlooi te evalueer wanneer proef-areas beskikbaar kom.

2.5.4 PROGRESS REPORT: Control of Asian Citrus Psyllid, vector of Huanglongbing

Project 1158 (2016/7 – 2017/8) by Aruna Manrakhan, Hennie Le Roux, Tim Grout, Sean Moore, Glynnis Cook, Fanie Van Vuuren (CRI), Herbert Wiehe (Domaine de Labourdonnais, Mauritius), Preeaduth Sookar (Entomology Division, Ministry of Agro-Industry and Food Security, Mauritius), Malini Alleck (Entomology Division, Ministry of Agro-Industry and Food Security, Mauritius)

Summary

The Asian citrus psyllid (ACP), *Diaphorina citri*, is an important pest of citrus in many parts of the world due to its effective ability to vector Huanglongbing (HLB) caused by the bacteria *Candidatus* Liberibacter asiaticus (Las). ACP was reported in Tanzania in 2016 and is an important threat to the citrus industry in South Africa. In view of the potential introduction of ACP in South Africa, the question being raised is whether the control measures which are recommended for the citrus psylla *Trioza erytreae* in South Africa will be as effective against ACP and against transmission of the greening disease. A study was therefore initiated in October 2016 in a Valencia orchard at Domaine de Labourdonnais, Mauritius, to test the efficacy of a South African citrus psylla treatment package on ACP. Mauritius was deemed a suitable study site since both ACP and citrus psylla are known to be present as well as both Las and *Candidatus* Liberibacter africanus (Laf). The South African citrus psylla treatment package consisted of a trunk treatment with acetamiprid 20% SL followed a month later by application of imidacloprid 305 SC through soil drenching followed 5 months later by a trunk treatment with acephate 350 AL. The South African citrus psylla treatment package was compared to untreated blocks. There were four replicates of treated and untreated blocks. Efficacy of control of ACP was determined by trapping. Three yellow sticky traps were placed in the middle of each block. Traps were replaced every week. *Diaphorina citri* specimens found on the traps were identified and counted. Results from October 2016 to March 2017 indicate that the numbers of *D. citri* were generally lower in the treated blocks (mean of 0.05 ± 0.01 per trap per week) compared to the untreated blocks (mean of 0.11 ± 0.03 per trap per week).

Opsomming

Die Asiatiese sitrus bladvlooi (ACP), *Diaphorina citri*, is ŉ belangrike pes van sitrus in baie dele van die wêreld weens sy effektiewe vermoë om as vektor vir Huanglongbing (HLB), veroorsaak deur die bakterie *Candidatus*

Liberibacter asiaticus (Las), op te tree. ACP is in 2016 in Tanzanië aangeteken en is ŉ belangrike bedreiging vir die sitrus-industrie in Suid-Afrika. In die lig van moontlike inbring van ACP in Suid-Afrika, moet die vraag gevra word of die beheermaatreëls wat vir die sitrus bladvlooi, *Trioza erytreae*, in Suid-Afrika aanbeveel word, ewe effektief teen ACP en teen oordraging van die vergroeningsiekte sal wees. ŉ Studie is gevolglik in Oktober 2016 in ŉ Valencia boord by Domaine de Labourdonnais, Mauritius, begin ten einde die effektiwiteit van ŉ Suid-Afrikaanse sitrus bladvlooi behandelingspakket teen ACP te toets. Mauritius is as ŉ geskikte studie-area gekies aangesien beide ACP en sitrus bladvlooi daar voorkom, asook beide Las en *Candidatus* Liberibacter africanus (Laf). Die Suid-Afrikaanse sitrus bladvlooi behandelingspakket het uit ŉ stambehandeling met acetamiprid 20% SL bestaan, ŉ maand later gevolg deur toediening van imidacloprid 305 SC deur gronddrenking, en 5 maande later gevolg deur ŉ stambehandeling met acephate 350 AL. Die Suid-Afrikaanse sitrus bladvlooi behandelingspakket is met onbehandelde blokke vergelyk. Daar was vier herhalings van behandelde en onbehandelde blokke. Effektiwiteit van beheer van ACP is deur middel van lokvalle bepaal. Drie geel klewende lokvalle is in die middel van elke blok geplaas. Lokvalle is elke week vervang. *Diaphorina citri* spesimens wat op die lokvalle gevind is, is geïdentifiseer en getel. Resultate vanaf Oktober 2016 tot Maart 2017 dui aan dat die getalle van *D. citri* oor die algemeen laer in die behandelde blokke was (gemiddelde van 0.05 ± 0.01 per lokval per week) in vergelyking met die onbehandelde blokke (gemiddelde van 0.11 ± 0.03 per lokval per week).

2.6 PROGRAMME: MINOR PESTS AND MITES

Programme Coordinator: Tim G Grout (CRI)

2.6.1 Programme summary

The only project receiving funding in this programme during 2016/7 was the release of the parasitoid *Cales noacki* for the control of woolly whitefly (2.6.2). This parasitoid has now become established in parts of the North-West Province, Mpumalanga and the Western Cape but natural dispersion appears to be slow. Further attempts will be made to try to get it established in the Eastern Cape.

Programopsomming

Die enigste projek wat in hierdie program gedurende 2016/7 befondsing ontvang het, was die vrylating van die parasitoïed *Cales noacki* vir die beheer van wollerige witvlieg (2.6.2). Hierdie parasitoïed het nou in dele van die Noordwesprovinsie, Mpumalanga en die Wes-Kaap gevestig, maar natuurlike verspreiding blyk stadig te wees. Verdere pogings sal aangewend word om dit in die Oos-Kaap gevestig te probeer kry.

2.6.2 PROGRESS REPORT: Importing and releasing *Cales noacki* for the control of woolly whitefly

Project 1082 (2014/5 – 2016/7) by T G Grout and P R Stephen (CRI)

Summary

Cales noacki, the parasitoid of woolly whitefly (WWF) that we imported from Spain, has become established in Mooinooi in the North-West Province and in Nelspruit, but not where releases were made in the Eastern Cape. Populations of WWF were low in the north due to the drought and numbers of parasitoids were therefore too low to make further releases in the Eastern Cape. Surprisingly, *C. noacki* has become established in the Western Cape, even though we made no releases there. These populations can then be used to make further releases in the Western and Eastern Cape.

Opsomming

Cales noacki, die parasitoïed van wollerige witvlieg (WWV) wat ons vanaf Spanje ingevoer het, het in Mooinooi in die Noordwesprovinsie en in Nelspruit gevestig, maar nie waar vrylatings in die Oos-Kaap gemaak is nie. Populasies van WWV was laag in die noorde weens die droogte, en parasitoïed getalle was gevolglik te laag om verdere vrylatings in die Oos-Kaap te maak. *C. noacki* het verbasend genoeg in die Wes-Kaap gevestig, hoewel ons geen vrylatings daar gemaak het nie. Hierdie populasies kan dan gebruik word om verdere vrylatings in die Wes- en Oos-Kaap te maak.

3 PORTFOLIO: DISEASE MANAGEMENT

3.1 PORTFOLIO SUMMARY

By Dr Jan van Niekerk (Portfolio Manager: Disease Management, CRI)

Effective management of soilborne, fruit and foliar diseases, postharvest diseases and graft transmissible diseases forms an integral part in the production of high quality export citrus. Within the Disease Management portfolio there are specific research programmes, each led by an expert researcher, in these specific research areas. The aim of all research programmes are to address current industry research needs within these different areas, while also being proactive in doing research in the management of diseases that are expected to be industry challenges in the future.

Within the Graft Transmissible Diseases (GTD) group "Huanglongbing" (HLB) or "Asian Greening" has been identified as an eminent threat to the South African industry. This was mainly due to the confirmed presence of '*Candidatus*' Liberibacter asiaticus (Las) and its vector, *Diaphorina citri,* on the African continent. However, due to many years' research done within CRI, the correct identification of the Liberibacter species present in East Africa was possible. Analysis of survey samples from this region identified less aggressive '*Candidatus*' Liberibacter africanus (Laf) species as being present in Uganda, Kenya and Tanzania, confirming that Las is only present in Ethiopia (3.2.7).

Despite the focus on Liberibacter species, strong focus remains within GTD on *Citrus tristeza virus* (CTV) due to the use of cross-protection to mitigate the effects of this virus within the Citrus Improvement Scheme (CIS). Due to the progress made in CTV strain identification, the specific strains involved in disease expression and those required to mitigate the disease, can now be characterized accurately. Single strain sources can be studied in glasshouse and field trials to determine their impact on the host and their potential for use in cross-protection. More specific CTV-host interaction studies and advanced virus diagnostics using bioinformatics are enabling researchers to more deeply study the biology of this virus (3.2.6).

Field trials assessing the various CTV sources indicated that the current LMS6 CTV pre-immunization source for sweet oranges are still the best option and its use in pre-immunization should be continued (3.2.2 and 3.2.4). Within grapefruit trials, results have shown that single strain CTV sources are more promising than mixed strain sources. Effect of climate on symptom expression is furthermore evident from these trials (3.2.2 and 3.2.5). Investigation into CTV and its role in soft citrus is also being done (3.2.8), while 4.2.9 results have already shown in a glass house trial that CIS material, that are free from viruses and viroids, are performing better than contaminated field-cut material.

Soilborne disease research are focusing on soilborne pest and diseases of citrus. Looking for alternatives that are more sustainable than chemicals are ongoing. Furthermore, there are studies also investigating the complex etiology of citrus decline and replant problems, looking at factors that are early warning signs. Then unknown diseases and their causes are getting attention, specifically what causes them and how to manage them.

Long term studies of pre-and postplant management of nematodes and *Phytophthora* are showing that preplant fumigation are having an effect with trees in these treatments being taller with thicker trunks than trees in other treatments (3.3.3). Evaluation of alternative, non-chemical treatments, to replace chemical nematicides, have shown that the products have variable results and that the best results are often achieved in regimes where the alternative is combined with one chemical application (3.3.4). Phosphonate applications on mandarin fruit were also shown to cause sever phytotoxic burn if these applications were done after colour break.

A complex of pathogens was found to be involved with a trunk rot and decline disease observed by growers in the Eastern Cape production region and that high soil and water pH in this region are possibly predisposing the Carrizo citrange and Swingle citromelo rootstocks to this pathogen complex. This postulation is currently under further investigation (3.3.5). Citrus decline is also seen as having a complex etiology of biotic and or abiotic factors and in Project 1092 (3.3.7) these are investigated further to look for any factors that can be used as early warning of decline problems. Citrus replant problems are more and more experienced due to old orchards being replaced and Project 1152 is investigating this to identify all the soilborne pests and pathogens that could possibly be involved. To date results indicated that oomycete and ascomycete (fungi and fungal-like) pathogens along with the citrus nematode are probably responsible for the stunted growth observed in replant orchards.

The rapid expansion in the industry as well as replant programmes implemented, is putting a strain on nursery production and in Project 1101 (3.3.6), the preventative and curative soilborne disease management practices followed in citrus nurseries are investigated through pathogen characterization and fungicide sensitivity work. Results from this project has already led to recommendations to nurseries.

Postharvest fungicides are applied in various manner during the packhouse procedure. Imazalil (IMZ) and thiabendazole are widely used as it provides curative control in sporulation inhibition of *Penicillium* spp. although it does not provide much control of sour rot. IMZ are therefore not recommended to be included in drench treatments and also as an anti-resistance measure. In the drench Pyrimethanil (PYR) and thiabendazole are seen as the man components. The addition of sanitisers (chlorine and HPPA) to fungicide mixtures contributed to sour rot control while still giving good control of *Penicillium* spp. HPPA were effective at short exposure times at high pH and can therefore easily be incorporated in a drench system where pH is not regulated. PYR residue loading were furthermore found to vary between top and bottom bins in a drench stack with fruit in top bine having higher residue values. Dip tank recommendations with regards to pH, temperature, exposure time and their effects on chemicals will be finalized in 2017 and provided to packhouses (3.5.4).

Postharvest fungicides in use in packhouses along with alternative fungicides were evaluated for their effect on latent CBS infection and the reproductive ability of CBS lesions. It was seen that the full packhouse treatment combined with cold storage significantly reduced development of latent infections. Reproductive ability of lesions were found to be low, with few of the lesions developing pycnidia. The combined epidemiological requirements for pycnidiospore release along with results from trials indicate that harvested citrus fruit is not an epidemiologically significant pathway for the spread of CBS (3.5.2). Essential oils (EO) are known to have an effect on postharvest pathogens and the EO are therefore being tested to encapsulating them into a slow-release nano-or micro particles. Specialized equipment and methods were developed for this work and extensive data was gathered with this that are under analysis (3.5.8).

Alternative and new products were tested and the most successful found to be an azoxystrobin formulation against *Penicillium digitatum*. Combination products of hydrogen peroxide and acetic acid were seen to have no effect on the sensitivity of pyrimethanil and stability of propiconzole. A powder formulation of PAA was developed and tested and seen not to have the phytotoxic problems of liquid formulations. Water sanitation is also under focus with several products being tested (3.5.3). Propiconazole has been registered in South Africa for sour rot control and this necessitated the determination of the baseline sensitivity of South African isolates of *G. citri-aurantii* causing sour rot and *Penicillium digitatum* causing green mould (3.5.6).

The role of phytochemicals, produced by citrus fruit, in inhibiting the infection of fruit by *Phyllosticta citricarpa* (citrus black spot, CBS), is investigated. Cultivars with varying susceptibility to CBS are included and investigated with respect to their apolar (waxes, lipids, oils) and polar (flavonoids, anthocyanidins, alkaloids, glycocides) fractions in their rind phytochemistry. Included are Bitter Seville, which is accepted have low susceptibility to CBS infection, Valencia orange with medium susceptibility, and highly susceptible lemon and kumquats as a resistant type (3.5.5).

Fungal decay of wooden pallet bases are in the spotlight as it soils cartons and contribute to decay for cartons and fruit. The causes of this phenomenon is unknown and Project 1165 (3.5.7) are investigating the fungal species involved as well as any other factors such as storage methods, and the possible role of environmental factors (for instance moisture, UV degradation, and insect infestation). Results of this study will enable manufacturers and users of the wooden pallet bases to prevent fungal contamination and other associated problems.

Citrus black spot (CBS) and Alternaria brown spot (ABS) are two major fruit and foliar diseases that hamper the export of citrus fruit to fresh markets by South African producers. The focus of the Fruit and Foliar (with CBS) programme is to study the epidemiology and control aspects of these diseases. The epidemiology of CBS is not fully understood and strict regulations have been implemented by certain of our existing export markets, on the use of certain products registered for the control of ABS.

Citrus black spot, caused by *Phyllosticta citricarpa*, occurs in many citrus growing areas but in the EU it is subject to phytosanitary legislation. The occurrence of *Phyllosticta* spp. in Europe were determined via surveys of citrus orchards, nurseries and in gardens in EU citrus regions (3.4.10). *Phyllosticta* spp. were found to occur with limited occurrence in the EU and never in association with infections. CBS symptoms were never observed, indicating that the fungi persist but does not cause disease.

In Project 977 (3.4.4) 383 isolates of *P. citricarpa* were genotyped and this along with mating type analysis revealed that both mating types were present in the populations from South Africa, China, Australia and Brazil at an approximately 1:1 distribution, but the USA population harboured only a single mating type. The populations of this pathogen in South Africa, Australia and Brazil were found to be highly connected which was contributed to plant material exchanges during establishment of the citrus industries in these countries.

Project RCE-6 (3.4.7) is addressing knowledge gaps with regards to the epidemiology of the CBS pathogen. It was seen that high spore germination is achieved with spores older than 1 day and from the second and following spore generations formed by pycnidia. Tolerance of lime trees to CBS was furthermore seen not to be due to the germination process and that even after 3 h of dryness pycnidiospores can still germinate as early as 4 h after inoculation. An optimized qPCR protocol was developed to quantify *P. citricarpa*

pycnidiospores obtained from spore traps. This protocol could however, not reliably quantify spore numbers below 1000 spores. Project RCE-7 (3.4.8) incorporated different CBS related epidemiological models along with weather data into a web-based platform (PhytRisk) that can assist growers with CBS decision making. A mobile phone version is under development while the CBS model validation is also in progress. Validation and improvement of the CBS models are done in RCE-8 (3.4.9) where new spore trap and weather data are used for this purpose. This follows on the termination of some of the objectives in RCE-8 due to the failure of planned and amended methodologies to yield any results for these objectives.

Due to the need to get complete control of pests and diseases that are of market access concern, high volume spray applications are used in South Africa. Although these are effective, they are costly and environmentally unsustainable. Projects 1132 (3.4.6) and 1089 (3.4.5) are therefore aimed at evaluating the efficacy of reduced volume applications for pest and disease control while also developing a canopy density based calibration model with the use of novel technology. Certain markets furthermore implemented strict regulations on mancozeb use for ABS control and Project 750 (3.4.2) are therefore continuously testing alternative fungicides and specifically RB-1. To date this new fungicide was shown to be ineffective as a replacement for mancozeb in ABS control programmes.

PORTEFEULJE OPSOMMING

Die effektiewe bestuur van grondgedraagde, blaar-en vrugsiektes, na-oes siektes en entoordraagbare siektes mak ŉ integrale deel uit van die produksie van uivoersitrus van ŉ hoë kwaliteit. Binne die Siektebestuur portfolio is daar dus navorsingsprogramme wat elk gelei word deur ŉ ekspert navorser en fokus op hierdie verskillende areas. Die doel van die navorsingsprogramme is om huidige industriebehoeftes aan te spreek terwyl daar ook proaktiewe navorsing gedoen word op siektes wat verwag word om binnekort uitdagings aan die industrie te bied.

Binne die Entoordraagbare Siektes groep is “Huanglongbing” (HLB) of “Asiatiese Vergroening” identifiseer as ŉ dreigende gevaar vir die Suid-Afrikaanse industrie. Dit was hoofsaaklik as gevolg van die bevestigde teenwoordigheid van ‘*Candidatus*’ Liberibacter asiaticus (Las) en die vektor, *Diaphorina citri*, op die Afrika kontinent. Bie jare se navorsing binne CRI het egter daartoe gelei dat die Liberibacter species teenwoordig in Oos-Afrika korrek identifiseer kon word. Analise van opname monsters uit hierdie areas het getoon dat ŉ minder aggressiewe *Candidatus*’ Liberibacter africanus (Laf) spesies teenwoordig is in Uganda, Kenia en Tanzanië end at Las dus huidiglik net in Etihopiëteenwoordig is (3.2.7).

Ten spyte van die fokus op Liberibacter spesies, is daar steeds ŉ sterk fokus op *Citrus tristeza virus* (CTV) as gevolg van die gebruik van kruisbeskerming binne die Sitrusverbeteringskema (SVS) om die effek van hierdie virus te verminder. As gevolg van die vordering wat daar gemaak is in die identifikasie van CTV rasse, kan die spesifiekte rasse betrokke by simptoomuitdrukking en die wat nodig is om hul effek te verminder, nou akkuraat gekarakteriseer word. Enkel ras bronne kan dus nou in glashuis en veldproewe bestudeer word ten einde hul impak op die gasheer te bepaal asook hul potensiaal vir kruisbeskerming. Meer spesifiekte CTV-gasheer interaksies en gevorderde virusdiagnostiek deur middel van bioinformatika maak dit nou vir naorsers moontlik om die biologie van die virus meer in diepte te bestudeer (3.2.6).

Veldproewe wat verskillende CTV bronne evalueer het, het aangedui dat LMS6 steeds die beste bron is vir die preïmmunisering van soetlemoene end us kan die grbuik daarvan voortgaan (3.2.2 en 3.2.4). In pomelo proewe is bevind dat enkel ras CTV bronne meer potensiaal het as gemende ras bronne. Die effek van klimaat op simptoomuitdrukking was ook duidelik uit hierdie proewe (3.2.2 en 3.2.5). CTV en die rol binne sagte sitrus word ook ondersoek (3.2.8) terwyl dit in glashuisproewe reeds bewys is dat SVS material, wat vry is van virusse en viroïdes, beter vertoon as vuil materiaal wat in boorde gesny is (3.2.9).

Grondgedraagde siektenavorsing fokus op grondgedraagde siektes en peste van sitrus. Die soektog na alternatiewe, meer volhoubare middels is voordurend. Sommige studies ondersoek ook die komplekse oorsake van sitrusagteruitgang en herplant problem en kyk na faktore wat vroeëwaarskuwings kan wees. Onbekende siektes en hul oorsaak word ook ondersoek asook hoe om hulle te bestuur.

Langtermyn studies van voor-en na plant bestuur van nematodes en *Phytophthora* dui aan dat voorplant beroking ŉ effek het met bome in hierdie behandelings wat hoër is met dikker stamme (3.3.3). Evaluering van alternatiewe, nie-chemiese behandelings om chemise nematisiedes te vervang het getoon dat die produkte variërende resultate lewer en dat die beste resultate dikwels verkry word waar hierdie middels kombineer word met een chemiese toediening (3.3.4). Dit is ook bevind dat fosfonaat toedienings op mandaryne fititoksiese skade op vrugte veroorsaak as dit na kleurbreuk gedoen word.

ŉ Patogeen kompleks is bevind om betrokke te wees in die stamvrot en agteruitgang wat deur produsente in die Oos-Kaap waargeneem is en dat hoë grond en water pH moontlik Carrizo citrange en Swingle citrumelo onderstamme predisponeer vir infeksie deur bogenoemde patogeenkompleks. Hierdie postulasie word tans verder ondersoek (3.3.5). Sitrusagteruitgang het ook ŉ komplekse oorsaak van biotiese en abiotiese faktore

en in Projek 1092 (3.3.7) word hierdie faktore ondersoek om was te stel of enige van hulle gebruik kan word as vroeëwaarskuwing van probleme. Sitrus herpplantprobleme word meer ondervind omdat ou boorde meer vervang word en Projek 1152 ondersoek dit om die grondgedraagde peste en patogene wat betrokke kan wees te ondersoek en identifiseer. Tot op hede toon resultate dat verskeie patogene saam met die sitrus nematode waarskynlik verantwoordelik is vir die verdwergde groei wat waargeneem word in herplant boorde.

Die snelle groei van die industrie asook herplantprogramme wat implimenteer word, plaas druk op kwekeryproduksie en Projek 1101 (3.3.6) ondersoek die voorkomende en kuratiewe grondgedraagte siekte bestuurspraktyke deur patogeen karakterisering en swamdodersensitiwiteits werk. Resultate van die projek het reeds gelei tot aanbevelings aan kwekerye.

Na-oes swamdoders word op verskillende maniere in die pakhuis aangewend. Imazalil (IMZ) en thiabendasool word algemeen gebruik omdat dit kuratiewebeheer en sporulasie-inhibisie van *Penicillium* spp. verskaf alhoewel dit nie beheer bied van suurvrot nie. Dit word dus nie aanbeveel dat IMZ ingesluit word in die die stortbehandelings nie wat ook bydra tot die teenweerstandsmaatreëls. In die stortbehandeling word Pirimetaniel en thiabendasool as die hoofkomponente beskou met die byvoeging van saniteerders, chloor en HPPA) wat bydra tot suurvrot beheer terwyl goeie beheer van *Penicillium* spp. steeds verkry word. HPPA is bevind om effektief te wees teen kort blootstellingstye by hoë pH waardes. Dit kan dus ingesluit word in ʼn stortsisteem waar die pH nie beheer word nie. Tussen die boonste en onderste kratte in ʼn stortbehandeling is bevind dat die vrugte in die boonste kratte meer PYR residue op het as die vrugte in die onderste kratte. Aanbevelings ten opsigte van pH, temperatuur, blootstellingstyd en die effek op chemikalië in die dompelbad, sal in 2017 voltooi word en aan pakhuise voorsien word (3.5.4).

Na-oes swamdoders tans in gebruik in pakhuise tesame met alternatiewe swamdoders is bestudeer om vas te stel wat hul effek is op latente sitrus swartvlek (SSV) letsels asook die reproduktiewe potensiaal van die letsels. Dit is gesien dat die volle pakhuisbehandeling tesame met koue opberging, die ontwikkeling van latente infeksies betekenisvol verminder end at baie min van die letsels uiteindelik piknidia ontwikkel. Die gekombineerde epidemiologiese vereistes vir piknidiospoor vrystelling, saam met die resultate van hierdie proewe, dui daarop dat ge-oesde sitrusvrugte nie 'n epidemiologies pad vir die verspreiding van SSV is nie (3.5.2). Esentiële olies (EO) is bekend daarvoor dat hulle ʼn effek het op na-oes patogene en dus word Eo getoets ten einde hulle te enkapsuleer in stadigvrystellende nano – of mikropartikels. Gespesialiseerde toerusting en metodes is vir hierdie werk ontwikkel en uitgebreide data is versamel wat tans onder analise is (3.5.8).

Alternatiewe en nuwe produkte is getoets en die beste is bevind om ʼn azoksiestrobien verbinding teen *Penicillium digitatum* te wees. Produkte wat waterstofperoksied en asynsuur (PPA) kombineer is gesien om geen effek te hê op die sensitiwiteit van pyrimethaniel en die stabiliteit van propikonasool nie. ʼn Poeierformulasie van PAA is ontwikkel en getoets en bevind om nie die fitotoksiese probleme te hê van vloeibare formulasies nie. Sanitasie van water word steeds op gefokus met verskeie produkte wat getoets word (3.5.3). Propikonasool is ook in Suid-Afrika geregistreer vir suurvrot beheer wat dit genoodsaak het dat die basissensitiwiteit van Suid-Afrikaanse suurvrot en *Penicillium digitatum* isolate vasgestel moes word (3.5.6).

Die rol van fitochemikalieë, wat deur sitrusvrugte gevorm word, in die inhibisie van vruginfeksies deur *Phyllosticta citricarpa* word ondersoek. Kultivars wat verskil ten opsigte van hul sensitiwiteit vir *Phyllosticta citricarpa* infeksies is ingesluit en word bestudeer ten opsigte van hul apolêre fraksies (was, olie, lipiede) en polêre fraksies (flavonoïede, antosianiene, alkaloïede, glikosiede) in die vrugskil. Huidiglik word Bitter Seville as ʼn lae vatbare tipe, medium vatbare navel en hoogsvatbare suurlemoen tipes gebruik, terwyl kumkwat as n nie-vatbare tipe bygevoeg is (3.5.5).

Swamverrotting van hout palletvoetstukke is onder die kollig geplaas weens die feit dat hulle kartonne besmet en bydra tot die verval van kartonne en na-oes bederf van vrugte. Die oorsake hiervan is onbekend en Projek 1165 (3.5.7) ondersoek die swamspesies wat betrokke is tesame met faktore soos opbergingsmetodes en die moontlike rol van omgewingsfaktore (bv. vog, UV afbraak en insekkolonisasie). Resultate van hierdie studie sal vervaardigers en gebruikers van hout palletvoetstukke in staat stel om swambesmetting en meegaande probleme te voorkom.

Sitrus swartvlek (SSV) en Alternaria bruinvlek (ABV) is twee belangrike vrug –en blaarsiektes wat nadelig is vir uitvoer van vars vrugte deur Suid-Afrikaanse produsente. Die fokus van die Vrug –en Blaarsiekte (met SSV) program is om die epidemiologie en beheer van hierdie siektes te bestudeer. Die epidemiologie van SSV word nie ten volle verstaan nie en streng regulasies is deur sekere bestaande markte ingestel ten opsigte van die produkte wat vir die beheer van ABV geregistreer is.

Sitrus swartvlek, veroorsaak deur *Phyllosticta citricarpa*, kom in baie sitrus produksieareas voor, maar in die EU is dit onderworpe aan fitosanitêre wetgewing. Die voorkoms van *Phyllosticta* spp. in die EU is bepaal deur opnames in sitrus boorde, kwekerye en in huistuine in die EU sitrusareas (3.4.10). *Phyllosticta* spp. is bevind

om beperk voor te kom in die EU, maar nooit in assosiasie met infeksies nie. SSV simptome is nooit waargeneem nie wat aandui dat die patogeen vestig, maar nie siekte veroorsaak nie.

In projek 977 (3.4.4) is 383 *P. citricarpa* isolate gegenotipeer en tesame met paringstipe analises is onthul dat beide paringstipes teenwoordig is in die populasies van Suid-Afrika, China, Australië en Brasilië in ŉ 1:1 verspreiding, maar dat die VSA populasie slegs een paringstipe bevat. Die populasies van hierdie patogeen in Suid-Afrika, Australië en Brasilië is bevind om hoogs verbind te wees wat toegeskryf is aan die uitruil van plantmateriaal tydens die vestiging van die sitrusindustrieë in hierdie lande.

Kennisgapings aangaande die epidemiologie van die SSV patogeen word in RCE-6 (3.4.7) aangespreek. Hoë spoorontkieming is behaal met spore ouer as 1 dag afkomstig van die 2de en 3de generasies spore afkomstig uit piknidia. Lemmetjie bome se toleransie teen SSV is bevind om onafhanklik te wees van die ontkiemingsproses en dat self na 3 h van droogte, piknidiospore kan ontkiem, ook so vroeg as 4 h na inokulasie. ŉ qPCR protokol is ontwikkel en optimiseer om *P. citricarpa* piknidiospore te kwantifiseer wat afkomstig is van spoorlokvalle. Hierdie protokol on egter nie spoorhoeveelhede minder as 1000 akkuraat kwntifiseer nie. Binne projek RCE-7 (3.4.8) is verskillende epidemiologiese modelle van SSV tesame met weerdata in ŉ webgebasseerde platform saamgevat (PhytRisk) wat produsente kan help met SSV besluitneming. A selfoon weergawe word ontwikkel terwyl validasie van bestaande SSV modelle ook aangaande is. Die toetsing en verbetering van SSV modelle word in projek RCE-8 (3.4.9) gedoen met behulp van nuwe spoorlokval en weerdata wat versamel is. Dit volg op die terminering van sommige doelwitte in die projek omdat die beplande en aangepaste metodes nie enige betekenisvolle resultate kon oplewer vir hierdie doelwitte nie.

Dit is noodsaaklik dat peste en siektes wat van fitisanitêre belang is ten volle beheer moet word. Daarom word hoë volume toedienings in Suid-Afrika gebruik. Alhoewel hierdie volumes effektief is, is hulle duur en nie-omgewingsvriendelik. Projekte 1132 (3.4.6) en 1089 (3.4.5) is dus gemik daarop om laer spuitvolumes te toets vir effektiewe pes-en siektebeheer terwyl ŉ boomdigtheid gebasseerde kalibrasiemodel ontwikkel word met behulp van nuwe tegnologie. Sommige markte het ook streng regulasies geplaas op die gebruik van mankoseb in die beheer van ABV wat meebring dat Projek 750 (3.4.2) op ŉ voordurende basis alternatiewe middels ondersoek. RB-1 word tans ondersoek, maar tot op datum is bevind dat hierdie nuwe swamdoder nie effektief is as ŉ mankoseb plaasvervanger in ŉ ABV beheerprogram nie.

3.2 PROGRAMME: GRAFT TRANSMISSIBLE DISEASES

Programme coordinator: Glynnis Cook (CRI)

3.2.1 Programme summary

A ‘Huanglongbing’ (HLB) or ‘Asian Greening’ incursion is a growing threat to citrus production in southern African with the confirmed presence of both ‘*Candidatus*’ Liberibacter asiaticus (Las) and *Diaphorina citri* on the African continent. Research, conducted over a number of years, investigating the alternate hosts of the African Greening pathogen, ‘*Ca*’ L. africanus (Laf), has enabled the correct analysis of the presence of Liberibacter species found in citrus in East Africa. Detailed investigation of survey samples from this region demonstrated the presence of a less aggressive Laf sub-species and not Las in Uganda, Kenya and Tanzania (3.2.7). These findings give some reprieve as Las has not been confirmed in Africa other than in Ethiopia.

A strong research focus is maintained on *Citrus tristeza virus* (CTV) due to the use of cross-protection to mitigate the effects of this virus and the necessity to understand both the mechanism and strains involved in cross-protection. Diagnostics to identify CTV strains has progressed significantly, enabling studies to identify strains involved in disease expression and those required to mitigate the disease. The most appropriate approach to study the disease is to identify single-strain sources and to apply them in glasshouse and field trials to evaluate their impact on the host. Additionally, studies of CTV-host interactions and virus diagnostics using new bioinformatics technologies are enabling a deeper understanding of the genetics driving the biology (3.2.6).
Field trials to assess the performance of various CTV sources in sweet orange for use as cross-protection sources were concluded. Trial results show good performance of LMS6, the current CTV pre-immunisation source for sweet oranges and confirm the continued use of this CTV source (3.2.3 and 3.2.4). Grapefruit CTV trials are mostly concluded and single-strain CTV sources show promise over sources consisting of strain mixtures. The effect of climate on symptom expression of the virus is evident from these trials (3.2.2 and 3.2.5). Two trials geared to investigating CTV in soft-citrus cultivars are underway (4.2.8).

A comparative trial to test the horticultural performance of pre-scheme material containing viroids and CTV compared to material supplied by the Citrus Improvement Scheme (CIS) is underway and a duplicate glass-house trial has already demonstrated the benefit of CIS propagation material over field-cut sources (3.2.9).

A delay in seed import has postponed field testing of commercial or potentially important rootstock selections for viroid sensitivity (3.2.10).

Programopsomming

Daar is ŉ toenemende bedreiging van 'Huanglongbing'(HLB) of 'Asiese Vergroening' vir sitrusproduksie in Suider-Afrika met die bevestigde teenwoordigheid van beide '*Candidatus*' Liberibacter asiaticus (Las) en *Diaphorina citri* op die Afrika-kontinent. Die ondersoek na alternatiewe gashere vir die Afrika-vergroeningspatogeen, '*Ca*' L. africanus (Laf), oor ŉ geruime tydperk, het die korrekte analise van die teenwoordigheid van Liberibacter-spesies in sitrus in Oos-Afrika moontlik gemaak. Indiepte ondersoek van monsters versamel vanuit hierdie streek het die teenwoordigheid van 'n minder aggressiewe Laf subspesie en nie Las uitgewys in Uganda, Kenia en Tanzanië (3.2.7). Hierdie bevindinge gee 'n mate van gerustheid, aangesien die teenwoordigheid van Las in Afrika net in Ethiopië bevestig is.

'n Sterk navorsingsfokus word gehandhaaf op *Citrus tristeza virus* (CTV) as gevolg van die gebruik van kruisbeskerming om die effekte van hierdie virus te beheer asook die noodsaaklikheid om beide die meganisme en rasse betrokke by kruisbeskerming te verstaan. Diagnostiek om CTV-rasse te identifiseer het aansienlik vordering gemaak en die studie van CTV rasse betrokke by siekte uitdrukking, asook die betrokke by die beperking van siekte uitdrukking, moontlik gemaak.Die mees geskikte benadering om die siekte te ondersoek is om enkel-ras bronne te identifiseer en in glashuis en veld proewe te evalueer. Daarbenewens, ondersoek na CTV-gasheer-interaksies en virusdiagnostiek met die gebruik van nuwe bioinformatika-tegnologie, bied 'n dieper begrip van die genetika wat die biologie dryf (3.2.6).

Veldproewe om die prestasie van verskeie CTV bronne vir gebruik as kruisbeskermingsbronne in soetlemoene, te beoordeel, is afgesluit. Resultate toon goeie prestasie van LMS6, die huidige CTV-kruisbeskermingsbron vir soetlemoene, en bevestig die voortgesete gebruik van die CTV-bron (3.2.3 en 3.2.4). Pomelo CTV-proewe word ook afgesluit en CTV-bronne bestaande uit enkele CTV rasse het meestal beter presteer as bronne bestaande uit rasmengsels. Die uitwerking van klimaat op simptoomuitdrukking van die virus is ook uitgewys in hierdie proewe (3.2.2 en 3.2.5). Twee proewe, gerig op die ondersoek van CTV in sagte sitruskultivars vorder goed (3.2.8).

'n Veldproef om die tuinboukundige prestasie van pre-skema materiaal, wat viroïede en CTV bevat, met materiaal voorsien deur die Sitrus Verbetering Skema (SVS) te vergelyk, vorder.'n Duplikaat glashuisproef het reeds die voordeel van die gebruik van SVS voortplantingsmateriaal getoon (3.2.9).

'n Vertraging in saadinvoer het veldevalueering van kommersiële of potensieël belangrike onderstamme vir viroid sensitiwiteit, uitgestel (3.2.10).

3.2.2 FINAL REPORT: *Citrus tristeza virus* cross-protection of Star Ruby using Beltsville sub-isolates of Nartia mild strain for the Orange River Valley

Project 738 (2004 - 2016) by J.H.J. Breytenbach, G. Cook and S.P. van Vuuren (CRI)

Summary

Indications of a severe *Citrus tristeza virus* component in the Nartia (GFMS12) cross-protecting source necessitated the separation of the strain populations into sub-isolates by single aphid transmissions (SAT). These sub-isolates were derived from two grapefruit Nartia sources (A = GFMS12, C = GFMS14) and a sweet orange, Mouton source. The GFMS14 and Mouton SAT sub-isolates were done in Beltsville, USA, and imported back to South Africa. Four sub-isolates showed potential for further evaluation after biological indexing; (GFMS14: B389-1, B389-4; Mouton: B390-3, B390-5). Two sub-isolates from the ARC-ITSC of GFMS12 (GFMS12-7, GFMS12-9) were included in the trial as well as GFMS12 and GFMS35 (current cross-protection source for grapefruit). Star Ruby trees were prepared and inoculated with the various CTV sources. An un-inoculated treatment served as a control and field-infection indicator. Trees were planted in the Kakamas region in the Northern Cape Province in 2004. This trial was a duplicate experiment of Project 679, planted in 2003 in Swaziland, to assess the effect of the CTV sources in different climatic regions. A similar trial was also planted in 2007 in Letsitele (Project 742). Tree canopy volumes were determined yearly and fruit was harvested and graded into export sizes for 4 consecutive seasons. Trees grew slower than would be the case in more favourable grapefruit production areas due to the cooler winter climate and also took longer to come into production compared to the trial planted in Swaziland. Significant differences in tree sizes were found between treatments. Trees inoculated with GFMS12 were the largest and trees inoculated with GFMS35 and B389-1 the smallest. Results with the GFMS12 treatment are in contrast to results obtained in Swaziland and Letsitele, where a significant reduction in tree size was associated with GFMS12 due to severe stem-pitting. Some trees inoculated with sub-isolate B389-1 did develop severe stem-pitting in this trial, in contrast to results

from the trials in Swaziland and Letsitele. No significant differences were found in cumulative yields between treatments over 4 years and no treatment was consistently associated with a high percentage of small fruit. Therefore, crop production did not differ significantly between CTV treatments. The control trees remained symptomless after 11 years in the field, indicating low pressure of aphid and/or severe CTV at the trial. Comparative analysis with similar trials in other grapefruit production regions therefore indicates that climate does impact symptom expression of certain CTV sources and demonstrates that potential CTV cross-protection sources should be trialled in various climatic regions to confirm suitability for the various regions.

Opsomming

Weens aanduidings van ʼn strawwe *Citrus tristeza virus (*CTV) komponent in die Nartia (GFMS12) 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=GFMS12, C=GFMS14) en ʼn Mouton bron verkry. Die GFMS 14 en Mouton sub-isolate is in Beltsville, VSA voorberei en terug na Suid Afrika ingevoer. Na biologiese evaluering van die sub-isolate, het slegs vier potensiaal getoon vir verdere evaluasie (GFMS 14: B389-1, B389-4; Mouton: B390-3, B390-5). Twee belowende Nartia sub-isolate afkomstig van die LNR-ITSG, GFMS12-7 en GFMS12-9, is ook by die proef ingesluit. GFMS12 en GFMS35 (huidige kruisbeskermingsbron) is as kontrole verwysings gebruik. Star Ruby boompies is voorberei en met die verskeie bronne gepreïmmuniseer. ʼn Virusvrye behandeling is as kontrole ingesluit om natuurlike besmettings aan te dui. Die boompies is in die Kakamas omgewing in die Noord Kaap Provinsie gedurende 2004 uitgeplant. Hierdie proef is ʼn herhaling van Projek 679 wat in Swaziland in 2003 aangeplant is, asook ʼn gedeeltelike herhaling van ʼn proef aangeplant in Letsitele (Projek 742). Die verskeie proewe dien om CTV bronne in verskeie klimaatstoestande te evalueer. Boomvolumes is bepaal en vrugte geoes en gegradeer volgens uitvoer vruggroottes vir 4 agtereenvolgende seisoene. Die bome het heelwat stadiger gegroei as bome in die gunstiger pomelo produksiegebied van Swaziland, as gevolg van die koeler winter klimaat. Die bome het ook langer geneem om in produksie te kom. Beduidende verskille in boom groottes is gevind tussen behandelings. Bome geïnokuleer met GFMS12 was die grootste en bome geënt met GFMS35 en B389-1 die kleinste. Resultate met die GFMS12 behandeling is in teenstelling met die resultate verkry in die Swaziland en Letsitele proewe, waar 'n aansienlike vermindering in boomgrootte gevind is as gevolg van ernstige stamgleuf. Van die bome geinokuleer met isolaat B389-1 het ernstige stamgleuf ontwikkel, ook in teenstelling met resultate verkry in Swaziland en Letsitele. Geen beduidende verskille is gevind in kumulatiewe opbrengste tussen behandelings oor 4 jaar nie en geen behandeling het gereeld hoë persentasie van klein vrugte gelewer nie. Produksie in hierdie proef, was dus nie beduidend beïnvloed deur die CTV behandelings nie. Die bome wat virus-vry geplant is, het simptoomloos gebly ná 11 jaar, wat op 'n lae plantluis en / of ernstige CTV druk dui. Vergelykings met soortgelyke proewe in ander pomelo produksiegebiede dui dus daarop dat die klimaat die simptoomuitdrukking van sekere CTV bronne beïnvloed en toon dat potensiële CTV-kruisbeskermingsbronne in verskillende klimaatstreke getoets moet word, om die geskiktheid vir die verskillende streke te bevestig.

Introduction

The severe impact that CTV stem-pitting had on grapefruit production necessitated the implementation of cross-protection with mild strain sources (Marais *et al*., 1996). The so-called breakdown in CTV cross-protection with the GFMS12 (Nartia A) source, possibly owing to the presence of severe strains within the source and probable strain segregation, motivated the separation of the strains in various sub-isolates, using single aphid transmission (SAT). Sub-isolates from two Nartia sources (A = GFMS 12, C = GFMS 14; van Vuuren *et al.*, 1993) and a Mouton source (derived from sweet orange) were obtained with the SATs. The sub-isolates from the GFMS14 and Mouton sources were made at the quarantine facility in Beltsville MD, USA and exported back to South Africa. These sub-isolates underwent biological assessment in glasshouse trials. Some sub-isolates induced severe stem-pitting, showed poor translocation of the virus or reached low virus titres in the citrus host and were excluded from further trials (Breytenbach *et al.*, 2002). Four sub-isolates showed potential as mild sources and were evaluated further in field trials together with promising SAT sub-isolates of GFMS12 (Nartia A) obtained from the ARC-ITSC (van Vuuren *et al.*, 2000). As host and climate both influence CTV disease expression, it was imperative that CTV isolates be evaluated in different climatic regions (da Graça *et al.*, 1984).

Objectives

To compare the effect of various CTV sub-isolates to existing sources on grapefruit, under field conditions in the Orange River Valley.

Performance evaluation was based on:

- Presence/severity of stem pitting
- Tree canopy volume

- Yield
- Fruit size

Materials and methods

Virus-free Star Ruby grapefruit was budded to MxT rootstocks in a glasshouse. When the scions had developed to approximately 5 mm thickness, they were bud inoculated with the sub-isolates of the GFMS14 or Mouton CTV sources (B389-1, B389-4, B390-3, B390-5), ARC-ITSC sub-isolates (GFMS12-7, GFMS12-9); GFMS12, previous CTV cross-protection source for white grapefruit and GFMS35, current CTV cross-protection source for all grapefruit. Control trees were not inoculated. CTV transmission was confirmed by ELISA and trees were planted in the Kakamas area with five replicates in each treatment. The trees were evaluated annually with regards to growth, production and tree health.

Tree canopy volumes were determined using the formula $V=S^2(\pi h - 1.046S)$, where S is canopy radius and h is the height of the fruit bearing canopy (Burger *et al*. 1970).

Fruit yield (kg/tree) was measured annually and a 5-year cumulative yield/tree obtained. Fruit size distribution was also determined at harvest according to export size categories.

Stem pitting was evaluated externally using a severity scale of 0 to 3, where (0) represents a smooth trunk with no visible pits, (1) represents one to three grooves on the stem, (2) indicates multiple grooves and (3) is severe stem pitting where the tree trunk has a knotted appearance.

Molecular determination of the CTV strains within the sources used for this trial was done within Project 1100. Next Generation Sequencing was also done on sources GFMS12, GFMS35, B389-1 and B390-5 to confirm conventional sequencing results.

Results and discussion

Tree size: Mean tree canopy volumes (m^3) per treatment are presented in Table 1. Significant differences in tree size were observed between treatments. Trees inoculated with GFMS12, GFMS12-7 and B390-3 were the largest and trees inoculated with GFMS35 and B389-1 and B389-4 the smallest. Results with the GFMS12 treatment are in contrast with results obtained in the Letsitele and Swaziland trials, where a significant reduction in tree size was associated with GFMS12 due to severe stem-pitting.

Tree health: Average stem-pitting ratings per treatment are presented in Table 3.2.2.1. Trees containing CTV sub-isolate B389-1 developed significantly more stem-pitting than other treatments. Although the average rating was mild (rating between 1 - 2), only half (3/6) of the trees of this treatment showed severe stem-pitting. Figure 1 shows the correlation of severe stem-pitting of 3 replicates to the reduction in canopy volume of the 3 trees. Severe stem-pitting obtained with this CTV source is in contrast to results obtained in Letsitele and Swaziland, where trees containing B389-1 were not associated with any stem-pitting. GFMS12 induced no visible stem-pitting in this trial and this is also in contrast to results obtained in Letsitele and Swaziland, where this CTV source induced severe stem-pitting. Trees planted virus-free remained symptomless after 11 years, indicating a low level of severe CTV challenge in the area.

Production: The average yield per tree for each treatment for the 2015 harvest as well as the cumulative yield over 4 years is presented in Table 3.2.2.2. No significant differences were found in cumulative yields between treatments, however, treatments B389-1, B390-3 and the virus-free plants produced the most of the small fruit (size 48), it the last season. No treatment was, however, consistently associated with a high percentage of small fruit during the evaluation of the trial. The 3 trees of CTV treatment B389-1 showing severe stem-pitting and reduced growth, were also associated with lower cumulative yields over 4-years compared to the 3 replicates not showing severe stem-pitting (Figure 3.2.2.2). It is expected that this difference in production will become more pronounced as the trees age.

Table 3.2.2.1. Mean canopy volumes and external stem-pitting ratings for Star Ruby trees inoculated with various CTV sources and sub-isolates, 11 years after planting in the Orange River Valley.

Treatment	Canopy volume (m^3)	Stem pitting**
B389-1	25.7 d*	1.5 a
B389-4	28.1 cd	0 b
B390-3	41.1 ab	0 b
B390-5	33.9 bcd	0.1 b
GFMS12-7	41.2 ab	0.3 b
GFMS12-9	33.4 bcd	0 b
GFMS12	45.9 a	0.1 b
GFMS35	24.3 d	0 b
Virus-free	36.6 abc	0 b

* Figures in each column followed by the same letter do not differ significantly at the 95% confidence level (Fisher's LSD).
** Stem pitting rating: 0 = Smooth trunk; 1 = Mild pitting; 2 = Moderate pitting; 3 = Severe pitting.

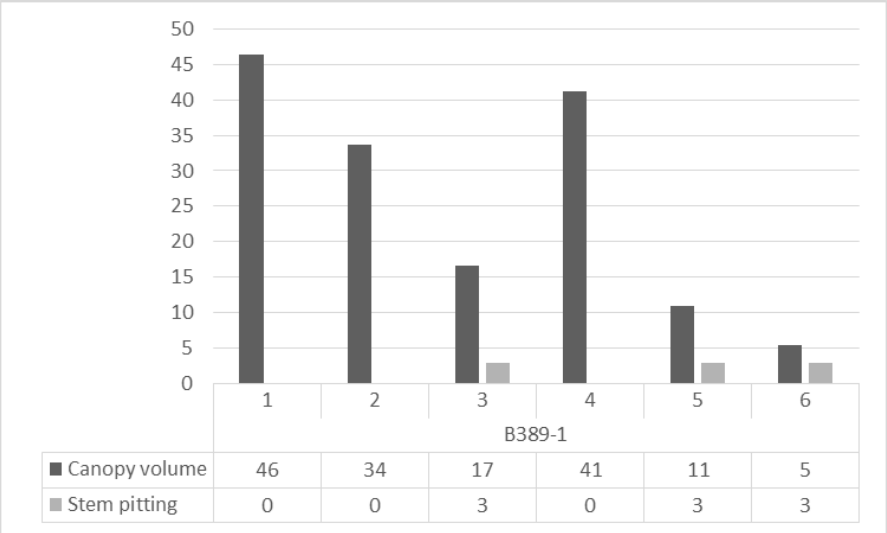

Figure 3.2.2.1. Canopy volume (m^3) and stem-pitting values for the 6 tree replicates of CTV treatment B389-1.

Table 3.2.2.2. Average yield (kg/tree), relative fruit size distribution and average 4-year cumulative yield per tree for Star Ruby grapefruit trees pre-immunised with different CTV sources and sub-isolates, 11 years after planting.

Treatment	Fruit size (kg)						2015 yield (kg/tree)	Cumulative yield: 2012-15
	27	32	36	40	48	64		
B389-1	3.3 a	3.0 a	11.8 a	20.6 ab	35.1 ab	6.7 a	80.7 a	338 a
B389-4	4.9 a	3.9 a	18.4 abcd	23.7 abc	33.8 a	8.2 a	93.3 ab	381 a
B390-3	4.0 a	5.6 ab	19.0 abcd	34.2 c	51.7 b	13.6 a	128.3 b	437 a
B390-5	9.3 ab	7.7 abc	20.9 bcd	27.0 abc	32.5 a	10.8 a	108.3 ab	407 a
GFMS12-7	15.2 b	12.1 bc	26.8 d	26.9 abc	28.4 a	8.8 a	118.3 a	397 a
GFMS12-9	8.9 ab	5.7 ab	16.4 abc	14.9 a	23.6 a	7.8 a	77.3 a	364 a
GFMS12	9.9 a	13.5 c	24.6 cd	24.1 abc	32.8 a	11.6 a	116.6 ab	386 a
GFMS35	3.4 a	2.6 a	13.3 ab	20.3 ab	32.3 a	8.1 a	80.1 a	389 a
Virus-free	6.8 a	7.7 abc	22.1 cd	30.2 bc	38.2 ab	12.1 a	117.2 a	394 a

* Figures in each column followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

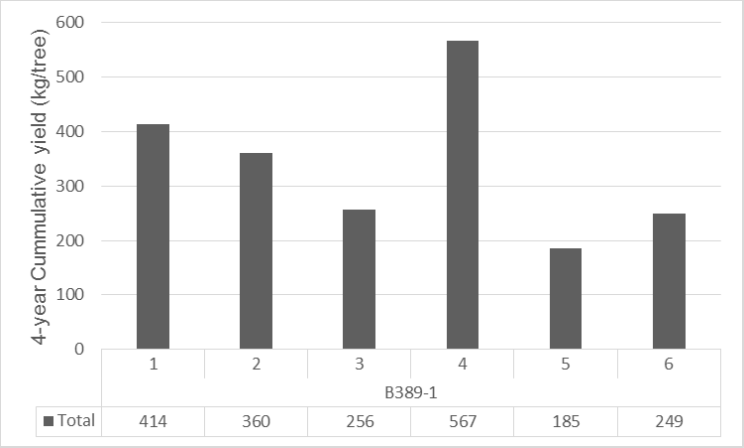

Figure 3.2.2.2. Four-year cumulative yield (kg/tree) for the 6 tree replicates of CTV treatment B389-1.

CTV strain analysis (Project 1100): Results regarding CTV strain analysis of the various sources are not presented in this report, but form part of analyses done within Project 1100. Conventional RT-PCR and sequencing indicated that the sub-isolates consist of single CTV strains, however, NGS data indicated a possible low-level presence of an additional strain in the B389-1 source. CTV strain analysis of the trial trees did indicate the presence of a second strain, which might be implicated with the severe stem-pitting observed in some of the trial trees inoculated with this source. It is possible that a strain segregation occurred with this source, resulting in the varied results, but these results require further investigation.

Conclusion

The cooler winter climate suppressed tree growth compared to the more favourable grapefruit production areas such as Letsitele and Swaziland. Three trees inoculated with sub-isolate B389-1 developed severe stem-pitting, whilst trees with GFMS12 developed very little stem-pitting. These results are in contrast to results in the Letsitele and Swaziland trials, where no stem pitting was associated with the B389-1 CTV source and severe stem-pitting was consistently obtained with the GFMS12 CTV source, indicating probable climatic influences. The control trees remained symptomless after 11 years in the field, demonstrating low pressure of aphid and/or severe CTV at the trial. Canopy volumes of trees containing GFMS35, the current cross-protection source for grapefruit, were the smallest in the trial, although cumulative yields were not lower than other treatments and no stem-pitting was observed with this source.

Comparative analysis with similar trials in other grapefruit production regions, indicates that climate does impact CTV symptom expression. These results demonstrate that potential CTV cross-protection sources should be trialled in various climatic regions to confirm suitability for the various regions.

Future research

The CTV strain profiles of trial trees will be compared to the strain profiles of the same treatments in the other climatic regions where variant results were obtained, in order to establish whether the original treatment populations changed by introductions of other strains by aphid transmission or whether climatic differences influenced symptom expression.

Technology transfer

Oral Presentation at Citrus Research Symposium:
Breytenbach, J. H. J., van Vuuren, S. P. and Cook, G. 2014. The effect of climate and host on cross-protection of grapefruit. 8th Citrus Research Symposium. Champagne Sports resort, Drakensberg.
Cook, G., van Vuuren, S.P., Breytenbach, J.H.J., Steyn, C. Burger, J.T. and Maree, H.J. *Citrus tristeza virus*: A journey of discovery. 2016. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.
Oral Presentation at Scientific Conference:
Cook, G, Breytenbach, J. H. J. & van Vuuren, S. P. Field performance of various *Citrus tristeza virus* cross-protection sources trialled in grapefruit in different climatic regions. 20th IOCV Conference, Chongqing, China, 10-15 April 2016.

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Marais, L.J., M.L. Marais and Rea, M, 1996. Effect of tristeza stem pitting on fruit size and yield of Marsh grapefruit in southern Africa. Proc.13th Conf. IOCV, 163-167. IOCV, Riverside, CA.

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3.2.3 FINAL REPORT: The effect of different CTV sources on 3 Valencia cultivars in the Orange River Valley

Project 739 (2004 - 2017) by J.H.J. Breytenbach, G. Cook and S.P. van Vuuren (CRI)

Summary

Disease expression of *Citrus tristeza virus* (CTV) is influenced by the CTV strains present, the specific citrus cultivar and various climatic factors. Although CTV symptoms such as stem pitting or decline may not always be visible on all citrus cultivars, different CTV sources do influence tree growth and yield. CTV sources, SM46, SM47, SM48 and SM49, derived from various good-performing, sweet orange trees, were used to inoculate virus-free 'Delta'-, 'Midknight'-, and 'Turkey' Valencia on C35 citrange rootstocks. These sources were compared to LMS6, the standard CTV pre-immunisation source for sweet oranges, and virus-free controls. The trees were planted in the Kakamas area in the Northern Cape Province in 2007. Trees were evaluated annually for disease symptoms and tree canopy volumes were determined. Fruit yield per tree was determined in the last 3 seasons of the trial. Nine years after planting, yield of all trial trees remained poor and conclusions regarding the effect of the various CTV sources on production could not be determined. The C35 rootstock is not the recommended rootstock for the area (not known at the time of planting) and this is likely the reason for the poor production. The influence of the different CTV sources are nonetheless evident on tree growth. Trees of all three cultivars inoculated with SM48 were noticeably smaller than those of other treatments. Determination of the CTV strain components of the source showed that SM48 contained the severe T3 strain. The LMS6 CTV source had a slight impact on the growth of the 'Delta' trees compared to un-inoculated trees, but had no significant influence on 'Midknight' or 'Turkey' tree growth. The CTV sources used in this trial were shown to be mixtures of CTV strains. These sources will be difficult to maintain as stable populations and are therefore not ideal candidates for cross-protection sources. The grower requested removal of the trial as the site was designated for production purposes and the trial was terminated. Results do not indicate any detrimental impact with the LMS6 source, the standard CTV pre-immunisation source for sweet oranges. Overall, none of the other CTV sources out-performed LMS6 and no change in pre-immunization source is required.

Opsomming

Simptoom uitdrukking van *Citrus tristeza virus* (CTV) word beïnvloed deur verskillende CTV rasse en verskil ook tussen sitrus kultivars. Verskillende klimaatstoestande beïnvloed ook CTV simptoom uitdrukking en alhoewel simptome soos stamgleuf nie altyd sigbaar is op alle sitrus variëteite nie, word boom groei en opbrengs wel beïnvloed deur CTV. Dit is dus nodig om verskillende CTV bronne in verskillende sitrus klimaatstreke te evalueer. Potensiële CTV preïmmuniseringsbronne wat oorspronklik vanaf soetlemoenbome versamel is, nl. SM46, SM47, SM48 en SM49, is gebruik om virusvrye 'Delta'-, 'Midknight'-, en 'Turkey' Valencia op C35 citrange onderstam te preïmmuniseer. Hierdie bronne is met LMS6, die standaard preïmmuniseringsbron vir soetlemoene vergelyk, asook met bome wat virusvry geplant is. Die boompies is gedurende 2007 in die Kakamas omgewing in die Noord-Kaap geplant. Bome is jaarliks geëvalueer vir siektesimptome en boomvolumes is bepaal. Vrugopbrengs per boom is in die laaste 3 seisoene van die proef bepaal. Nege jaar na plant was die opbrengs van alle proefbome nog steeds swak en gevolglik kon geen gevolgtrekkings rakende die effek van die verskillende CTV bronne op produksie gemaak word nie. Die C35 onderstam is nie die aanbevole onderstam vir die gebied nie (nie bekend tydens proef beplanning nie) en dit is waarskynlik die rede vir die swak produksie. Die invloed van die verskillende CTV bronne is egter duidelik op boomgroei. Bome van al drie kultivars wat met SM48 gepreïmmuniseerwas, was merkbaar kleiner as dié van ander behandelings. Bepaling van die CTV ras komponente van hierdie bron het getoon dat SM48 die strawwe T3 ras bevat. Die LMS6 CTV-bron het 'n effense impak gehad op die groei van die 'Delta'-bome in

vergelyking met ongeïnokuleerde bome, maar het geen beduidende invloed op die groei van' Midknight 'of' Turkey' gehad nie. Die CTV bronne wat in hierdie proef gebruik is, bestaan uit mengsels van CTV-rasse. Hierdie bronne sal moeilik wees om as stabiele populasies te handhaaf en is dus nie ideale kandidate vir kruisbeskermingsbronne nie. Die produsent het versoek om die proefblok te verwyder omdat die boord geoormerk was vir kommersiële produksie en die proef is beëindig. Resultate dui nie op enige nadelige impak met die gebruik van die LMS6 bron, die standaard CTV preimmuniseringsbron vir soet lemoene nie. Oor die algeheel, het geen van die ander CTV bronne beter resultate gelewer as LMS6 nie en ŉ verandering in preïmmuniseringsbron is nie nodig nie.

Introduction

Citrus tristeza virus (CTV) is the causal agent of destructive diseases of citrus such as 'Tristeza' disease and stem-pitting decline. The virus is spread mechanically by infected propagation material and is vectored by various aphid species of which *Toxoptera citricida* is the most efficient and abundant on citrus in South Africa. Seven strains of CTV have been identified and variants within these strains have been characterised (Harper, 2013). These stains and variants naturally co-exist as mixtures in most field sources. Symptoms induced by CTV range from mild, with no noticeable effect on the host to severe stem pitting and decline, resulting in reduced yields. Severe strains that adversely affect sweet orange exist (Broadbent *et al*, 1992; Müller *et al*, 1968; Roistacher,1988), but are less prevalent locally (Marais, 1994).

The only practical means of controlling the effects of severe CTV strains, where the virus occurs endemically, is by mild strain cross-protection. Mild isolates are normally sourced from healthy-looking trees in old orchards, severely affected by CTV. Successful use of cross protection involves comprehensive evaluation of the effects of the CTV sources on specific hosts (Müller, G.W. & Costa, A.S, 1987). Since CTV symptom expression is influenced by factors including the citrus host and climate, evaluation of potential CTV 'cross-protection' sources in different climatic regions and on various hosts is required.

All citrus cultivars in the Southern African Citrus Improvement Scheme (CIS) are rendered virus-free by means of shoot-tip grafting (von Broembsen & Lee, 1988). To prevent detrimental effects of field CTV infection, virus-free plants are deliberately infected with mild strains (syn. cross-protection) before plant multiplication at the Citrus Foundation Block (CFB) (von Broembsen & Lee, 1988). The current 'cross-protective' CTV source used for all sweet orange citrus cultivars is LMS6. In a 'Palmer' navel trial on four rootstocks in the Eastern Cape, LMS6 was associated with significantly higher yields compared to other treatments and the average tree size was also larger than other treatments (van Vuuren *et al*, 2009).

The purpose of this experiment was to evaluate selected CTV sources in various Valencia cultivars in order to identify a suitable cross-protecting CTV source for these selections in the Orange River Valley.

Objectives

To compare the influence of various CTV sources in four Valencia cultivars to the existing cross-protection source, LMS6, under field conditions in the Orange River Valley production region.

Performance evaluation was based on:

- Presence/severity of decline
- Tree canopy volume
- Yield

Materials and methods

Three virus-free Valencia scions; Delta, Midknight, and Turkey, were budded on C35 citrange rootstocks. When the scions had developed sufficiently, each Valencia cultivar was bud-inoculated with one of five selected CTV sources namely; SM46, SM47, SM48, and SM49 and LMS6. SM46 was derived from a Shamouti sweet orange tree in Musina, SM47 from a 100-year old Valencia tree in Grahamstown, SM48 from a tree in Citrusdal and SM49 from a Valencia tree in Nelspruit. Trees inoculated with these sources were compared to trees inoculated with LMS6 (the standard cross-protection source for sweet oranges, originally obtained from a 'Mexican' lime tree) and trees planted virus-free. Inoculation was confirmed by ELISA, where after the trees were planted at Karsten Boerdery in the Kakamas area in the Northern Cape Province in September 2007. Horticultural performance i.e. growth (canopy volume), yield data (kg/tree) and tree health (decline) were evaluated annually. Tree canopy volumes were determined using the formula $V=S^2(\pi h - 1.046S)$, where S is canopy radius and h is the height of the fruit bearing canopy (Burger *et al*. 1970). Fruit yield (kg/tree) was measured annually and a 3-year cumulative yield/tree obtained.

Note: McClean Seedless trees were originally included in the trial, but had to be removed during 2009 due to poor drainage in the orchard.

The sources used in this study were additionally characterised to determine the CTV strains present in these sources, using the genotype (strain) specific tests previously developed (Cook *et al*. 2016).

Results and discussion

Tree size: Final canopy size (m^3) of each trial tree was determined in 2015 (8th year of the field trial). The average sizes for each treatment are presented in Table 3.2.3.1.

Delta Valencia: Trees containing CTV sources SM46, SM47, SM48 and LMS6 were smaller than the control trees, however, SM48, had the greatest influence on tree size and trees were significantly smaller than all treatments apart from SM46.

Midknight Valencia: Trees containing SM48 were stunted and significantly smaller compared to other treatments.

Turkey Valencia: No significant differences in tree size was observed between the treatments although on the SM48 inoculated trees were the smallest on average.

In summary, 'Delta' and 'Midknight' trees inoculated with SM48 were significantly smaller than most other treatments and 'Turkey' trees containing SM48 were, on average, smaller than trees of other treatments, but this difference was not significant.

Production: The 2016 season was the 3rd harvest of the trial and similar to the 2014 and 2015 harvests, overall fruit production was very poor and variable for the age of the trees. This was probably due to the C35 rootstock that is not recommended for deep sandy and high pH soils as found at the trial site. C-35 was a newly introduced rootstock to the industry at the time that the trial was planned and very little information was available regarding its suitability for various soil conditions. The 2016 yield data and the 3-year cumulative yield data are nonetheless presented in Table 3.2.3.2 and Table 3.2.3.3 respectively. Although significant differences are indicated, yields are so small, no inferences can be drawn from this data. There is no definitive correlation between the yield data and tree size.

Tree health: No decline symptoms such as twig die-back was observed in any of the trial trees and no bud-union incompatibilities were observed.

CTV strains: The CTV sources that were evaluated in this trial were all shown to consist of mixtures of strains. The only source that showed detrimental effects, SM48, contained the T3 severe CTV strain, which was not a component of any other CTV source used in this trial (Table 3.2.3.4).

Table 3.2.3.1. Average tree canopy volumes (m^3) of 3 Valencia scions inoculated with the various CTV sources, 8 years after planting.

CTV source	**Canopy volume of scions (m^3)**		
	Delta	**Midknight**	**Turkey**
SM46	14.1 ab[1]	15.3 b	7.4 a
SM47	17.9 bc	13.7 ab	8.7 a
SM48	11.8 a	8.2 a	7.2 a
SM49	23.6 cd	16.9 b	12.7 a
LMS6	19.1 bc	15.7 b	12.2 a
Control (un-inoculated)	25.6 d	16.8 b	11.7 a

[1] Figures in each column followed by the same letter do not differ significantly at the 95% confidence level (Fisher's LSD).

Table 3.2.3.2. Average yield (kg/tree) of 3 Valencia scions inoculated with the various CTV sources, 9 years after planting.

CTV source	**Average yield (kg/tree)**		
	Delta	**Midknight**	**Turkey**

SM46	13.5 a[1]	12.8 b	9.1 ab
SM47	17.2 a	14.8 b	14.5 bc
SM48	14.9 a	8.6 ab	6.2 a
SM49	16.9 a	3.3 a	13.4 bc
LMS6	13.3 a	9.0 ab	13.4 bc
Control (un-inoculated)	18.0 a	12.1 b	19.3 c

[1] Figures in each column followed by the same letter do not differ significantly at the 95% confidence level (Fisher's LSD).

Table 3.2.3.3. Average 3-year cumulative yields (kg/tree) of 3 Valencia scions inoculated with the various CTV sources for 2014-2016.

CTV source	**Cumulative yield 2014-2016 (kg/tree)**		
	Delta	**Midknight**	**Turkey**
SM46	32.4 a[1]	34.2 b	35.5 b
SM47	29.7 a	39.3 b	42.2 b
SM48	26.6 a	33.5 ab	17.9 a
SM49	50.5 ab	21.9 a	43.3 b
LMS6	48.1 ab	21.1 a	47.5 b
Control (un-inoculated)	68.2 b	29.1 ab	48.2 b

[1] Figures in each column followed by the same letter do not differ significantly at the 95% confidence level (Fisher's LSD).

Table 3.2.3.4. *Citrus tristeza virus* (CTV) strains detected in various field derived sources including LMS6, the current CTV cross-protection source for sweet oranges.

CTV source	CTV strain							
	T68	RB1	RB2	HA16-5	VT	T30	T3	T36
SM46	√	-	√	-	√	-	-	-
SM47	-	√	√	-	√	√	-	-
SM48	√	-	√	√	√	√	√	-
SM49	√	√	√	-	√	-	-	-
LMS6	√	√	√	√	-	-	-	-

Conclusion

Slight stunting of trees is not necessarily detrimental, but can be useful for high density plantings. However, severe stunting of trees, induced by certain CTV sources, is generally associated with reduced yields and/or small fruit. Unfortunately, due to the C35 rootstock, being the wrong rootstock selection for the area, production data was unreliable. SM48 was associated with severe stunting and therefore precludes this source for further use. The T3 severe CTV strain was found to be a component of this source and is the probable cause of the stunting obtained with SM48. The CTV sources used in this trial were shown to be mixtures of CTV strains. These sources will be difficult to maintain as stable populations and are therefore not ideal candidates for cross-protection sources. Results do not indicate any detrimental influence with the LMS6 source, the standard CTV pre-immunisation source for sweet oranges. Overall, none of the other CTV sources out-performed LMS6 in the evaluation period and no change is in pre-immunization source is required.

The grower requested removal of the trial as the site was designated for commercial production purposes and the trial was terminated.

Technology transfer

None

Further objectives and work plan

The effect of single strain CTV isolates are being evaluated in new field trials in various citrus types, including sweet orange (Project 1173).

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3.2.4 FINAL REPORT: Identification of suitable *Citrus tristeza virus* sources for pre-immunising Turkey Valencia

Project 789 (2005 - 2017) by, J.H.J. Breytenbach, G. Cook and S.P. van Vuuren (CRI)

Summary

'Turkey' Valencia is an early ripening Valencia and an important component of the citrus export portfolio. There were indications that the cultivar might be more sensitive to *Citrus tristeza virus* (CTV) as bud-union crease was noted with certain rootstocks. It was therefore important to determine whether LMS6, the standard CTV cross-protection source for sweet oranges is the most suitable CTV source for this variety. Virus-free scions were budded to 'Troyer' citrange rootstocks and inoculated with various CTV sources including LMS6, SM46, SM47, SM48 and SM49, previously obtained from good-performing sweet orange trees. Trees inoculated with GFMS12 and un-inoculated trees were included as controls. The trial was established in the Malelane area in Mpumalanga province in 2007. Trees were evaluated annually and tree canopy volumes for each tree determined. Each year substantial fruit was lost to baboon foraging due to the trial location. No reliable harvest data could therefore be obtained for this trial and the trial was terminated. Significant stunting was associated with the SM48 CTV source. Stunting was similarly found in a trial in the Orange River Valley with 'Delta'-'Midknight'- and 'Turkey' Valencia trees inoculated with SM48 (Project 739). Results do not indicate any detrimental effects associated with the LMS6 source and none of the other CTV sources out-performed LMS6.

Opsomming

'Turkey' Valencia is 'n vroeë kultivar en is 'n belangrike komponent van die sitrus uitvoer mark. Daar was aanduidings dat 'Turkey' Valencia meer gevoelig vir *Citrus tristeza virus* (CTV) is, vergeleke met ander soet lemoen kultivars. Dit was dus belangrik om te bepaal of LMS6, die standaard preïmmuniseringsbron vir soetlemoene, ook 'n geskikte CTV bron is vir 'Turkey'. Virusvrye 'Turkey' geënt op 'Troyer' citrange onderstam, is in 'n glashuis voorberei en geïnokuleer met verskeie CTV bronne insluited LMS6, SM46, SM47, SM48, SM49, wat almal voorheen vanaf soetlemoene versamel is. Bome wat met die GFMS12 CTV bron geïnokuleer is en virusvrye bome is onderskeidelik as positiewe en negatiewe kontroles ingesluit. Die bome is gedurende 2007 in die Malelane omgewing geplant. Die bome was jaarliks geëvalueer en boomgrootte bepalings gedoen. As gevolg van verlies van vrugte, elke oesjaar, weens bobbejane by die proefperseel kon geen betroubare oesdata verkry word vir hierdie proef nie, en die proef is beëindig. Boomgroottes is bepaal en betekenisvolle verdwerging was geassosieer met die SM48 CTV bron. Soortgelyk, was verdwerging ook gevind in 'n proef in die Oranjerivierdal met 'Delta'-' Midknight'- en 'Turkey' Valencia-bome wat met SM48 ingeënt is (Projek 739). Resultate dui aan dat die huidige kruisbeskermingsbron, LMS6, geen negatiewe invloed op hierdie soetlemoen kultivar gehad het nie en nie een van die ander CTV bronne het beter as die LMS6 bron presteer nie.

Introduction

Cross-protection to control *Citrus tristeza virus* (CTV) is specific with regard to the citrus type, i.e. the most effective protecting CTV isolate for a given citrus type is usually obtained from the same citrus type (Müller & Costa, 1987). With the initiation of the Citrus Improvement Scheme, all citrus, including sweet oranges, were pre-immunised with a CTV source originating from grapefruit until suitable sources were found for the different types (von Broembsen & Lee, 1988). Subsequently, a suitable source, LMS6, was identified for lime (van Vuuren *et al*., 1993). LMS6 contains a mild form of seedling yellows, which the grapefruit source does not have, and it was therefore approved to replace GFMS12 with LMS6 as the pre-immunising source for sweet oranges (van Vuuren *et al*., 2000).

The suitability of LMS6 as a CTV source for sweet orange has been demonstrated in a Palmer navel trial (van Vuuren, 2009), but further evaluations are required on different sweet orange cultivars and in different climatic regions. Midseason cultivars and other sweet oranges are known to be affected by a transmissible factor causing abnormal bud-union on Rough lemon rootstock (McClean, 1974). The transmissible factor is not insect- or seed transmissible and was not correlated to any known citrus disease. It has been shown that it can be removed by shoot tip grafting (Navarro *et al*., 1993).

Recently, it was found that 'Turkey' Valencia trees on Rough lemon and Volckameriana rootstocks developed bud-union creasing symptoms (personal observation; Beeton *et al*., 2000). Various sources of 'Turkey' Valencia had stem pitting symptoms and it appears that this cultivar is more sensitive to CTV than other Valencia cultivars (CRI Group Annual Research Report, 2003). 'Turkey' Valencia is an early ripening cultivar and an important component for the export market. The identification of a suitable CTV source for cross-protection is therefore essential.

Objectives

To evaluate CTV sources to identify a suitable cross-protecting source for 'Turkey' Valencia.

Tree performance criteria:

- Presence/severity of decline
- Tree canopy volume
- Yield
- Fruit size

Materials and methods

Virus-free 'Turkey' Valencia scions on Troyer citrange rootstocks were prepared in the greenhouse according to normal nursery practices. When the scions were approximately 5 mm in diameter, they were inoculated with various CTV sources (Table 1) by budding two buds containing the required CTV source onto each scion. Each treatment was replicated five times and trees with GFMS12 as well as un-inoculated virus-free trees served as positive and negative controls respectively. CTV transmission was confirmed by ELISA, 3 months post inoculation. The trial was planted at Riverside in the Malelane area of Mpumalanga Province in 2007 and evaluations were done annually on growth and tree health.

Table 3.2.4.1. Treatments for 'Turkey' Valencia on Troyer citrange rootstock to identify a suitable CTV source for pre-immunisation.

CTV sources	Origin and comments
LMS6	Mexican lime, Tzaneen (current CTV cross-protection source for sweet orange)
SM46	Shamouti Midseason, Musina
SM47	Valencia, Grahamstown (sourced from tree > 100 years old)
SM48	Midseason, Citrusdal (first planting of citrus in the area)
SM49	Valencia, Nelspruit (possible greening tolerance)
GFMS12	Nartia grapefruit (previous cross-protection source for sweet orange)
Control (un-inoculated)	

Results and discussion

Tree size: The average canopy volumes for each treatment are presented in Table 3.2.4.2. Trees inoculated with SM48 were significantly smaller than trees of other treatments. This was similarly found at a trial in the Orange River Valley where SM48 reduced the canopy volumes of Delta -, Midknight - and Turkey Valencia trees (Project 739).

Production: Due to extensive fruit losses by baboon foraging, trees were not harvested for most of the trial. Data from one harvest in 2014 was unreliable and production data could therefore not be obtained.

Tree health: No decline was observed in any of the trial trees.

Table 3.2.4.2. Average tree canopy volumes of 9 year-old 'Turkey' Valencia trees inoculated with various CTV sources.

Treatment	Canopy volume (m^3)
SM46	28.8 bc*
SM47	31.7 c
SM48	7.9 a
SM49	22.4 b
GFMS12	34.9 c
LMS6	33.4 c
Virus-free control	37.6 c

* Figures in the columns followed by the same letter do not differ significantly at the 95% confidence level (Fisher's LSD).

Conclusion

Significant stunting of the 'Turkey' trees was associated with the SM48 CTV source. This was similarly found in a trial in the Orange River Valley with stunting of 'Delta'- 'Midknight'- and 'Turkey' Valencia trees, inoculated with the same source (Project 739). The CTV sources used in this trial were shown to be complex mixtures of numerous CTV strains (Project 739). These sources will be difficult to maintain as stable populations and are therefore not ideal candidates for cross-protection sources. Results do not indicate any negative impact with the LMS6 source, the standard CTV pre-immunisation source for sweet oranges and no change in pre-immunization source is required for 'Turkey' Valencia.

Technology transfer

None

Further objectives and work plan

No further trials are planned for Turkey Valencia specifically.

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3.2.5 PROGRESS REPORT: *Citrus tristeza virus* cross-protection of Marsh and Star Ruby grapefruit using the best field isolates collected in the different grapefruit production areas in southern Africa

Project 742 (2004 - 2017) by J.H.J. Breytenbach, G. Cook and S.P. van Vuuren (CRI)

Summary

Budwood was collected from 108 superior grapefruit trees from different grapefruit production areas of southern Africa to obtain mild CTV sources for cross-protection studies. These CTV sources were established in the glasshouse at CRI and inoculated to virus-free 'Mexican' lime indicator plants to evaluate the severity of the CTV sources. Nineteen sources were selected for further evaluation and were inoculated again to Mexican lime plants and compared to CTV sources; GFMS12, GFMS35, GFMS12-7, GFMS12-9, and four Beltsville sub-isolates (GFMS14: B389-1, B389-4; Mouton: B390-3, B390-5). The four most promising field sources, Tabankulu 1, New Venture 41-2, ORE 8 and Tshipise 19-5, indexed free for citrus viroids are being evaluated as pre-immunising agents for Marsh and Star Ruby trees in field trials. The sources are compared to GFMS12, previous standard CTV cross-protection source for white grapefruit at the time, GFMS35, current standard cross-protection source for all grapefruit, as well as the four best Beltsville sub-isolates; B389-1, B389-4, B390-3 and B390-5, the ITSC sub-isolates; GFMS12-7 and GFMS12-9 and virus-free controls. The Star Ruby trees were planted in the Letsitele area in Limpopo province and the Marsh trees in the Malelane area in Mpumalanga province in 2007. This was the 6th crop from the Marsh trees and the 4th crop for Star Ruby. Both the Marsh and Star Ruby trees inoculated with GFMS12 developed severe stem-pitting resulting in suppressed tree growth and smaller fruit. Star Ruby and Marsh trees containing the sub-isolated, single-strain sources, generally performed better than trees inoculated with the field sources, including the current grapefruit cross-protection source, GFMS35. CTV source B390-5 was associated with good tree size, higher yields and good fruit size in both Marsh and Star Ruby and shows the most potential for further assessment.

Opsomming

Enthout is vanaf 108 uitstaande pomelo bome versamel uit verskillende pomelo gebiede in suider Afrika, vir moontlike kruisbeskermingsbronne. Die bronne is in die glashuis by CRI gevestig en afsonderlik op Meksikaanse lemmetjie geïnokuleer om te bepaal of die bome moontlik ligte rasse van C*itrus tristeza virus* (CTV) huisves. Slegs 19 bronne het potensiaal getoon en is verdere evalueer en ŉ tweede keer op Meksikaanse lemmetjie geïnokuleer om met bestaande bronne GFMS12, GFMS35, GFMS12-7, GFMS12-9 en die Beltsville sub-isolate (GFMS14: B389-1, B389-4; Mouton: B390-3, B390-5) te vergelyk. Die geïnokuleerde plante is vir groei en voorkoms van stamgleuf asook die virus titer d.m.v. ELISA geëvalueer. Die 4 mees belowende bronne, wat vry was van viroïede, was Tabankulu 1, New Venture 41-2, ORE 8 en Tshipise 19/5. Hierdie bronne is verder gebruik om virus-vrye Marsh en Star Ruby boompies vir boord evaluasie te preïmmuniseer. Die bronne word met GFMS12 (vorige CTV bron vir wit pomelos), GFMS35 (huidige CTV bron vir alle pomelos), asook die Beltsville sub-isolate (B389-1, B389-4, B390-3, B390-5) en LNR-ITSG sub-isolate (GFMS12-7, GFMS12-9) vergelyk. Die Star Ruby boompies is in 2007 in die Letsitele omgewing in Limpopo provinsie en die Marsh boompies in die Malelane omgewing in Mpumalanga provinsie geplant. Dit was die sesde oes van die Marsh bome en die vierde oes vir Star Ruby. Beide die Marsh en Star Ruby bome wat met GFMS12 geïnokuleer is, het ernstige stamgleuf getoon wat boom groei onderdruk het en kleiner vruggrootte opgelewer het. Star Ruby en Marsh bome met die sub-isolaat CTV bronne (enkelras bronne), het oor die algemeen beter presteer as bome met die veldbronne, insluitend die huidige pomelo kruisbeskermingsbron, GFMS35. CTV bron B390-5 was met goeie boomgroottes, hoër opbrengste en goeie vruggrootte in beide Marsh en Star Ruby geassosieer en toon ook die meeste potensiaal vir verdere ondersoek as ŉ alternatiewe kruisbeskermingsbron.

3.2.6 PROGRESS REPORT: Characterisation of *Citrus tristeza virus* variants and their influence on the symptom expression in the grapefruit host.

Project 1100 (2014/15 – 2016/2017) by G. Cook (CRI), T. Jooste (SU), Marike Visser (SU), C. Steyn (CRI), J.H.J. Breytenbach (CRI), J.T. Burger (SU), H.J. Maree (SU)

Summary

To ultimately identify the required *Citrus tristeza virus* (CTV) strain components for cross-protection, it is necessary to genetically and biologically characterise CTV single-strain isolates and evaluate their individual and combined effects on specific citrus hosts. Seven single-strain CTV isolates were identified and biologically characterised on a host range of various citrus types. Complete viral genomes of six CTV strains were determined. Two commercial grapefruit varieties, 'Star Ruby' and 'Marsh', were then used, in a glasshouse trial, to evaluate the ability of specific CTV strains to induce stem-pitting in single or mixed infections. An

additional trial, still in progress, aims to test the ‘cross-protection’ ability of various CTV strains characterised in the first trial by challenging with a severe stem-pitting strain of grapefruit. Initial results obtained from the first trial suggest possible interactions between different strains, as diminished symptom expression was noted in some treatments containing strain mixtures. The final relative quantitative analysis of the various CTV strains in the ‘Marsh’ and ‘Star Ruby’ trial plants has not been completed, but the protocols have been developed and the extractions prepared. Small RNA sequencing was used to investigate the citrus host response to CTV infection. Differential regulation between infected and the healthy samples of three plant miRNAs was shown. The source was, however also shown to contain a viroid and differentially expressed sRNA and genes will be further validated by RT-qPCR. Additionally, Next Generation Sequencing (NGS) was applied to diagnostics in citrus viral pathology. A bioinformatic pipeline for virus diagnostics based on NGS data was optimized and packaged in a user-friendly interface named Truffle (http://truffle.sourceforge.net). This work will benefit future viral diagnostics once certain standards and methodologies are in place. There are a number of objectives that are still in progress and a final report will be submitted in 2018.

Opsomming

Om die geskikte *Citrus tristeza virus* (CTV)-raskomponente vir kruisbeskerming te identifiseer, is genetiese en biologiese karakterisering van CTV enkel-ras isolate nodig asook evaluering van hul individuele en gekombineerde invloed op spesifieke sitrusgashere. Sewe enkel-ras CTV isolate is geïdentifiseer en biologies gekarakteriseer op 'n gasheerreeks van verskillende sitrus tipes. Volledige virus genoom basisvolgordes van ses CTV-rasse is bepaal. Twee kommersiële pomelo variëteite, 'Star Ruby' en 'Marsh', is gebruik in 'n glashuisproef, om die vermoë van sekere CTV-rasse te bepaal om stamgleuf te veroorsaak, aleenlik of in gemengde infeksies. 'n Verdere proef is daarop gemik om die kruisbeskermings-vermoë van verskeie CTV-rasse, gekarakteriseer in die eerste proef, te toets deur inokulasie met 'n strawwe CTV ras van pomelos. Voorlopige resultate van die eerste pomelo proef dui op moontlike interaksies tussen die verskillende CTV rasse, vanweë verlaagde simptoom uitdrukking waargeneem in behandelings met sekere ras-mengsels. Die finale relatiewe kwantifisering van die verskillende CTV rasse in die ‘Marsh’ en ‘Star Ruby’ proef plante is nog nie voltooi nie, maar die toetse is ontwikkel en die ekstraksies voorberei. Die voorkoms van klein RNA (sRNA) is gebruik om die sitrus gasheer se reaksie op CTV-infeksie te ondersoek. Differensiële uitdrukking van plant-miRNAs is gewys tussen besmette en gesonde monsters. Die bron is egter ook aangetoon om 'n viroid te bevat en differensiaal uitgedrukte sRNA en gene sal verder deur RT-qPCR bevestig word. Verder, was metagenomiese volgende-generasie volgordebepaling (NGS) toegepas vir diagnostiese doeleindes in sitrus. ʼn Bioinformatiese pyplyn vir virus diagnose, gebaseer op die NGS data is geoptimiseer en verpak in 'n gebruikersvriendelike program, genoem Truffle (http://truffle.sourceforge.net). Hierdie werk sal tot groot voordeel wees vir toekomstige diagnostiek sodra sekere standaarde in plek gestel is. Daar is 'n aantal doelwitte wat nog onvoltooid is en 'n finale verslag sal in 2018 ingedien word.

3.2.7 PROGRESS REPORT: Detection of ‘*Candidatus* Liberibacter asiaticus’ and biological characterization of Liberibacter species from South Africa.

Project 1157 (1 April, 2016 – 31 March, 2018) by Ronel Roberts (ARC-PPRI)

Summary

‘*Candidatus* Liberibacter asiaticus’ (Las), the causal agent of citrus huanglongbing (HLB), was reported from Uganda in 2015. Following this report, CRI and ICIPE conducted surveys in south-eastern Uganda, southern Kenya and eastern Tanzania of citrus showing mottling symptoms, typical of both HLB and African Greening. Total DNA was extracted from these samples and assessed for the presence of Liberibacter species by Las- and ‘*Ca.*’ L. africanus (Laf) -specific real-time PCR tests. Liberibacter positive samples were further characterised to species level by conventional PCR targeting three conserved genes within the Liberibacter genome and the amplification products sequenced. The genes assessed were the 16S rDNA, ribosomal protein J gene (*rplJ)* and the outer membrane protein gene *(omp).* Phylogenetic analyses of the sequences demonstrated that a number of samples from Uganda, Kenya and Tanzania were infected with Laf, the bacterial agent associated with African Greening. Additionally, samples from these three countries were shown to contain Liberibacter sequences characteristic of ‘*Ca.*’ L. africanus subsp. clausenae’ (LafCl) which was first described from indigenous *Clausena anisata* (Horsewood) trees in South Africa. We were not able to verify the presence of Las in Uganda, nor from samples collected from Kenya or Tanzania. However, this is the first report of natural infection of citrus with LafCl. This study demonstrated the need for a Las-specific test and various published and newly-designed real-time PCR assays were assessed. A single primer set differentiated LafCl and Laf in High Resolution Melt (HRM) analysis. Graft transmission studies of LafCl from *C. anisata* to sweet orange seedlings were conducted, but transmission of LafCl has not been confirmed as yet.

Opsomming

‘*Candidatus* Liberibacter asiaticus’ (Las) die oorsaakende organisme van sitrus huanglongbing (HLB) was vir die eerste keer in 2015 gerapporteer vanuit Uganda. Na aanleiding van hierdie verslag het CRI en ICIPE opnames van sitrus vir kenmerkend simptome van beide HLB en Afrika vergroening geloots in suid-oos Uganda, die suide van Kenya en oostlike gebiede van Tanzania. Totale DNS was geëkstraeer uit die versamelde monsters en die teenwoordigheid van Liberibakters was bepaal met Las en ‘*Ca.*’ L. africanus (Laf)-spesifieke toetse. Liberibakter positiewe monsters is verder tot op spesie vlak gekarakteriseer met konvensionele PKR van drie gekonserveerde gene binne die Liberibakter genoom, waarvan die produkte se nukleotied volgorde bepaal is. Die volgende gene is bestudeer, 16S rRNA, ribosomale protein J (*rplJ*) en die buite membraan protein (*omp*). Vanaf filogenetiese resultate is Laf, die bakteriële agent van Afrika vergroening, geïdentifiseer vanaf ŉ aantal monsters uit Uganda, Kenya en Tanzania. Monsters uit hierdie drie lande het Liberibakter DNS kenmerkend van ‘*Ca.*’ L. africanus subsp. clausenae’ (LafCl) bevat, wat geassosieër word met inheemse *Clausena anisata* (Perdepis) uit Suid Afrika. Die teenwoordigheid van Las in Uganda asook Kenya en Tanzania kon nie bevestig word deur die opnames nie. Hierdie is die eerste keer dat LafCl vanaf sitrus geïdentifiseer word. Hierdie studie het duidelik die tekort aan ŉ Las-spesifieke toets gedemonstreer. Verskeie gepubliseerde asook nuwe PKR toetse is ondersoek, maar slegs ŉ enkele toets kon tussen LafCl en Laf differesieer met ‘High Resolution Melt’ (HRM) analise. Oordragingings toetse met LafCl is gedoen en is vanaf *C. anisata* na soet lemoen saailinge ge-ent, maar oordraging is tot op hede nog nie bevestig nie.

3.2.8 PROGRESS REPORT: Searching for a *Citrus tristeza virus* source suitable for cross-protecting soft citrus

Project 968 (2004 - 2020) by J.H.J. Breytenbach, G. Cook and S.P. van Vuuren (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 pre-immunisation with the LMS6 CTV source. This caused concern as the budwood that was multiplied from these mother trees and supplied to the commercial nurseries, were virus-free, rendering the trees unprotected against natural CTV infection with severe strains introduced by aphids. A change to another CTV source compatible with mandarin types was required. The GFMS12 CTV source was approved 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 soft citrus cultivars. The current field trials are extensions of the glasshouse trial. Trees of two Clementine selections (‘Clemenluz’, ‘Esbal’) and two mandarin selections (‘Valley Gold’, ‘Morr 22’) on Troyer citrange rootstock were prepared and pre-immunized with CTV sources; i.e. CTVSC, SM47, SM48 and SM49. Trees with these sources are compared to trees pre-immunised with GFMS12 (standard) and trees planted virus-free. Trees were planted during 2010/11 at two localities in different climatic regions suitable for the production of soft citrus, i.e. Groblersdal in Mpumalanga and Citrusdal in the Western Cape. Trees at both trials sites were lost due to frost and poor drainage one year after planting. New trees were prepared and the trials re-planted at two new sites. The first trial was planted in the Citrusdal area in 2012 and the second was planted in spring 2014 at Burgersfort. Tree canopy volumes were recorded at Citrusdal and Burgersfort this season. The trees at both sites are still young and this early tree growth is not definitive, but divergent results between the areas may either indicate influences of climate on symptom expression or that different strains of the original CTV source were transmitted during pre-immunisation. Transmission of all components contained in a mixed population source can be problematic. These trials were also re-established at different times, which could have influenced the CTV population structures. The CTV challenge populations in the two areas would be another influencing factor. If significant differences are observed later in the trial, it will be necessary to determine the CTV strain components found in individual trees.

Opsomming

Tydens die her-indeksering van die Grondvesblok se moederbome gedurende 2003 is gevind dat ŉ groot aantal Clementine en mandaryn bome geen CTV bevat het nie, ten spyte van preïmmunisering met die LMS6 bron. Dit het kommer gewek dat enthout, wat aan die kommersiële kwekerye verskaf word, virusvry is en nie beskerming bied teen natuurlike CTV rasse wat in die veld deur plantluise oorgedra word nie. Die GFMS12 CTV bron is goedgekeur om tydelik gebruik te word tot ŉ geskikte bron vir sagte sitrus gevind is. ŉ Glashuis proef is gedurende 2006 gedoen om CTV bronne in vier verskillende kultivars te evalueer. Die veldproewe is ŉ uitbreiding van die glashuis proef. Twee Clementine seleksies (‘Clemenluz’, ‘Esbal’) en twee mandaryn hibried seleksies (‘Valley Gold’, ‘Morr 22’) is op ‘Troyer’ citrange onderstamme ge-okuleer en gepreïmmuniseer met verkillende CTV bronne, nl. CTVSC, SM47, SM48 en SM49. Bome met hierdie bronne word met GFMS12 (standaard) en bome wat virusvry geplant is, vergelyk. Die bome is gedurende 2010/11 in twee verskillende klimaatstreke, wat vir sagte sitrus geskik is, geplant, nl. Groblersdal in Mpumalanga en Citrusdal in die Wes Kaap. As gevolg van dreineringsprobleme en koue skade is proefbome in beide persele verloor. Nuwe bome is voorberei en gedurende 2012 in die Citrusdal omgewing geplant en gedurende 2014 is die proef te

Burgersfort geplant. Groei data is by die Citrusdal en Burgersfort proefbome geneem. Die bome op albei persele is nog jonk en hierdie vroeë boomgroei is nie noodwendig aanduidend nie, maar verskillende resultate verkry uit die twee gebied kan óf die invloede van die klimaat op simptoomuitdrukking aandui of dat verskillende rasse van die oorspronklike CTV-bron tydens preimmunisering oorgedra is. Oordrag van alle komponente in 'n bron, bestaande uit verskeie CTV rasse , kan problematies wees. Hierdie proewe is ook op verskillende tye hervestig, wat moontlik die CTV-bevolkingstrukture beïnvloed het. Die endemiese CTV rasse in die twee gebiede sal ook verskil en inpak hê. As daar later belangrike verskille in die proewe waargeneem word, sal dit nodig wees om die CTV-raskomponente, wat voorkom in individuele bome, te bepaal.

3.2.9 PROGRESS REPORT: Comparison of shoot tip grafted citrus with old clone material

Project 1074 (2013 - 2023) by G. Cook, S.P. van Vuuren, J.H.J. Breytenbach and C. Steyn (CRI)

Summary

Claims that the use of old clone material of cultivars is more profitable than that supplied by the Citrus Improvement Scheme (CIS) is investigated in a glasshouse trial and a field trial. Graft transmissible pathogens are removed by shoot tip grafting from accessions submitted to the CIS. Thereafter this material is inoculated with an approved *Citrus tristeza virus* (CTV) source for cross-protection. The objective of this study is to compare tree and fruit characteristics of shoot tip grafted material with that of old clone material. Three cultivars were selected for this investigation; *viz.* 'Benny' Valencia, 'Cambria' navel and 'Glen Ora Late' navel. Budwood was collected from original sources of the cultivars and budded according to normal nursery practices to 'Swingle' citrumelo, 'Carrizo' citrange and 'C35' citrange rootstocks. The same was done with equivalent material obtained from the CFB. Strict sterilisation measures of cutting tools were maintained during budding and inoculation. The planting of the trial was delayed by problems with the source material and then drought conditions. The trial was eventually planted at Burgersfort in 2016 and the trial trees have established well. Two trial visits were done this season and maintenance pruning done. The second set of trial trees is kept in an insect free tunnel as a glasshouse trial as a second trial site could not be found. Stem-pitting on sweet orange is not commonly observed in South Africa due to the mild CTV pre-immunisation sources applied in the cross-protection programme, however, old-clone, field derived inoculum sources used, contained CTV components that caused mild to severe stem-pitting on the young trees. This result already demonstrates the value of using bud-wood supplied by the CFB, rather than field-cut material. Use of field-cut budwood has the potential to disseminate severe strains of CTV.

Opsomming

Aannames dat ou kloon materiaal meer winsgewend is as Sitrus Verbeteringskema (SVS) materiaal vanaf die Grondvesblok (GVB) word tans ondesoek in beide ŉ glashuis en ŉ veld proef. GVB materiaal is skoongemaak van alle entoordraagbare siektes deur middel van groeipunt-enting en is daarna geïnokuleer met ŉ goedgekeurde *Citrus tristeza virus* (CTV) bron vir kruisbeskerming. Die doel van die studie is om boom en vrug eienskappe van GVB materiaal met die van ou kloon materiaal te vergelyk. Drie kultivars is betrokke, nl. 'Benny' Valencia, 'Cambria' nawel en 'Glen Ora Late' nawel. Okuleerhout is van oorspronklike bronne van die kultivars versamel en volgens normale kwekery praktyke op 'Swingle citrumelo', 'Carrizo citrange' en 'C35 citrange' onderstamme ge-okuleer. Dieselfde is gedoen met materiaal wat vanaf die GVB ontvang is. Streng sterilisasie voorsorgmaatreëls van snygereedskap is gevolg tydens okulering. Die aanplant van die proef is vertraag deur probleme met die bronmateriaal en ook droogte toestande. Die proef is uiteindelik in Burgersfort in 2016 geplant en die proef bome het goed gevestig. Twee proefbesoeke is gedoen in hierdie seisoen. Die tweede stel proefbome word in 'n insekvrye tonnel gehou as 'n glashuisproef omdat 'n tweede proefperseel nie gevind kon word nie. Stamgleuf op soet lemoene word nie algemeen in Suid-Afrika waargeneem nie as gevolg van die matige CTV- preïmmuniseringsbron wat in die kruisbeskermingsprogram gebruik word, maar die ou kloon veldbronne wat as inokulum gebruik was, bevat CTV-komponente wat stamgleuf veroorsaak het op die jong proefbome. Hierdie resultaat toon reeds die waarde van die gebruik van enthout afkomstig van die GVB eerder as ou kloon materiaal. Gebruik van veldgesnyde enthout het die potensiaal om erge rasse van CTV te versprei.

3.2.10 PROGRESS REPORT: Field testing of commercial or potentially important rootstock selections for viroid sensitivity

Project 1155 (2016/7 – 2024/5) by Glynnis Cook, Kobus Breytenbach, Chanel Steyn and Johan Joubert (CRI)

Summary

The choice of rootstock is a critical factor in establishing any citrus orchard. Apart from the productive advantage imparted by commercial rootstocks, they should be compatible to certain soil and climatic

environments as well as demonstrate resistance or tolerance to diseases and pests. Sound rootstock systems also enable growers to topwork an orchard to a different scion, ensuring a quicker return to economic production. *Citrus tristeza virus* (CTV) tolerance is essential for rootstocks used in southern Africa as CTV and its' aphid vector are endemic. Due to their CTV sensitivity, sour orange, its hybrids, and *Citrus macrophylla* cannot be used locally. Viroids are graft-transmitted agents that are pathogenic on sensitive scions and rootstocks and are unlikely to be problematic if disease-free budwood is used, but given the ease of mechanical transmission via cutting tools, they are sometimes unintentionally introduced into nurseries and orchards. However, viroids have recently been associated with disease outbreaks where field-cut budwood was used to top-work sensitive rootstocks. New rootstocks emerging on the commercial scene over the past two decades include 'C35' citrange and the Minneola x trifoliate hybrid, 'MxT'. Additional to these, other rootstock selections from the USA will be trialled under local conditions. Apart from their horticultural suitability and performance, it is important to know the viroid sensitivities of these rootstocks. A field trial is being prepared to test the sensitivity of these newer commercial or potentially commercial rootstocks to *Citrus dwarfing viroid* and *Hop stunt viroid* (IIa). All seed sources were obtained, but release from Post Entry Quarantine was delayed, which resulted in a delay in the trial preparation. The various seed sources were supplied to a nursery for tree production. All seed sources germinated well and seedlings were planted out for scion budding in the spring of 2017.

Opsomming

Die keuse van onderstam is belangrik in die vestiging van enige sitrus boord. Afgesien van die produksie voordele wat kommersiële onderstamme bied, moet hulle geskik wees vir sekere grond- en klimaatomgewings, asook weerstand of verdraagsaamheid toon teenoor siektes en plae. Gesonde onderstamme stel ook ŉ produsente in staat om 'n boord oor te werk na ŉ ander bostam, wat 'n vinniger terugkeer tot ekonomiese produksie moontlik maak. *Citrus tristeza virus* (CTV) verdraagsaamheid is noodsaaklik vir onderstamme wat in Suider-Afrika gebruik word omdat CTV en sy plantluisvektor endemies is. Weens die vatbaarheid vir CTV, kan bitter serville, sy hibriedes asook *Citrus macrophylla* nie lokaal gebruik word nie. Viroïdes is oordraagbare entiteite wat patogenies is op sensitiewe bo- en onderstamme, en is meestal nie problematies as siektevrye enthout gebruik word nie, maar weens maklike meganiese oordraging deur snygereedskap word viroïdes soms, per ongeluk, in kwekerye en boorde versprei. Viroïede is wel onlangs geassosieer met uitbrake van siektes waar veld gesnyde enthout gebruik was om sensitiewe onderstamme oor te werk. Nuwe onderstamme wat die afgelope twee dekades op die kommersiële toneel verskyn het, sluit in 'C35' citrange en die Minneola x trifoliate baster, 'MxT'. Bykomend tot hierdie, sal verskeie ander onderstamme, afkomstig van die VSA, onder plaaslike toestande getoets word. Afgesien van tuinboukundige geskiktheid en prestasie, is dit belangrik om die viroïed sensitiwiteit van hierdie nuwe onderstamme te ondersoek. 'n Veldproef word voorberei om die sensitiwiteit van hierdie nuwer kommersiële of potensieel kommersiële onderstamme teen *Citrus dwarfing viroid* en *Hop stunt viroid* (IIa) te toets. Alle saadbronne is verkry, maar vrystelling van die saad uit kwarantyn is vertraag, wat 'n vertraging in die proefvoorbereiding veroorsaak het. Die verskillende saadbronne is aan 'n kwekery vir boomproduksie verskaf. Alle saadbronne het goed ontkiem, saailinge is uitgeplant en boompies sal in die lente van 2017 gemaak word.

3.3 PROGRAMME: SOILBORNE DISEASES

Programme coordinator: Jan van Niekerk (CRI)

3.3.1 Programme summary

The projects within the soilborne diseases portfolio address diverse research questions related to soilborne diseases and pests of citrus. Attention is given to finding alternative, softer and more sustainable chemicals that can be used in the management of *Phytophthora* and citrus nematode problems. Together with this the problem of citrus decline and replant disease are also being investigated, specifically looking at factors that could be used as early indicators of tree decline. Unknown diseases with unknown causal organisms and epidemiology is furthermore studied to determine the causes of the observed disease and which management practices have potential to lessen the impact of the disease.

Projects 762 (3.3.3) and 1030 (3.3.4) are specifically aimed at finding alternative means of control for *Phytophthora* and citrus nematode. Data have been recorded in project 762 since 2011 and the juvenile and female nematode counts still do not clearly show up differences between treatments. However, preplant fumigation treatments are starting to emerge as having taller trees with thicket trunks compared to the other treatments. Preplant fumigation is therefore clearly beneficial. In the 2016/2017 season a field trial continued to test PL Gold Plus, a *Paecilomyces lilacinus* containing fungal nematicide and OMV-JJ1, a garlic derivative, according to respectively seven and nine different treatment regimes. Results from nematode counts indicated that the PL Gold Plus gave the best results in terms of control of citrus nematode juveniles and females. The specific regimes that gave the best results were regime 4 where the PL Gold Plus was applied in September

and October 2016 followed by a Rugby application in November 2016. The other regime that also gave good results were regime 7 where the regime started with a Rugby application in September 2016 followed by a PL Gold Plus application in October and November. The OMV-JJ1 unfortunately did not give very good results. It was furthermore discovered in this project that phosphonate applications for brown rot control on Nadorcott mandarin fruit, leads to severe phytotoxicity on the fruit when the applications are done at or after colour break phase of fruit development.

Two distinct diseases have recently been observed in orchards in the Kirkwood and Patensie areas of the Eastern Cape province and in orchards in Swaziland and Hoedspruit (3.3.5). Extensive sampling and isolations from diseased material have led to the conclusion that a complex of pathogens are involved in the disease in the Eastern Cape while in the Swaziland/Hoedspruit areas only one pathogen is at work. Pathogenicity trials did show some lesion development by the inoculated pathogens in the inoculated rootstocks. However, these lesions were not big enough to explain the severe trunk rot observed in the field. Further investigation revealed that the soils in the affected orchards have high pH while the irrigation water has, apart from high pH, also a high bicarbonate content. All the affected orchards are planted to Carrizo citrange or Swingle citrumelo rootstock trees. These rootstocks are very sensitive to high pH conditions and the high pH soils and water could therefore have predisposed the trees to pathogen infection, ultimately leading to the trunk rot and dieback. These factors are now being investigated further.

Following on project 910, project 1092 was started in April 2015 to further investigate the factors involved in citrus decline (3.3.7). Four orchards, showing various degrees of decline, were selected in the Nelspruit area. In each orchard, 20 trees per decline category (1-3) were selected and marked. Soil, root and leaf samples were collected from these trees. Chemical leaf and soil analyses were done along with physical soil analysis, nematode counts in the soil and roots, *Phytophthora* incidence in the soil and starch content of the roots. Due to outstanding results, in depth data analyses using multivariate and principle component analyses were not possible. These will be completed once all data is available and reported on in 2018.

In project 1152 (3.3.8) results indicated that in soils from old orchards there are oomycete and fungal pathogens present that reduces the growth of citrus seedlings. This indicates that orchards replanted on these soils can expect to experience replant problems and stunted growth due to the pathogens present. The pathogens identified include *Phytophthora nicotianae, P. citrophthora, Pythium* spp. and *Fusarium* spp along with citrus nematodes. The interaction between these biological agents need to be investigated further to determine how they combine to cause replant disease problems, specifically stunted growth.

The last project in the programme, 1101 (3.3.6), focusses on the soilborne pathogens in citrus nurseries. A large number of *Phytophthora* and *Pythium* isolates were collected during the project. These were characterized using molecular techniques. This indicated that a variety of *Pythium* species are present in citrus nurseries. These need to be studied further to determine their pathogenicity along with mefenoxam and chlorine sensitivity. *Phytophthora* isolates were found to have varying degrees of sensitivity to mefenoxam. Chlorine sensitivity tests also indicated that *Phytophthora nicotianae* and *P. citrophthora* isolates varies in their sensitivity to chlorine and that the propagules of some isolates are eliminated from water only after 60 minutes' exposure at 6 ppm. Standard potting medium treatments with mefenoxam and captan showed variable effectiveness in different potting media that need to be investigated further. Phosphonates were furthermore applied to citrus seedlings according to three regimes, using two application methods. The results from this trial showed that foliar applications lead to bigger, healthier trees as *Phytophthora* infections in treated trees were also less than in untreated trees.

Programopsomming

Die projekte binne die grondgedraagde siekteprogram spreek diverse navorsingsvrae aan wat verband hou met grondgedraagde siektes en peste van sitrus. Aandag word geskenk om alternatiewe, meer omgewingsvriendelike en volhoubare middels te vind wat gebruik kan word in die bestuur van *Phytophthora* en sitrus nematode probleme. Die verskynsel van sitrus agteruitgang ("citrus tree decline") en herplant probleme word ook ondersoek ten einde spesifieke faktore te identifiseer wat gebruik kan word as vroeë indikators van agteruitgang en/of herplant probleme. Onbekende siektes waarvan die oorsakende organismes en epidemiologie onbekend is, word bestudeer om vas te stel wat die oorsake is en watter bestuurspraktyke die potensiaal het om die impak van die siekte te verminder.

Projek 762 (3.3.3) en 1030 (3.3.4) se doel is spesifiek om alternatiewe chemiese middels vir die beheer van *Phytophthora* en sitrus nematode te vind. Binne projek 762 word data al sedert 2011 versamel en die nematode tellings toon steeds geen duidelike verskille tussen behandelings nie. Die voorplant berokingsbehandelings begin toon dat hulle beter doen in dat hulle bome bevat wat hoër is met dikker stamme as ander behandelings. Voorplant beroking van grond in herplant situasies is dus duidelik voordelig. Gedurende die 2016/2017 seisoen

is ŉ veldproef voortgesit vir die evaluasie van PL Gold Plus, ŉ *Paecilomyces lilacinus* bevattende nematisied en OMV-JJ1, ŉ knoffel ekstrak produk in onderskeidelik sewe en nege verskillende programme. Resultate het aangedui het dat PL Gold Plus die beste resultate gelewer het in terme van beheer van sitrus nematode J2's en wyfies. Die spesifieke programme wat die beste vertoon het was program 4 waar PL Gold Plus toegedien is in September en Oktober 2016 gevolg deur ŉ Rugby aanwending in November 2016. Die ander program wat ook goeie resultate gegee het was program 7 waar Rugby aangewend is in September 2016 gevolg deur PL Gold Plus toedienings in Oktober en November 2016. Die OMV-JJ1 het ongelukkig nie bevredigende resultate gegee nie. In twee klimatiese diverse produksie areas is fosfonaat vrugtoedienings op Nadorcott mandaryne geëvalueer. Resultate van die evaluasies het getoon dat indien die aanwendings gemaak word na die kleurbreuk fase van vrugontwikkeling, ernsitige fitotoksiese skade op vrugskille voorkom.

Twee duidelik verskillende siektes is onlangs in boorde in Kirkwood en Patensie in die Oos-Kaap en boorde in Swaziland en Hoedspruit waargeneem (3.3.5). Intensiewe monsterneming van simptomatiese materiaal tesame met isolasies uit hierdie materiaal het tot die gevolgtrekking gelei dat in die Oos-Kaap ŉ kompleks van patogene teenwoordig is. In die Swaziland/Hoedspruit areas is slegs een patogeen geassosieer met die waargenome simptome. Patogenisiteitsproewe het getoon dat die patogen wel letsels veroorsaak in geïnokuleerde onderstamme, maar dat die letsels nie groot genoeg is om die ekstreme stamvrot in die veld te verklaar nie. Verdere ondersoek het aangetoon dat die grond en water van geaffekteerde boorde hoë pH waardes het met die water wat ook hoog is in bikarbonaat inhoud. Hierdie faktore is nadelig vir Carrizo citrange en Swingle citrumelo wat die bome op hierdie onderstamme kon predisponeer vir patogeen infeksie wat eindelik lei tot stamverrotting en terugsterwing. Hierdie faktore word nou verder ondersoek.

In opvolg van projek 910 is projek 1092 in April 2015 begin (3.3.7). Die doel was om die faktore betrokke in sitrus agteruitgang verder te ondersoek. Vier boorde wat verskillende vlakke van agteruitgang toon is in die Nelspruit area geïdentifiseer. In elke boord is 20 bome per kategorie (1-3) gekies en gemerk. Grond, blaar en wortelmonsters is versamel van hierdie bome. Chemiese ontledings van blare en grond, blaargrootte en fisiese grondontledings is gedoen. Hiermee tesaam is ook nematode ontledings van die grond en wortels gedoen asook *Phytophthora* in die grond en styselontledings van die wortels. As gevolg van uitstaande data kon in diepte statistiese ontledings nog nie gedoen word nie.

Resultate van projek 1152 (3.3.8) het aangedui dat in gronde van ou boorde daar oomycete en swampatogene teenwoordig is wat die groei van saailinge inhibeer. Dit toon dat boorde wat herplant word in hierdie grond kan affekteer deur herplant probleme en geïnhibeerde groei van bome as gevolg van hierdie patogene. Patogene wat identifiseer is sluit in *Phytophthora nicotianae, P. citrophthora, Pythium* spp. en *Fusarium* spp tesame met sitrus nematodes. Die interaksie tussen hierdie biologiese agente moet bestudeer word ten einde vas te stel hoe hulle kombineer om herplant problem en inhibisie van groei te veroorsaak.

Die laaste projek in die program, 1101 (3.3.6), fokus op grondgedraagte patogene in sitruskwekerye. ŉ Groot getal *Phytophthora* en *Pythium* isolate is versamel en d.m.v. molekulêre metodes gekarakteriseer. Dit het getoon dat verskeie *Pythium* spp. in kwekerye voorkom. Hierdie sal verder ondersoek word t.o.v. hul mefenoxam en chloor sensitiwiteit. *Phytophthora* isolate is bevind om wisselende vlakke van mefenoxam sensitiwiteit te toon en verdere ondersoek is nodig om te bepaal wat die vlak van weerstand is. Chloorsensitiwiteit proewe het aangetoon dat *Phytophthora nicotianae* en *P. citrophthora* isolate ook varieer ten opsigte van hul sensitiwiteit vir chloor en dat propagules van sommige isolate eers elimineer word in water na blootstelling van 60-minute teen ŉ konsentrasie van 6 dpm. Fosfonate is verder ook toegedien op sitrussaailinge in drie verskillende programme deur gebruik te maak van twee verskillende aanwendingstegnieke. Die proefresultate het getoon dat blaarbespuitings gelei het tot groter en gesonder bome omdat die fosfonaat aanwendings ook die *Phytophthora* infestasie in behandelde saailinge verminder het.

3.3.2 FINAL REPORT: Training of dogs for the detection of citrus trunk and branch canker.

Project 1136 (2016/2017) by Tian Schutte, Jan van Niekerk, Charl Kotze (CRI) and Brig. Stan Gillham (specialist in working dogs and dog technology)

Summary

Phytophthora citrophthora causes trunk and branch cankers on citrus and causes severe losses in Clementine mandarin orchards especially. If detected early a trunk and branch treatment with contact fungicides are effective in treating this disease. However, early detection is often difficult as the disease starts with small gumming spots on the trunks of infected trees. Disease scouts therefore easily miss early signs of the disease. Due to infected trees excreting a distinctive smell, training of sniffer dogs was investigated in this study to determine if they could be trained to detect this disease and help with early detection of the disease. This will then help with early treatment of the disease, reducing the loss of trees. Results from this study showed that

dogs can be trained successfully to detect infected trees and that they can be used in a preventative disease management strategy.

Opsomming

Phytophthora citrophthora veroorsaak stam-en takkankers van sitrus en veroorsaak swaar verliese in veral Clementine mandaryn boorde. Kontakswamdoder behandelings van besmette bome is uiters effektief indien die siekte vroegtydig opgespoor word. Vroeë opsporing is egter moeilik aangesien die eerste tekens van besmetting klein gomkolle op die stamme van besmette bome is. Hierdie eerste tekens word dus maklik gemis deur werkers in die boord. Besmette bome gee ŉ kenmerkende reuk af en as gevolg van hierdie feit is die opleiding van honde om die bome vroeg op te spoor in hierdie studie ondersoek. Vroeë opsporing van die siekte sal help met tydige behandeling met kontakswamdoder wat dus boomverliese sal verminder. Resultate verkry uit hierdie studie dui aan dat honde suksesvol geleer kan word om besmette bome op te spoor wat dan gebruik kan word in ŉ voorkomende siektebestuurstrategie.

Introduction

South Africa cultivates more than 57 000 ha citrus trees and is the world's second largest exporter of citrus as it exports more than 90 million cartons (15 kg carton-1) of citrus worldwide. About 1.7 million Clementine mandarin trees were planted in South Africa with an average of 743 trees ha-1 (Anonymous, 2007). South African citrus growing areas are scattered over the winter and summer rainfall regions of South Africa, but 70% of all the Clementine trees are planted in the Western Cape province of South Africa, which has a Mediterranean climate.

Gum diseases of citrus trees worldwide are associated with *Phytophthora* spp. that can affect roots, trunks, branches, fruit and shoots (Graham & Menge, 2000). However, the most widespread and important are *P. nicotianae* Breda de Haan (syn. *P. parasitica*) and *P. citrophthora* (R.E. Sm. & E.H. Sm.) Leonian (Erwin & Ribeiro, 1996). They have distinct temporal and climatic requirements, so that their relative distribution and influence also vary in different production areas (Matheron et al., 1997). *P. nicotianae* is more common in subtropical areas of the world and causes foot rot and root rot and occasionally attacks aerial parts of the tree and causes a brown rot of fruit (Graham & Menge, 1999).

P. citrophthora causes gummosis and root rot in Mediterranean climates and is the most common cause of brown rot in these areas as well. Concomitantly, foot rot and gummosis occur when *Phytophthora* propagules are splashed onto susceptible trunks near ground level, infect through wounds or growth cracks and produce lesions, which extend down to the bud union (Graham & Menge, 1999). Citrus is grafted on rootstocks that are resistant to biotic or abiotic constraints, associated to a scion selected for its fruit quality. *Phytophthora* spp. can induce different diseases on citrus such as root rot and gummosis on the rootstock as well as gummosis and brown rot of fruit on the scion. Gummosis is a bark disease resulting in the degradation of cambium and phloem tissues. Gum exudation at the surface of the affected bark generally accompanies further development of the disease and extensive lateral invasion of the cambium through injuries or growth cracks results in girdling and weakening or death of the tree (Klotz & Calavan, 1969; Whiteside, 1971). *P. citrophthora* was identified to be the predominant species in orchard soils in Spain as well as the causal organism for branch cankers on Clementine mandarins (Alvarez et al., 2008).

Similar die-back with canker lesions was observed for the first time during 2002 in the Knysna region of the Western Cape on the Clementine mandarin (Citrus reticulata) cv. 'nules' on Troyer citrange (C. sinensis L. Osb. x Poncirus trifoliata L. Raf.) rootstock. Inspections of orchards revealed that dark water-soaked lesions develop on the trunk of the tree accompanied with excessive gumming. In the advanced stages of the disease, diseased bark may fall off to reveal a brown stain on the diseased wood. The bark surrounding the diseased part yellows and softens. The bark then dries and cracks and decaying inner bark can begin to smell. Cankers on the trunk were mainly found on the trunks as a V-shaped lesion starting from the funnel shaped scaffold branches expanding downwards towards the bud union showing a line of demarcation between healthy and unaffected rootstock tissue and the discoloured tissue of the affected scion. From the trunk infections, the lesions also expand upwards to affect the main branches. Advanced infections progressed to a point that resulted in total girdling of the trunk, wilting of the trees resulting in leaf drop of all leaves and fruit that is still retained on the trees. On certain farms in the Eastern Cape, they lose between 200 - 300 trees per year.

The infection on the trunk is superficial and a unique smell is excreted when scratched, the thought is to train dogs to identify only affected trees, screen trees at various intervals to determine if treatment is necessary and to check on efficacy of operators after trunk applications.

Objectives

1. Training of dogs in Pretoria.
2. Imprinting of dogs in Pretoria.
3. Field training of dogs in Pretoria.
4. Practical detection and identification of dogs in the Eastern Cape.

Materials and methods

1. Training:

Training of dogs, two Jack Russels, was completed in three phases as outlined below.

2. Imprinting

This phase of the training was conducted in a closed environment where no distractions were present and it was also protected from any adverse weather conditions. Samples of canker affected trees were collected from orchards in the Eastern and Western Cape and sent to the trainers in Pretoria. This period of imprinting took 2 weeks.

3. Field training.

After the initial imprinting of the dogs, the next step in training was initiated. This entailed field training where the dogs were taught to use their natural abilities to search "wind scent" to identify and locate woody material infected with branch and trunk canker. In this exercise, fresh samples from affected orchards were used. Initially this work was done in an area with sparse natural vegetation and with increased training the training was done in more and more dense vegetation. This work was done on a farm outside Pretoria where the handler and dogs could work safely.

4. Practical detection and identification.

In the final stage of training the dogs were taken to orchards in the Sunday's River Valley where it is known trunk and branch canker problems are experienced. In these orchards the dogs were successful in detecting trees affected by trunk and branch canker.

Results and discussion

In this project two small Jack Russel dogs was trained successfully to detect trunk and branch canker of citrus caused by *Phytophthora citrophthora*. The results obtained showed that dogs can be employed successfully to detect this disease at an early stage of development that will aid in early treatment of the disease.

Conclusion

Results showed that dogs can be used in the preventative management of trunk and branch cankers of citrus.

Future research

In order to gather quantitative data on the effectivity of the dogs in detecting this disease a new research proposal might be submitted for funding in the 2018/2019 year.

Technology transfer

Tian Schutte presented at the CRI symposium in 2016 a presentation on trunk and branch canker caused by *P. citrophthora.* As part of this presentation, the dogs gave a display in detecting infected material in glass jars.

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3.3.3 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 by Jan van Niekerk, MC Pretorius and C Kotze (CRI)

Summary

The aim of this project is to find preplant treatments that are effective in keeping orchard soils free from citrus nematode and Phytophthora for as long as possible after planting. The trial has been going on since January 2010. The various treatments were applied prior to planting in January 2010 with some treatments still being applied annually in January and November. Tree stem diameter, tree height, nematode soil and root analysis, Phytophthora status in the soil and a visual tree rating, are the parameters that have been monitored yearly since the start of the trial. Based on the average percentage reduction in juvenile nematode counts in the soil and female counts in the roots, no clear conclusions as to the best treatments are possible. However, in terms of average percentage Phytophthora infested leaf discs resulting from soil baiting from treated soil and the physical tree measurements, it is becoming clearer that the better treatments are the preplant fumigation treatment. After 7 years of monitoring the trial it is becoming clear that the preplant fumigation treatments are beneficial in that the trees in these treatments are taller, with thicket trunks, compared to the untreated control.

Opsomming

Die doel van hierdie projek is om voor plant behandelings te vind wat grond in boorde vir so lank as moontlik na plant vry sal hou van sitrusaalwurm en Phytophthora. Die proef is al sedert 2010 aan die gang. Verskeie grondbehandelings is gedoen voor plant in Januarie 2010 terwyl sommige behandelings jaarliks in Januarie en November toegedien word. Parameters wat jaarliks gemonitor is sedert die begin van die proef sluit in stam deursnee, boom hoogte, nematode tellings in die grond en wortels, Phytophthora status in die grond en ŉ visuele boom gradering. Op grond van nematode tellings in die grond en boomwortels staan geen behandeling duidelik uit nie. Die gemiddelde persentasie Phytophthora besmette blaarskyfies tesame met die fisiese boommetings begin aantoon dat die beter behandelings die voorplant berokingsbehandelings is. Na afloop van 7 jaar se monitering begin dit duidelik word dat die voor plant berokingsbehandelings se bome groter, met dicker stamme is as die onbehandelde kontrole. Hierdie behandelings het dus ŉ positiewe effek op boomgroei.

3.3.4 PROGRESS REPORT: Evaluation of alternative products for control of citrus nematode and *Phytophthora* spp. in citrus

Project 1030 (2008 – 2018/19) by Jan van Niekerk, MC Pretorius and C Kotze (CRI)

Summary

In the 2016/2017 season a field trial continued to test PL Gold Plus, a *Paecilomyces lilacinus* containing fungal nematicide and OMV-JJ1, a garlic derivative, according to respectively seven and nine different treatment regimes. Also included were Cropguard from Illovo along with Rugby 10ME (cadusafos) from Philagro as the industry standard. Results from nematode counts done in January and March 2017 indicated that the PL Gold Plus gave the best results in terms of control of citrus nematode juveniles and females. The specific regimes that gave the best results were regime 4 where the PL Gold Plus was applied in September and October 2016 followed by a Rugby application in November 2016. The other regime that also gave good results was regime 7 where the regime started with a Rugby application in September 2016 followed by a PL Gold Plus application in October and November. The OMV-JJ1 unfortunately did not give very good results. Phosphonate

applications on fruit were evaluated on Nadorcott mandarins in two climatically diverse production areas. Results from these evaluations showed that if applications were done from colour break phase onwards, severe phytotoxic burn occurs on the fruit rind. Potassium and ammonium phosphite were seen to cause the same damage and growers were therefore cautioned to avoid these applications during the post colour break phases of mandarin fruit development. In the 2017/2018 season potential alternatives will be evaluated for late season control of Phytophthora brown rot on mandarin fruit.

Opsomming

Gedurende die 2016/2017 seisoen is ŉ veldproef voortgesit vir die evaluasie van PL Gold Plus, ŉ *Paecilomyces lilacinus* bevattende nematisied en OMV-JJ1, ŉ knoffel ekstrak produk in onderskeidelik sewe en nege verskillende programme. Cropguard van Illovo en Rugby 10 ME (kadusafos) van Philagro is ook ingesluit as die industrie standaard behandeling. In Januarie en Maart 2017 is nematode tellings gedoen wat aangedui het dat PL Gold Plus die beste resultate gelewer het in terme van beheer van sitrus nematode J2's en wyfies. Die spesifieke programme wat die beste vertoon het was program 4 waar PL Gold Plus toegedien is in September en Oktober 2016 gevolg deur ŉ Rugby aanwending in November 2016. Die ander program wat ook goeie resultate gegee het was program 7 waar Rugby aangewend is in September 2016 gevolg deur PL Gold Plus toedienings in Oktober en November 2016. Die OMV-JJ1 het ongelukkig nie bevredigende resultate gegee nie. In twee klimatiese diverse produksie areas is fosfonaat vrugtoedienings op Nadorcott mandaryne geëvalueer. Resultate van die evaluasies het getoon dat indien die aanwendings gemaak word na die kleurbreuk fase van vrugontwikkeling, ernsitige fitotoksiese skade op vrugskille voorkom. Kalium en ammonium fosfonaat is bevind om dieselfde skade te veroorsaak en produsente is dus vermaan om hierdie aanwendings te vermy na kleurbreuk fase van mandaryn vrugontwikkeling. Tydens die 2017/2018 seisoen sal potensiële alternatiewe getoets word vir die laatseisoen beheer van Phytophthora bruinvrot op mandaryn vrugte.

Introduction

Nematodes are a diverse group of invertebrates, abundant as parasites or free living forms in soil, freshwater and marine environments. Soils are a particularly rich environment for nematodes, with about 26% of described genera inhabiting soil as bacterivores, fungivores, omnivores, predators or plant parasites (McSorley, 2005).

The citrus nematode, *Tylenchulus semipenetrans* Cobb, infects citrus worldwide (Van Gundy and Meagher, 1977; Heald and O'Bannon, 1987) and is the most abundant and frequent plant-parasitic nematode in citrus groves. Yield losses are estimated at about 10% worldwide. The citrus nematode is associated with poor growth of young citrus trees planted in infested groves and with poor performance of mature citrus trees. The host range of *T. semipenetrans* includes all *Citrus* species and most hybrids of citrus with other members of the rutaceous family such as trifoliate orange (*Poncirus trifoliate* L.Raf*)*. Non-rutaceous plants such as grape (*Vitis vinifera*, L*),* olive (*Olea europea*, L*)* and persimmon (*Diospyrus spp.)* are also hosts (Verdejo-Lucas, 2002).

Damage thresholds, nematode population densities that suppress tree growth and yield, are influenced by several factors including aggressiveness of the nematode population, soil type, rootstock, other diseases and grove management practices (Garabedian *et a*l. 1984). Threshold values in South Africa have been set at 10 000 juveniles/250 cc soil and a 1000 females/10 g roots in samples.

T. semipenetrans migrates very slowly on its own power and therefore does not readily spread from tree to tree in existing orchards. Infestation of new orchards occurs mainly through infested planting material and contaminated irrigation water (Tarjan, 1971; Baines, 1974). It is recorded that the sheath nematode, *Hemicycliophora* spp. occurs in combination with the citrus nematode in certain citrus producing countries in the world (Van Gundy, 1959) but the effect of the nematode on yields is not known. The sheath nematode was also detected in certain citrus producing regions in South Africa (L. Huisman, personal communication, CRI Diagnostic Centre, Nelspruit, 2007).

In the past, the nematicide 1,2 dibromo-3-chloropropane (DBCP) was widely used in the irrigation water against this nematode with great success. The nematode was effectively controlled while yields were also substantially increased (O'Bannon *et al.,* 1963; Philis, 1969). The activity on eggs is the most important difference between the soil fumigants used to control nematodes earlier this century, and today's non-fumigant chemicals. Following the withdrawal of DBCP, non-fumigant post-plant nematicides (carbamate or organophosphate acetylcholinesterase inhibitors) were introduced. These chemicals, however, could not eliminate, or greatly reduce, nematode populations even if applied every year. Fenamiphos is translocated systemically in the vascular system of plants, whilst the other nematicides are non-systemic and reduce nematode populations through their initial contact action only. This explains the quick recovery of nematode

populations once the nematicide has been degraded in soil and emphasizes the adverse effect of enhanced degradation, as eggs hatching after the nematicide has been degraded can continue the nematode's life-cycle. The following nematicides are currently registered on citrus in South Africa: aldicarb, cadusafos, fenamiphos, terbufos, ethoprophos, fosthiazate and furfural (Nel *et al*., 2002). When multiple nematicide applications were introduced on a commercial scale to citrus orchards in South Africa, situations occurred where growers were not successful in disrupting the nematode's life cycle despite adhering strictly to prescribed procedures. In an investigation to determine the efficacy of cadusafos in soil where aldicarb and fenamiphos failed as a result of accelerated degradation, it was found that in the absence of sufficient irrigation water none of the nematicides were distributed thoroughly through the soil profile and they consequently failed to eliminate the citrus nematode (Le Roux *et al*., 1998).

Due to safety, environmental concerns and market pressure, only a few registered chemical nematicides remain worldwide for utilization by farmers, and the use of these products is highly restricted. Developing alternatives to chemical nematicides is essential and a great concern to researchers worldwide. Recent attempts to develop alternative methods to manage plant-parasitic nematodes include the use of entomopathogenic nematodes and various biologically derived nematicides and other organic compounds. The aim of this experiment is to: evaluate less toxic compounds for the control of the citrus nematode as well as for the control of *Phytophthora* spp. in citrus orchards.

In the field trial conducted, a range of alternative products such as non-toxic and organic compounds for the control of the citrus nematode have been evaluated. International pressure from various market organizations and governments to reduce the use of highly toxic and environmentally unfriendly products along with the final withdrawal of aldicarb in South Africa, justifies the continued testing of alternative chemicals for the control of nematodes and *Phytophthora* in South African citrus orchards.

Objectives 2015/2016

1. The development and evaluation of new products for the control of soilborne pests and diseases in citrus orchards and nurseries.
2. Investigate grower reports of phosphonate phytotoxic damage on mandarin fruit when applied for Phytophthora brown rot control.

Materials and methods

Nematicide evaluation

A nematode infested citrus orchard with nematode female counts in excess of 5000 females per 10 g of roots was identified in 2014. This was regarded as a suitable trial site, as the standard threshold value of 1000 females per 10 g of roots was exceeded. The 17-year-old Late Valencia on Rough Lemon citrus orchard with a 10 m^2 drip zone is situated east of Nelspruit at Crocodile Valley Citrus Co. Single tree plots were randomly selected and replicated eight times for each treatment. The treatments included were PL Gold Plus, a *Paecilomyces lilacinus* containing fungal nematicide from Becker Underwood and OMV-JJ1, a garlic derivative, was applied according to seven and nine different regimes respectively. Also included were Cropguard from Illovo along with Rugby 10ME (cadusafos) from Philagro that was included as an industry standard treatment. The different dates of applications and dosages are presented in Table 3.3.4.1.

The liquid formulated products were applied by means of a 10 litre watering can to ensure an even distribution of the products under the drip zone of the trees. Cadusafos served as the standard chemical control. Protective clothing was worn to protect the researcher and assisting staff during application of these products. All the applications were executed in good weather conditions with an average day temperature of 29°C. In each year of 2014, 2015 and 16 the first applications were done in September, with soil and root samples collected during December 2014, 2015 and 2016 as well as February and April 2016 and 2017. These were analysed at the CRI DC using the standard techniques for *Phytophthora* and nematode analyses.

Table 3.3.4.1. Dosages and dates of application of the different products applied to determine the effect of these treatments on the citrus nematode populations in the orchard at Crocodile Valley Citrus Co. during the 2016/2017 season.

Product/application regime	**Rate of application**	**Application month**							
Untreated control		**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**May**

PL+ (Regime 1)	10g PL Gold plus 10ml Gold Starter	X	X						
PL+ (Regime 2)	10g PL Gold plus 10ml Gold Starter			X	X				
PL+ (Regime 3)	10g PL Gold plus 10ml Gold Starter					X	X		
PL+ (Regime 4)	10g PL Gold plus 10ml Gold Starter/150ml	X	X	Rugby					
PL+ (Regime 5)	10g PL Gold plus 10ml Gold Starter	X	X	X	X	X	X	X	
PL+ (Regime 6)	10g PL Gold plus 10ml Gold Starter	X	X		X	X			
Rugby/PL+ (Regime 7)	150ml/10g PL Gold plus 10ml Gold Starter	Rugby	X	X					
OMV-JJ1 (Regime 1)	0.5 ml			X					
OMV-JJ1 (Regime 2)	0.5 ml (x 4 consecutive)			X	X	X	X		
OMV-JJ1 (Regime 3)	0.5 ml (x 4 every 2nd month))			X		X		X	X
OMV-JJ1 (Regime 4)	1,0 ml			X					
OMV-JJ1 (Regime 5)	1,0 ml (x 4 consecutive)			X	X	X	X		
OMV-JJ1 (Regime 6)	1,0 ml (x 4 every 2nd month))			X		X		X	X
OMV-JJ1 (Regime 7)	2,0 ml			X					
OMV-JJ1 (Regime 8)	2,0 ml (x 4 consecutive)			X	X	X	X		
OMV-JJ1 (Regime 9)	2,0 ml (x 4 every 2nd month))			X		X		X	X
Cropguard	75ml			X					
Rugby 10 ME	150ml			X		X		X	

Evaluation of phosphonate phytotoxicity on "Nadorcott" *mandarin fruit*

Citrus reticulata Blanco cv. Nadorcott was selected for the study as it makes up the majority of the mandarin plantings in South Africa (CGA, 2016). The first trial site was located outside Nelspruit in the Mpumalanga province of South Africa. This area is characterized by little or no rain during the Nadorcott harvest period. The second trial site was located at Riviersonderend in the Western Cape province of South Africa. This area is prone to getting rain during the Nadorcott harvest period, making this an area with a high citrus brown rot risk. The different trials sites were selected to determine if climatic conditions during fruit development played any role in the phytotoxic damage observed or if it was an occurrence related to the specific cultivar.

Potassium phosphite (555 g/L a.i. = 350 g/L phosphorous acid equivalent) [Fighter, Agchem, South Africa] and ammonium phosphite (386 g/L a.i. = 300 g/L phosphorous acid equivalent) [Brilliant, Arysta LifeScience, South Africa] were applied according to label rates to Nadorcott trees. In the case of the potassium phosphite it was 570 ml/100 L water and for ammonium phosphite the rate was 666 ml/100L water with trees sprayed from both sides to just before the point of run-off. A Stihl® SR 420 motorized backpack mist blower was used in all applications. Application times were immature green, colour break and full colour stages of fruit development. At the Nelspruit trial site, abovementioned treatments were at each application time applied to 6 replicate trees. These 6 trees were randomly laid out in 3 experimental blocks with 2 trees per block. At the Riviersonderend site the layout was the same with the only difference that there were only 3 trees per treatment x application time combination which was divided into 1 tree in each of 3 experimental blocks.

When fruit reached commercial harvest maturity, 20 fruit were picked from each tree representing a different treatment x application time combination and evaluated for phytotoxic damage according to a 0 – 3 index which was quantified as a rating 0 being fruit with no damage, rating 1 fruit with <10% of fruit rind damaged, rating 2 fruit having 11% – 30% of the fruit rind damaged and a rating 3 were fruit with >30% of the fruit rind displaying damage. These ratings were for each replicate tree transformed into a phytotoxic index according to the following equation:

Phytotoxic index = $\sum \frac{[\text{Rind surface damge \%}(0-3)\text{ x number of fruit within each category}]}{\text{Total number of fruit in replicate (N=10)}}$

The phytotoxic index data was subsequently subjected to statistical analyses using analyses of variance (ANOVA) and Fisher's test for least significant difference (LSD) were used to compare means.

Results and discussion

Objective / Milestone	**Achievement**
Apr –Jun 2015 1. Annual report	1. Annual report was written and submitted.
Jul – Sept 2015 1. Trial planning 2. First applications according to trial layout	1. Trial was planned and products obtained. 2. The first applications were done according to the trial layout in Table 3.3.4.1.
Oct – Dec 2015 1. Do applications according to trial layout. 2. Collect soil and root samples. 3. Start with phosphonate trial on mandarins	1. Applications were done according to trial layout. 2. Soil and root samples were collected. 3. Phosphonate applications were done according to the trial protocol.
Jan – Mar 2016 1. Do applications according to trial layout. 2. Collect soil and root samples. 3. Phosphonate applications on mandarins	1. Applications were done according to trial layout. 2. Soil and root samples were collected. 3. Phosphonate applications were done according to the trial protocol.

Nematicide evaluation

In the 2017 season the juvenile citrus nematode counts in this trial was very low (Table 3.3.4.2). In the January 2017 sampling the mean count per 250 cc soil in the untreated control was 731 and in March 2017 this mean was 494. This could be a result of the drought experienced. Due to the low counts, significant differences between treatments were not observed (Table 3.3.4.2). During the January 2017 sampling only 3 treatments reduced the mean counts in comparison to the untreated control. The Rugby treatment reduced counts by 38% compared to the control while the PL+ (R1) treatment reduced the counts by 20% and the OMV-JJ1 (R4) treatment reduced the counts by 15%. From the March 2017 sampling results were much better. Although no significant differences were observed between treatments, several treatments led to a reduction of 60% or more compared to the untreated control (Table 3.3.4.2). The PL+ (R7) where Rugby formed the first application, reduced the juvenile counts by 82% compared to the Rugby alone treatment that caused a reduction of 68% in comparison to the untreated control. The OMV-JJ1 (R3) treatment led to a reduction of 62% and the PL+ (R4) treatment in turn led to a reduction of 60% (Table 2).

Mean female citrus nematode counts were also low making significant results difficult (Table 3.3.4.3). Soil samples taken during January 2017 indicated that none of the treatments led to a decline in mean counts compared to the untreated control. In fact, some treatments had markedly to significantly higher mean counts (Table 3.3.4.3). The picture changed somewhat with the March 2017 sampling. These results revealed that 6 of the treatments reduced the mean counts in comparison to the untreated control (Table 3.3.4.3). The best treatment was PL+ (R7) that reduced the mean counts by 67%. This was followed by the PL+ (R5) with a reduction of 55%, PL+ (R4) with a 40% reduction and the OMV-JJ1 treatment with a reduction of 39%. The Rugby treatment which is the industry standard led to a reduction of 27% in the mean female counts (Table 3.3.4.3).

Phosphonate phytotoxicity on mandarins

Analysis of variance (ANOVA) of the phytotoxic index data indicated that there was no significant location x colour development phase x phosphonate treatment interaction ($P = 0.094$). The phytotoxic damage observed on the fruit was, therefore not influenced by trial site and the difference in climate between the two trial sites. A significant ($P < 0.0001$) colour development phase x phosphonate treatment interaction was observed. This indicated that the most severe phytotoxic damage was caused by potassium phosphite application at the full colour stage of fruit development. On these fruit, the mean phytotoxic index was 2.63, which was significantly higher than the mean index (2.34) on full colour fruit to which ammonium phosphite was applied (Figure 3.3.4.1). Ammonium phosphite applications to fruit at the colour break phase of fruit development led to a

mean phytotoxic damage index of 1.23, which was significantly higher than the mean index (1.12) observed when potassium phosphite was applied to colour break fruit (Figure 1). In contrast to applications on colour break or full colour fruit, no phytotoxic damage was observed on fruit at harvest when applications were done on immature green fruit (Figure 1).

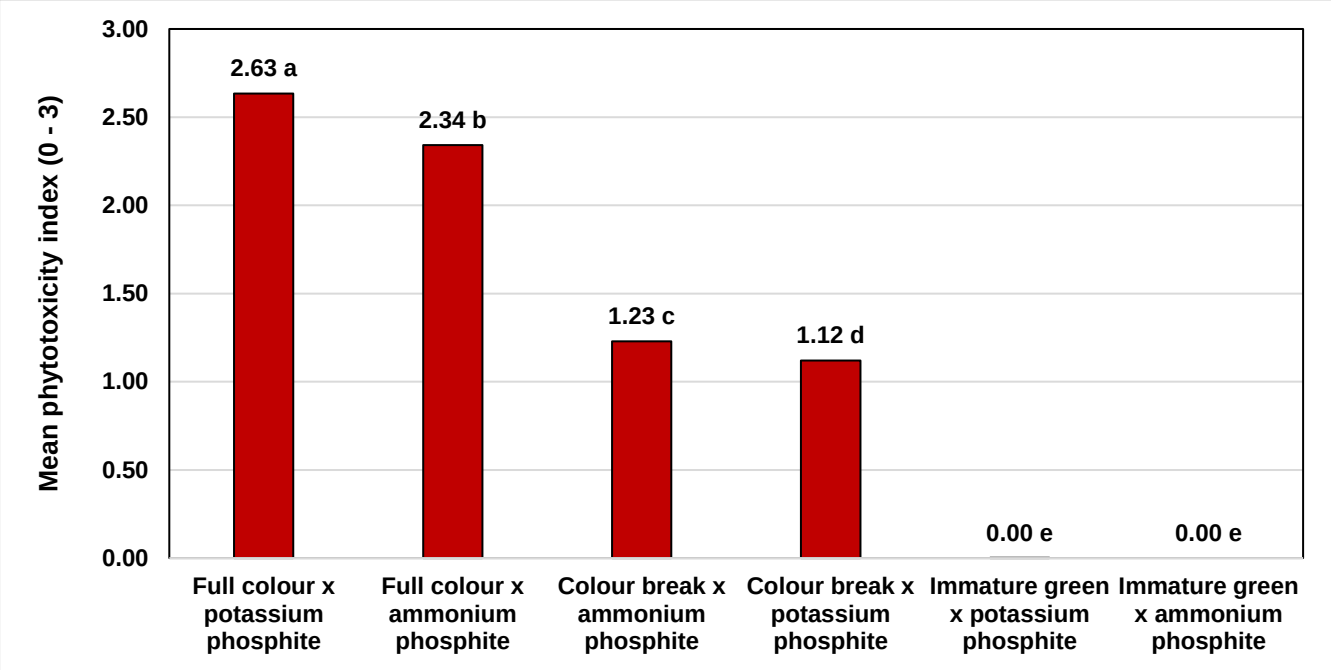

Figure 3.3.4.1. Mean phytotoxic index as observed on fruit at commercial harvest after applications of potassium phosphite or ammonium phosphite applied at immature green, colour break or full colour developmental phase.

Conclusions to date

1. The PL Gold Plus product has some potential as a non-chemical nematicide if applied in combination with cadusafos
2. Applications of phosphonates to mandarin fruit past colour break phase, leads to phytotoxic damage to the rind. Late season phosphonates for brown rot control are therefore not recommended for mandarins.

Table 3.3.4.2. The evaluation of various post-plant treatments for the control of the citrus nematode and their effect on citrus nematode 2nd stage juvenile populations at Crocodile Valley Citrus Co. based on analysis of soil and root samples collected 2016/2017

Treatments	**Rate prod/10m^2**	**January 2017**		**March 2017**	
		L2/250 cc soil	**% Increase(+)/ Decrease(-) vs control**	**L2/250 cc soil**	**% Increase(+)/ Decrease(-) vs control**
Untreated control		731 bc	–	494 ab	–
PL+ (x 2) (R1)	10g PL Gold plus 10ml Gold Starter	581 c	-20	294 ab	-41
PL+ (x 2) (R2)	10g PL Gold plus 10ml Gold Starter	838 a-c	15	625 ab	27
PL+ (x 2) (R3)	10g PL Gold plus 10ml Gold Starter	738 a-c	1	713 a	44
PL+ (x 2)/Rugby (R4)	10g PL Gold plus 10ml Gold Starter/150ml	669 c	9	194 ab	-60
PL+ (x 6) (R5)	10g PL Gold plus 10ml Gold Starter	1575 a	115	619 ab	25
PL+ (x 4) (R6)	10g PL Gold plus 10ml Gold Starter	1250 a-c	71	356 ab	-27
Rugby/PL+ (x 2) (R7)	150ml/10g PL Gold plus 10ml Gold Starter	763 a-c	4	88 b	-82
OMV-JJ1 (R1)	0.5 ml	850 a-c	16	481 ab	-3
OMV-JJ1 (R2)	0.5 ml (x 4 consecutive)	1513 ab	107	381 ab	-22
OMV-JJ1 (R3)	0.5 ml (x 4 every 2nd month))	1088 a-c	49	188 ab	-62
OMV-JJ1 (R4)	1,0 ml	619 c	-15	250 ab	-49
OMV-JJ1 (R5)	1,0 ml (x 4 consecutive)	1250 a-c	71	513 ab	4
OMV-JJ1 (R6)	1,0 ml (x 4 every 2nd month))	1119 a-c	53	456 ab	-8
OMV-JJ1 (R7)	2,0 ml	1000 a-c	36	463 ab	6
OMV-JJ1 (R8)	2,0 ml (x 4 consecutive)	756 a-c	3	338 ab	-32
OMV-JJ1 (R9)	2,0 ml (x 4 every 2nd month))	763 a-c	4	469 ab	-5
Cropguard	75ml	1038 a-c	42	588 ab	19
Rugby	150ml	450 c	-38	156 ab	-68

[1]Means followed by the same letter are not significantly different at a $P \leq 0.05$ confidence level.

Table 3.3.4.3. The evaluation of various post-plant treatments for the control of the citrus nematode and their effect on citrus nematode female populations at Crocodile Valley Citrus Co. based on analysis of soil and root samples collected during 2016/2017.

Treatments	Rate prod/m²	January 2017		March 2017	
		♀ / 10g roots	% Increase(+)/ Decrease(-) vs control	♀ / 10g roots	% Increase(+)/ Decrease(-) vs control
Untreated control		650 b	–	800 c-g	–
PL+ (x 2) (R1)	10g PL Gold plus 10ml Gold Starter	1475 b	126	1238 a-f	55
PL+ (x 2) (R2)	10g PL Gold plus 10ml Gold Starter	1225 b	88	1500 a-d	88
PL+ (x 2) (R3)	10g PL Gold plus 10ml Gold Starter	2400 a	269	950 a-g	19
PL+ (x 2)/Rugby (R4)	10g PL Gold plus 10ml Gold Starter/150ml	813 b	25	475 fg	-40
PL+ (x 6) (R5)	10g PL Gold plus 10ml Gold Starter	1188 b	83	363 g	-55
PL+ (x 4) (R6)	10g PL Gold plus 10ml Gold Starter	763 b	17	675 d-g	-16
Rugby/PL+ (x 2) (R7)	150ml/10g PL Gold plus 10ml Gold Starter	663 b	2	263 g	-67
OMV-JJ1 (R1)	0.5 ml	1000	54	488 fg	-39
OMV-JJ1 (R2)	0.5 ml (x 4 consecutive)	2700 a	315	1425 a-e	78
OMV-JJ1 (R3)	0.5 ml (x 4 every 2nd month))	1075 b	65	1613 a-c	102
OMV-JJ1 (R4)	1,0 ml	838 b	29	800 c-g	0
OMV-JJ1 (R5)	1,0 ml (x 4 consecutive)	788 b	21	863 b-g	8
OMV-JJ1 (R6)	1,0 ml (x 4 every 2nd month))	863 b	33	1388 a-e	73
OMV-JJ1 (R7)	2,0 ml	675 b	4	1713 ab	114
OMV-JJ1 (R8)	2,0 ml (x 4 consecutive)	1113 b	71	988 a-g	23
OMV-JJ1 (R9)	2,0 ml (x 4 every 2nd month))	763 b	17	1788 a	123
Cropguard	75ml	1163 b	79	1000 a-g	23
Rugby	150ml	900 b	38	588 e-g	-27

[1]Means followed by the same letter are not significantly different at a $P \leq 0.05$ confidence level.

Technology transfer

Cutting Edge 218 on the phytotoxic damage of phosphonate applications on Nadorcott mandarins.
Data will be presented at the biennial CRI Symposium in August 2018.

Further objectives and work plan

Continue to search for alternative products for the control of the citrus nematode and *Phytophthora* spp in citrus orchards. Any reports of phytotoxic damage caused by existing applications to control *Phytophthora* on new cultivars will be investigated along with any new products to use in the citrus nursery industry for the control of soilborne pathogens.

Quarterly milestones for Apr-Jun, Jul-Sep, Oct-Dec 2017 and Jan-Mar 2018

April – June 2018

1. Conclude field trial from the 2017/2018 season.
2. Write annual report.
3. Plan new field trials for *Phytophthora* and nematode control.

July – September 2018

1. Plan new field trials for *Phytophthora* and nematode control.
2. Start new trials.

October – December 2018

1. Trial applications.
2. Soil and root sample collection.

January – March 2019

1. Trial applications.
2. Soil and root sample collection.

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3.3.5 PROGRESS REPORT: The status of Armillaria root rot and its management in South African citrus orchards

Project 1068 (2012/3 – 2017/18) by Jan van Niekerk, MC Pretorius and C Kotze (CRI)

Summary

A decline and death of citrus trees have been reported from Swaziland, Hoedspruit and the Gamtoos and Sunday's River valleys for a number of years. Several fungal genera were isolated from diseased material. Molecular identification of the isolated fungi identified *Kretzschmaria deusta* as being the only pathogen associated with the symptoms observed in Swaziland and Hoedspruit. In the two Eastern Cape areas a complex of pathogens were found to be associated with the observed symptoms. In this case the dominant ones were *Fusarium solani*, *Diaporthe neotheicola*, *Coprinellus micaceus* and *Eutypella* sp. *Phaeoacremonium parasiticum* and *Schizophyllum commune* were also isolated but at levels much lower than the abovementioned group. Pathogenicity trials on four different rootstocks indicated that, although all pathogens caused lesions in the vascular tissue of the inoculated rootstocks, these lesions were all smaller than 5 mm. Chemical analyses of soil, leaves and irrigation water indicated that there could be some predisposing factors involved in this disease and that these need to be investigated further. More soil and water analyses will be conducted to confirm initial findings with regards to the predisposing factors present.

Opsomming

Agteruitgang en afsterwe van sitrus bome is vir 'n aantal jare al in Swaziland, Hoedspruit en die Gamtoos en Sondagsriver valleie gerapporteer. Verskeie swam genera is geïsoleer uit simptomatiese materiaal. Molekulêre identifikasie van die geïsoleerde swamme het *Kretzschmaria deusta* as die enigste patogeen geassosieer met die simptome in Swaziland en Hoedspruit geïdentifiseer. In die twee Oos-Kaap areas is bevind dat 'n kompleks van patogene met die waargenome simptome verbind word. Die dominante patogene was *Fusarium solani*, *Diaporthe neotheicola*, *Coprinellus micaceus* en *Eutypella* sp.. *Phaeoacremonium parasiticum* en *Schizophyllum commune* is ook geïsoleer, maar teen baie laer vlakke as bogenoemde groep. Patogenisiteitsproewe met vier verskillende onderstamme het aangedui dat, hoewel die patogene wel letsels veroorsaak het in die vaatweefsel van geïnokuleerde onderstamme, die letsels almal kleiner was as 5 mm. Chemiese analise van grond, blare en besproeiingswater het aangedui dat predisponerende faktore moontlik betrokke is be die siekte en dat dit verder ondersoek moet word. Meer water en grond analises sal dus gedoen word om te bevestig dat chemiese grondfaktore wel die onderliggende stresfaktor is.

3.3.6 PROGRESS REPORT: Preventative and curative management of soilborne pathogens in citrus nurseries

Project 1101 (2014 - 2017) by Jan van Niekerk, MC Pretorius, E Basson and C Kotze (CRI)

Summary

To date this project has delivered some exiting and practical results. Metham sodium drench treatment of potting media has been shown to be an effective alternative to MeBr for the elimination of soilborne pathogens. These results were consequently transformed into practical media treatment guidelines for use by accredited nurseries. Phosphonate foliar applications was furthermore found to significantly increase the size of treated seedlings while also reducing the level of *Phytophthora* infection in the roots. With regards to mefenoxam and chlorine sensitivity of *Phytophthora nicotianae* and *P. citrophthora* some interesting discoveries were made. These species and the isolates within these species vary greatly in terms of their mefenoxam EC_{50} values, with some isolates having values above 11 ppm. It was furthermore seen that these species and their isolates also vary in sensitivity to active chlorine and that in some cases exposure of at least 60 minutes at 6 ppm is needed to eliminate all pathogen propagules from contaminated water.

Opsomming

Tot op hede het hierdie projek opwindende en praktiese resultate opgelewer. ʼn Metam-natrium behandeling van plantmedium is bevind om effektief te wees as alternatief tot MeBr vir die eliminering van grondgedraagde patogene. Hierdie bevinding is omskryf in praktiese medium behandelingsriglyne vir gebruik deur geakkrediteerde kwekerye. Blaarbespuitings met fosfonate het gelei tot betekenisvolle groter saailinge met minder *Phytophthora* infestasie van hul wortels in vergeleke met onbehandelde saailinge. Interessante ontdekkings is ook gemaak met betrekking tot mefenoxam en chloor sensitiwiteit van *Phytophthora nicotianae* and *P. citrophthora*. Hierdie spesies en die isolate binne die spesies is bevind om baie te varieer ten opsigte van hul ED_{50} waardes, met sommige waardes bo 11 dpm. Dit is ook verder gesien dat hierdie spesies en hul isolate verskil in hul sensitiwiteit teenoor aktiewe chloor en dat in sommige gevalle blootstelling van minstens 60-minute teen 6 dpm nodig is om alle patogeen propagules uit besmette water te verwyder.

3.3.7 PROGRESS REPORT: Factors associated with citrus decline and spatial tempo distribution

Project 1092 (2016 - 2018) by MC Pretorius, Charl Kotze (CRI), Nico Labuschagne (UP), Driekie Fourie (NWU) and Adele McLeod (SU)

Summary

During the 2016-17 season the last of the measuring factor samples were collected and the data analysed. Soil and root samples analysed for the presence of nematode infestations indicated in most cases a decrease in counts from category 1 trees to category 3 trees, female counts ranged between 2330 to 200/10g roots. Leaf chlorophyll content also showed a decrease in SPAD units from healthy to declining trees. Although certain trends could be identified from the initial analysis, to determine their full effect on citrus tree decline the data should be analysed along with all of the previously collected data using a multivariate analysis program. The potential of hyper- spectral imaging was also evaluated as a rapid means of identifying declining trees. Initial results in this regard were very promising with the imaging software correctly identifying 77% of the different categories of declining trees.

Opsomming

Monsters van die die laaste maatstaf faktore is gedurende die 2016-17 seisoen geneem en geanaliseer. Grond en wortel monsters wat vir die teenwoordigheid van sitrusaalwurm geanaliseer is het getoon dat daar in meeste van die gevalle ʼn afname in getalle was van kategorie 1 bome na kategorie 3 bome. Wyfie tellings vanuit die wortel analises het tussen 2330 en 200/100g wortels gewissel. Blaar chlorofil inhoud het dieselfde afnemende tendens in SPAD eenhede getoon. Alhoewel daar sekere tendense vanuit die data afgelei kon word, sal die totale effek vanhierdie faktore tot sitrus agteruitgang eersbepaal kan word nadatal die versamelde data deur middel van ʼn multi-veranderlike analiserings program geanaliseer is. Daar is ook gekyk na die moontlikhied om hiper spektriese fotografie te gebruik as ʼn spoedige wyse om terugsterwende bome te identifiseer. Die inisiele resultate wat bekom is was baie positief deurdat die sagteware program tot op n akkuraatheidsvlak van 77% die verskillende agteruitgangs kategorieë kon identifiseer.

3.3.8 PROGRESS REPORT: Understanding citrus replant disease in South Africa with the aim of developing a methyl bromide free management strategy.

Project 1152 (2016 – 2018) by Jan van Niekerk, Charl Kotze (CRI), Laurika Swart and Prof Adele McLeod (USPP)

Summary

In this study, soil samples were collected from four citrus orchards all 45 years or older. The sampled soil was subjected to different analyses and treatments to identify the possible abiotic (physical or chemical) or biotic (pathogenic fungi, oomycetes or nematodes) present in these soils that could pose a risk when orchards are replanted. After treatment, Carrizo citrange seedlings were planted in the soil and grown for 7 months before trial termination. Seedlings measurements indicated that seedlings grown in steam treated soil were the biggest compared to other seedlings from other treatments. This indicated that a biological factor was present in the soils that were eliminated by the steam treatment. Seedlings from the other treatments that were aimed at eliminating oomycete, fungal pathogens or nematodes were smaller than the untreated control or steam treatment. There are thus different pathogens and pests present in the old orchard soils that work in unison to cause reduction in seedling growth that can ultimately manifest as replant problems in new orchards. Several oomycete (*Pythium* and *Pytophthora* spp.) and *Fusarium* spp. were isolates from seedling roots grown in the untreated control. The potential interaction of these pathogens and their potential to cause seedling growth reduction, needs to be investigated further.

Opsomming

Grond monsters is in hierdie studie versamel in boorde wat almal ouer as 45 jaar is. Die versamelde grond is onderwerp aan verskillende analises en behandelings om moontlike abiotiese (fisies of chemise) of biotiese (patogeniese swamme, oomycete of nematodes) in hierdie gronde te identifiseer wat moontlik ‘n risiko kan inhou as boorde herplant word. Carrizo citrange saailinge is na behandeling in die gronde geplant en vir ‘n periode van 7 maande daarin gekweek voordat die proef getermineer is. Metings van die saailinge het aangedui dat saailinge wat in die stoombehandelde grond gekweek is, groter is as die saailinge wat gekweek is in gronde van die ander behandelings. Dit dui aan dat ‘n biologiese faktor teenwoordig was in die grond wat deur die stoombehandeling geëlimineer is. Binne die ou boordgronde is daar dus verskillende patogene en peste teenwoordig wat saamwerk om saailing groei te verminder. Hierdie interaksie manifesteer uiteindelik dan as herplant probleme in nuwe boorde. Uit die wortels van saailinge wat in onbehandelde grong gekweek is, is verskillende oomycete (*Pythium* en *Pytophthora* spp.) en *Fusarium* spp. geïsoleer. Moontlike interaksie tussen hierdie patogene en hul potensiaal om groei van saailinge te inhibeer, moet verder ondersoek word.

3.4 PROGRAMME: FRUIT AND FOLIAR DISEASES (WITH CBS)

Programme coordinator: Providence Moyo (CRI)

3.4.1 Programme summary

Citrus black spot (CBS) and Alternaria brown spot (ABS) are two major fruit and foliar diseases that hamper the export of citrus fruit to fresh markets by South African producers. The focus of the Fruit and Foliar (with CBS) programme is to study the epidemiology and control aspects of these diseases. The epidemiology of CBS is not fully understood and strict regulations have been implemented by certain of our existing export markets, on the use of certain products registered for the control of ABS.

Citrus black spot, caused by *Phyllosticta citricarpa*, is widespread in citrus-growing regions but is absent within countries of the European Union (EU), where it is subject to phytosanitary legislation. In order to determine the occurrence and persistence of *Phyllosticta* spp. in Europe, surveys were conducted in citrus orchards, nurseries and gardens in the major citrus production areas of the EU (3.4.10). *Phyllosticta* spp. were found to occur in the EU but were not widespread and never associated with infections. Symptoms of CBS were also not observed during the surveys indicating that the fungi persisted but did not cause disease.

The population structure, mode of reproduction and introductory pathways of *P. citricarpa* were determined in Project 977 (3.4.4). New polymorphic simple sequence repeat (SSR) markers developed were used, in combination with published markers, to genotype 383 isolates of *P. citricarpa* obtained from different citrus growing areas of the globe. Mating type analysis revealed that both mating types were present in the populations from South Africa, China, Australia and Brazil at an approximately 1:1 distribution, but the USA population harboured only a single mating type. High levels of connectivity among *P. citricarpa* populations in South Africa, Australia and Brazil found during the study were attributed to exchanges of plant material during the establishment of citrus industries in these countries.

Project RCE-6 (3.4.7) aimed at bridging the knowledge gap that exist with regards to maturation of fruiting structures and spore germination requirements of both the sexual and asexual morphs of the CBS pathogen. The effect of spore age, generation, temperature, germination medium and wetness interruption was investigated. High germination percentages were obtained with spores older than 1 day old and from the second and subsequent generations of spores oozing from pycnidia. Confocal laser microscopy images showed very similar trends for pycnidiospore germination and appressorium formation on lemon and lime leaves, which indicate that lime trees’ tolerance to CBS is not linked to the germination process. Wetness interruption trials showed that pycnidiospores are able to germinate and survive a 3 h period of dryness, even as early as 4 h after inoculation. A qPCR protocol has been optimised to quantify *P. citricarpa* pycnidiospores and the protocol will eventually be used to quantify spores obtained from spore traps from orchards. However, less than 1000 spores could not reliably be quantified by the qPCR protocol.

In an effort to assist growers with better decision making in terms of timing of spray application and choice of fungicides, Project RCE-7 (3.4.8) integrated ascospore and pycnidiospore production, release and infection models together with accurate weather data into a web-based platform (PhytRisk). This platform is already in use and provides farmers with information about CBS risks, weather forecasts and suitable weather for spraying. The mobile phone version will be developed in 2017 but validation of the CBS model is also ongoing.

Methods described in the original proposal for Project RCE-8 (3.4.9), as well as amended methods, failed to generate data for 4 out of the 5 objectives set in the project. Consequently, the four objectives were discontinued and all focus was on the one objective in which data could be generated. Volumetric ascospore trapping was conducted, in geographically different citrus production areas in South Africa, and monitoring is complete. Ascospore release data for CBS was generated and it is currently being captured and will be used to validate and/or improve existing CBS models.

Because of the need to get 100% control of diseases and pests of market access concern, including CBS and false codling moth, high volume citrus spray applications are often used in South Africa. Although these high volume sprays are effective in disease and pest control as well as improved deposition, they are also costly and have negative impacts on the environment. Subsequently, research aimed at the use of reduced volume fungicide application in citrus orchards in South Africa was initiated (3.4.5 and 3.4.6). Project 1132 (3.4.5) evaluated the effect of reduced spray volumes on the control of CBS and false codling moth, while 1089 (3.4.6) aimed at the development of a tree canopy characteristic calibration formula for reduced volume fungicide application in citrus orchards.

Certain markets have implemented strict regulations on the use of mancozeb for the control of ABS and as a result, alternative fungicides are needed to replace this fungicide in ABS spray programmes. Project 750 (3.4.2) focused on evaluating an experimental fungicide, RB 1, and results of trials conducted showed that this fungicide is ineffective as a replacement of mancozeb in ABS spray programmes. The project will continue to evaluate other alternative fungicides.

Programopsomming

Sitrus swartvlek (SSV) en Alternaria bruinvlek (ABV) is twee belangrike vrug-en blaarsiektes wat die uitvoer van vars sitrusvrugte deur Suid-Afrikaanse produsente inhibeer. Die fokus van die Vrug-en Blaarsiekteprogram is om die epidemiologie en beheeraspekte van hierdie siektes te bestudeer. Die epidemiologie van SSV word nie ten volle verstaan nie en streng regulasies is deur sekere bestaande uitvoermarkte ingestel op die gebruik van sekere produkte wat geregistreer is vir die beheer van ABV.

SSV, veroorsaak deur *Phyllosticta citricarpa*, is wydverspreid in sitrusverbouingsareas, maar is afwesig in lande van die Europese Unie (EU) waar dit onderworpe is aan fitosanitêre wetgewing. Ten einde vas te stel wat is die voorkoms van *Phyllosticta* spp. in Europa is opnames onderneem in boorde, kwekerye en huistuine in die belangrikste sitrusareas in die EU (3.4.10). Dit is bevind dat *Phyllosticta* spp. in die EU voorkom, maar dat dit nie wydverspreid is nie en nooit geasssosieer is met infeksies. SSV simptome is ook nie tydens die opnames waargeneem nie wat aandui dat die swam voorkom daar, maar nie siekte veroorsaak nie.

In Projek 977 (3.4.4) is die populasiestruktuur, metode van reproduksie en introduksieroetes van *P. citricarpa* bepaal. Nuwe polimorfiese “simple sequence repeat” (SSR) merkers is ontwikkel en gebruik in kombinasie met gepubliseerde merkers om 383 isolate van *P. citricarpa*, verkry uit verskillende globale sitrus produksieareas, te genotipeer. Paringstipe analises het aangetoon dat beide paringstipes in die populasies van Suid-Afrika, China, Australië en Brasilië teenwoordig is teen ongeveer ŉ 1:1 verdeling. Die VSA populasie bevat egter net een paringstipe. Hoë verbindtenisvlakke is gevind tussen P. citricarpa populasies in Suid-Afrika, China, Australië en Brasilië. Dit word toegeskryf aan die uitruiling van plantmateriaal tydens die vestiging van sitrusbedrywe in hierdie lande.

Die doelwit van projek RCE-6 (3.4.7) is om die kennisgaping te oorbrug ten opsigte van vrugstruktuurrypwording en spoorontkiemingbehoeftes van beide die seksuele en aseksuele vorme van die SSV patogeen. Die effek van spoorouderdom, generasie, temperatuur, ontkiemingsmedium en natheidonderbreking is ondersoek. Hoë ontkiemingspersentasies is verkry met spore ouer as 1 dag en van die tweede en opvolgende generasies afkomstig van piknidia. Konfokale lasermikroskopie beelde het baie soortgelyke neigings getoon vir piknidiospoorontkieming en appresoriumvorming op suurlemoen en lemmetjie blare. Dit dui aan dat die toleransie van lemmetjie teen SSV nie gekoppel is aan die ontkiemingsproses nie. Natheidonderbreking periodes is in proewe ondersoek en het getoon dat binne 4 h na inokulasie, piknidiospore nog oorleef en ontkiem na ŉ 3 h periode van uitdroging. ŉ qPCR protokol is geoptimiseer om P. citricarpa piknidiospore te kwantifiseer wat uiteindelik gebruik sal word om spore afkomstig van spoorvangers in die veld, te kwantifiseer. Ongelukkig kon minder as 1000 spore nie akkuraat gekwantifiseer word deur die qPCR protokol nie.

In ŉ poging om produsente te help om beter besluite te neem ten opsigte van die tyd van spuittoedienings en keuse van swamdoder, het Projek RCE-7 (3.4.8) askospoor en piknidiospoor produksie, vrystelling en infeksiemodelle geïntegreer met akkurate weerdata in ŉ webgebasseerde platform (PhytRisk). Hierdie platform is reeds in gebruik en verskaf aan produsente inligting omtrent SSV risikos, weervoorspellings en

gunstige spuittoestande. Die selfoon weergawe word in 2017 ontwikkel terwyl validasie van die SSV model ook voorgaan.

Metodes wat in die oorspronklike projekvoorstel van Projek RCE-8 (3.4.9) beskryf is, asook aangepaste metodes, het nie enige data gegenereer vir 4 uit die 5 oorspronklike doelwitte van die projek. Hierdie 4 doelwitte is gevolglik gestaak en die fokus is geskuif na die een doelwit waar data wel versamel kan word. Volumetriese askospoor vangste is gedoen in geografies verskillende sitrus produksieareas in Suid-Afrika. Hierdie monitering is voltooi en die SSV vrystellingsdata is gegenereer wat huidiglik ingevoer word vir gebruik in die validasie en/of verbetering van bestaande SSV modelle.

Omdat 100% beheer vekry moet word vir siektes en peste wat van marktoegang belang is, insluitend SSV en valskodlingmot, word hoë volume spuitvolumes dikwels in Suid-Afrika gebruik. Ten spyte daarvan dat hierdie volumes effektief is vir siekte en pesbeheer, is hulle duur en nie omgewingsvriendelik nie. Navorsing, gerig op die vermindering van spuitvolumes in Suid-Afrikaanse sitrusboorde, is dus begin (3.4.5 en 3.4.6). Projek 1132 (3.4.5) evalueer die effek van verminderde spuitvolumes op die beheer van SSV en valskodlingmot terwyl 1089 (3.4.6) gemik is op die ontwikkeling van ŉ spuitkalibrasiemodel wat gebasser is op boomblaardigtheid.

Sekere markte het streng regulasies op die gebruik van mankoseb vir ABV beheer ingestel. Alternatiewe swamdoders is dus nodig vir hierdie swamdoder in ABV spuitprogramme. Projek 750 (3.4.2) fokus dus op die evaluering van ŉ eksperimentele swamdoder, RB 1. Resultate van proewe dui aan dat hierdie swamdoder nie effektief is as vervanging van mankoseb in ABV spuitprogramme nie. Ander alternatiewe swamdoders moet dus ondersoek word.

3.4.2 PROGRESS REPORT: Evaluation of new spray programmes for the control of Alternaria brown spot in the summer rainfall regions of South Africa

Project 750 (Ongoing) by G.C. Schutte and C. Kotze (CRI)

Summary

Because all “Nova” mandarin orchards in the Lowveld have been removed, trial sites were searched for elsewhere. A suitable site was found in the Swellendam district of the Western Cape, a winter rainfall region. Although there were no new products to be evaluated, evaluation of experimental product RB 1 continued as a replacement for mancozeb in Alternaria brown spot spray programmes. RB 1 performed poorly when sprayed on its own and when sprayed in tank mixtures with pyroclostrobin. However, results showed a marked improvement when RB 1 in a tank mixture with pyroclostrobin was alternated with Copper oxychloride.

Opsomming

Weens die verwydering van al die “Nova” mandaryn boorde in die Laeveld, moes daar elders na proefpersele gesoek word. ŉ Geskikte perseel is toe gevind in die Swellendam omgewing van die Wes Kaap, ŉ winterreenval gebied. Alhoewel daar geen nuwe produkte beskikbaar was vir evaluering nie, kon daar egter voortgegaan word om die eksperimentele produk RB 1 as n plaasvervanger vir mancozeb in Alternaria bruinvlek spuitprogramme te evalueer. Resultate wat hieruit bekom is het getoon dat RB 1 se effektiwiteit baie swak was wanneer dit op sy eie, of in tenkmengsels met pyroclostrobin toegedien is. Die resultate was egter aansienlik beter indien die tenk mengsels van RB 1 en pyroclostrobin met koperoksichloried afgewissel is.

Introduction

Alternaria brown spot (ABS) is a serious disease of tangerines (*Citrus reticulata*) and their hybrids in all citrus producing regions of South Africa. Susceptibility to ABS is a dominant trait that is transferred from ‘Dancy’ mandarin to its progeny. Dancy mandarin hybrids and some cultivars of unknown origin, such as ‘Murcott’, ‘Emperor’ and ‘Ponkan’, are affected by the disease. The presence of ABS in South Africa is still a serious problem on all cultivars derived from crosses with Dancy tangerine such as the ŉnova’, ‘Minneola’ and ‘Mor’.

ABS attacks young leaves, twigs and fruit, causing small black necrotic spots after a 24 to 36 h incubation period. ABS sporulates most abundantly on lesions on mature leaves remaining in the canopy. The pathogen produces a host-specific toxin that causes lesions to expand, often resulting in leaf and fruit drop and twig dieback. On more mature fruit, lesions may vary from small necrotic spots to large, sunken pockmarks. Leaves are susceptible until they are fully expanded and hardened whereas fruits are susceptible from petal fall until harvest. In the USA, however, fruit are only susceptible from petal fall until they reach about 5 cm in diameter. Thus, this disease may affect tree growth, cause considerable crop loss, and produce blemishes on fruit that are unacceptable to the consumer.

Cultural measures, such as wider tree spacing and pruning to allow air movement and dry-off of trees, the elimination of overhead irrigation and avoidance of excess nitrogen fertilizer, can assist in reducing disease severity in some orchards. However, fungicide applications are essential for disease control and production of blemish-free fruit. In South Africa, it is important to protect fruit and flushes of cultivars such as tangerines and their hybrids with fungicides from September to April/May, often requiring 8+ spray applications. The number of sprays and the products being used are not economically sustainable and may result in unacceptable residues on fruit.

The aim this season was to evaluate the experimental fungicide RB 1 as an alternative to mancozeb in ABS spray programmes, since there is increasing pressure on the use of the product from global markets. Certain markets such as the US and Canada has already implemented strict regulations on its use. Mancozeb makes up an integral part of not only our ABS spray programmes but also our citrus black spot spray programmes; it is, therefore, imperative that a substitute be identified in this regard.

Objectives

Different spray programmes consisting of an experimental fungicide was evaluated in the winter rainfall area of South Africa, to determine its effectiveness as a substitute to mancozeb. The product was evaluated in programmes where it was alternated with copper oxychloride.

Materials and methods

A susceptible 'nova' mandarin orchard at Indigo farms near Swellendam was selected as a trial site during the 2015-16 season. The efficacy of an experimental product, RB 1, was evaluated as an alternative to mancozeb in ABS spray programmes (Table 3.4.2.1). The product was evaluated on its own and in combinations with pyroclostrobin (Cabrio) and copper oxychloride (Demildex). For each of the treatments, three rows of a 10 year old 'nova' orchard were sprayed using a high volume hydraulic citrus sprayer at 8000l/ha. In total, seven applications occurred at monthly intervals during the period of 20 October 2015 to 19 April 2016. Five data trees were randomly selected from the centre row of each of the treated three-row-blocks. One isolated untreated row served as the control.

Evaluation consisted of inspecting 100 fruit per data tree and rating them according to an infection scale where: 0 = fruit with no brown spot lesions, 1 = fruit with one to five lesions and 2 = fruit with six or more lesions. Data accumulated were analysed using the statistical package XLStat and the mean percentages compared using the Fischer's student t-test of least significant difference (LSD).

Results and discussion

Objective / Milestone	**Achievement**
A. Evaluation of new fungicides	
A.1. Spraying field trial	All treatments were applied according to the experimental protocol and the trial was successfully completed.

RB 1 applied at rates of 100 and 200ml/100L water
The disease pressure for the period was particularly high as only 37% (Table 3.4.2.2) of the untreated fruit were lesion free. When comparing this to RB 1 at two different application rates of 100ml and 200ml per 100L (Table 3.4.2.2) of water, there was statistically no difference with each yielding 48 and 42% clean fruit, respectively. Furthermore, when comparing the different rates, there was statistically no difference in percentage of clean fruit, indicating that irrespective of the application rate, RB 1 applied on its own is ineffective in the control of ABS.

RB 1 in tank mixtures with pyroclostrobin and mineral oil
Alternating RB 1 with tank mixtures of RB 1, pyroclostrobin (Cabrio) and mineral spray oil at their standard and 2X rates (Table 3.4.2.1) resulted in only 45.5 - 47.5% clean fruit. These results were statistically not different from the untreated control (37%) (Table 3.4.2.2) and therefore, suggest that RB 1 does not control ABS effectively and cannot be considered as a direct replacement for mancozeb in standard ABS spray programmes.

RB 1 in tank mixtures with pyroclostrobin and mineral oil alternated with copper oxychloride

When copper oxychloride (Demildex) was alternated with tank mixtures of RB1, Cabrio and mineral spray oil at two different rates (1X and 2X) there was a marked improvement in ABS control. The standard 1X rate yielded 61% clean fruit while the higher 2X rate resulted in 69% clean fruit. The higher rate resulted in statistically more clean fruit than all the spray programmes where mancozeb was substituted with RB1.

Table 3.4.2.1 Application dates and rates of fungicides applied for the control of Alternaria brown spot at Indigo Farming, Swellendam, South Africa for the period 20 October 2015 to 19 April 2016.

No	20 October 2015	17 November 2015	29 December 2015	26 January 2016	23 February 2016	22 March 2016	19 April 2016
1	RB 1 (100ml)	RB 1 (100ml)	RB 1 (100ml)	RB 1 (100ml)	RB 1 (100ml)	RB 1 (100ml)	RB 1 (100ml)
2	RB 1 (200ml)	RB 1 (200ml)	RB 1 (200ml)	RB 1 (200ml)	RB 1 (200ml)	RB 1 (200ml)	RB 1 (200ml)
3	RB 1 (100ml)	RB 1+ Cabrio+Oil (100ml+ 10ml+250ml)	RB 1 (100ml)	RB 1+ Cabrio+Oil (100ml+ 10ml+250ml)	RB 1 (100ml)	RB 1+ Cabrio+Oil (100ml+ 10ml+250ml)	RB 1 (100ml)
4	RB 1 (200ml)	RB 1+ Cabrio+Oil (200ml+ 20ml+250ml)	RB 1 (200ml)	RB 1+ Cabrio+Oil (200ml+ 20ml+250ml)	RB 1 (200ml)	RB 1+ Cabrio+Oil (200ml+ 20ml+250ml)	RB 1 (200ml)
5	Demildex (200g)	RB 1+ Cabrio+Oil (100ml+ 10ml+ 50ml)	Demildex (200g)	RB 1+ Cabrio+Oil (100ml+ 10ml+250ml)	Demildex (200g)	RB 1+ Cabrio+Oil (100ml+ 10ml+ 250ml)	Demildex (200g)
6	Demildex (400g)	RB 1+ Cabrio+Oil (200ml+ 20ml+250ml)	Demildex (400g)	RB 1+ Cabrio+Oil (200ml+ 20ml+250ml)	Demildex (400g)	RB 1+ Cabrio+Oil (200ml+ 20ml+250ml)	Demildex (400g)
7	Untreated Control	-	-	-	-	-	-

Table 3.4.2.2. Evaluation of RB 1 as an alternative to mancozeb in standard spray programmes applied from October 2015 to April 2016 for the control Alternaria brown spot at Indigo Farming, Swellendam, South Africa.

	Treatment	Dosage (g/ml per 100L water tank mixture)	Percentage of fruit in each class		
			Lesions/fruit[x]		
			0	1-5	≥6
1	Experimental product (RB 1)[y] x 7	100ml	48.0 bc	25.0 ab	27.0 ab
2	Experimental product (RB 1) x 7	200ml	42.0 c	30.0 a	28.0 ab
3	RB 1/RB 1 + Cabrio + Oil/ RB 1/RB 1 + Cabrio + Oil	100ml/100ml +10ml + 250ml/ 100ml/100ml + 10ml + 250ml	45.5 c	22.5 ab	32.0 a
4	RB 1/RB 1 + Cabrio + Oil/ RB 1/RB 1 + Cabrio + Oil	200ml/200ml +20ml + 250ml/ 200ml/200ml + 20ml + 250ml	47.5 bc	17.5 b	35.0 a
5	Copper oxychloride/RB 1 + Cabrio + Oil/ Copper oxychloride/RB 1 + Cabrio + Oil	200g/100ml +10ml + 250ml/ 200g/100ml + 10ml + 250ml	61.0 ab	18.0 b	21.0 ab

6	Copper oxychloride/RB 1 + Cabrio + Oil/ Copper oxychloride/RB 1 + Cabrio + Oil	400g/100ml +20ml + 250ml/ 400g/100ml + 20ml + 250ml	69.0 a	18.0 b	13.0 b
7	Untreated control	–	37.0 c	27.0 ab	36.0 a

[x]Means followed by the same letter in the same column do not differ significantly
[y]Spray dates were: 20 October 2015; 17 November 2015; 29 December 2015; 26 January 2016; 23 February 2016; 22 March 2016 and 19 April 2016

Conclusion to date

Results show that irrespective of dosage and tank mixture, RB 1 as a replacement to mancozeb yielded very low levels of clean fruit. However, when alternated with copper oxychloride there was a marked increase in control, with 61 and 69% clean fruit achieved in the standard and 2X programmes, respectively. This is still not a satisfying level of control and could only be due to the copper oxychloride being applied every other month and not due to the effect of the RB 1. Therefore, it should be concluded that RB 1 is ineffective as a replacement to mancozeb and other alternatives should be investigated in future.

Technology transfer

This research will be included in the annual research report to be distributed to citrus growers and will be included in various talks to citrus growers. Certain detail of the work cannot be presented as the programmes and fungicides are not registered.

Future objectives and work plan

Future research will focus on the identification of alternative fungicides to mancozeb in ABS spray programmes and the optimisation of copper applications without the known side effects.

<u>Quarterly milestones for Apr-Jun, Jul-Sep, Oct-Dec 2016 and Jan-Mar 2017</u>
Apr-Jun:

- Evaluate previous season's field trial.
- Annual progress report.

Jul-Sep:

- Collect fungicides from the different chemical companies earmarked for the trial in August.
- Layout of trial in a susceptible 'nova' orchard.
- First applications will commence with the onset of the first spring flush.

Oct-Dec:

- Continue at pre-determined intervals as registered/recommended.

Jan-Mar:

- Continue at pre-determined intervals as registered/recommended.

Apr-Jun:

- Evaluate current field trial.

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3.4.3 PROGRESS REPORT: Development of new spray programmes for the control of citrus black spot

Project 970 (Ongoing) by G.C. Schutte and C. Kotze (CRI)

Summary

A series of different experimental fungicides and spray programmes were applied on a “Valencia” orange orchard during the 2015-16 season at Crocodile Valley Co (PTY), Nelspruit, Mpumalanga. Unfortunately, the orchard was harvested before the trial evaluations happened. Therefore, there is no reportable data available for this season.

Opsomming

Verskeie eksperimentele swamdoders en spuitprogramme is gedurende 2015-16 seisoen op ŉ “Valencia” lemoen boord by Crocodile Valley Co (PTY), Nelspruit, Mpumalanga toegedien. Ongelukkig is die boord ge-oes nog voordat die proef evalueer kon word. Daar is dus geen data waarop daar hierdie seisoen geraporteer kan word nie.

3.4.4 PROGRESS REPORT: The global population structure and reproductive biology of the fungal pathogen, *Phyllosticta citricarpa* Kiely

Project 977 (2010/11 – 2018) by E Carstens (CRI)

Summary

Genetic markers such as microsatellite markers are very useful and widely used in population genetic studies. Fifteen markers were used to genotype 383 *P. citricarpa* isolates from five South African populations and seven international populations from four countries including the United States of America (USA), China, Brazil and Australia. The study confirms that *P. citricarpa* populations in China and Australia had the highest genetic diversities, whereas populations from Brazil, USA and South Africa exhibited characteristics of founder populations. Mating type analysis revealed that both mating types were present in the populations from South Africa, China, Australia and Brazil at an approximately 1:1 distribution. The USA population harboured only a single mating type. Most populations exhibited sexual reproduction, but linkage disequilibrium analyses indicated that asexual reproduction is important in the pathogen’s life cycle.

Opsomming

Genetiese merkers soos mikrosateliete is geskik en word baie in genetiese studies gebruik. Vyftien merkers is gebruik om 383 *P. citricarpa* isolate van vyf Suid-Afrikaanse populasies en sewe internasionale populasies afkomstig van vier lande, insluitend die Verenigde State van Amerika (VSA), China, Brasilië en Australië te genotipeer. Die studie het bevestig dat *P. citricarpa* populasies in China en Australië die hoogste genetiese

diversiteit het, terwyl populasies in Brasilië, VSA en Suid-Afrika eienskappe toon van “founder” populasies. “Mating-type” analises het getoon dat beide “mating-types” in populasies van Suid-Afrika, China, Australië en Brasilië in 'n 1:1 verhouding teenwoordig is. Slegs een van die “mating-types” is in die VSA populasie gevind. Meeste van die populasies het geslagtelike voortplanting ondergaan maar “linkage disequilibrium” analises het getoon dat ongeslagtelike voortplanting ook belangrik is in die patogeen se lewenssiklus.

3.4.5 PROGRESS REPORT: Development of a tree canopy characteristic calibration formula for reduced volume fungicide application in citrus orchards

Project 1089 (2014/04 – 2015/04) by Jan van Niekerk (CRI), Tertia van Wyk (USPP) and P.H. Fourie (CRI)

Summary

Given the complexities of the computer programming of the LiDAR data, a collaboration was established with researchers at Department of Oenology and Viticulture, University of Stellenbosch to assist with LIDAR work and data processing. An MSc student has furthermore taken on the project to fill the void created by Gideon van Zyl’s resignation from CRI. Progress has been made in programming to get meaningful output. This progress allowed for preliminary scans with the LIDAR in a Nules Clementine orchard with variable canopy densities. Initial practical problems with the LIDAR height was sorted out and subsequent scans of the Nules orchard indicated that the LIDAR scans can detect differences in canopy densities between trees. Future trials in 2017 will include pruning trees to varying degrees to create spray windows. After pruning, trees will be scanned with the LIDAR, and sprayed with fluorescent pigment to compare different densities and spray deposition and quality in trees with different canopy densities created by pruning.

Opsomming

Gegewe die kompleksiteit van rekenaarprogrammering van die LiDAR data, is samewerking bewerkstellig met navorsers van die WIngerd-en Wynkunde department by Stellenbosch Universiteit, ten einde te help met LIDAR werk en data verwerking. ʼn MSc student werk ook nou binne die projek ten einde die gaping te vul wat veroorsaak is deur Gideon van Zyl se bedanking by CRI. Vordering is gemaak in die programmmering van die LIDAR data ten einde betekenisvolle resultate te verkry. Hierdie vordering het voorlopige skanderings met die LIDAR in ʼn Nules Clemetine boord, met variërende loofdigthede, moontlik gemaak. Praktiese probleme wat aanvanklik ondervind is met die LIDAR se hoogte is reggestel en daaropvolgende skanderings in die Nules boord het aangedui dat die LIDAR wel verskille in loofdigtheid tussen bome kan uitwys. Verdere proewe in 2017 gaan insluit die snoei van bome in verskillende mates vir die skepping van spuitvensters. Na afloop van die snoei, sal die bome met die LIDAR skandeer word en ook met ʼn fluoruserende pigment bespuit word ten einde verskillende loofdigthede en spuitdeposisie en kwaliteit te vergelyk in bome met verskillende loofdigthede wat deur snoei bewerkstellig is.

3.4.6 PROGRESS REPORT: Evaluation of reduced volume fungicide and pesticide sprays for control of citrus black spot and false codling moth.

Project 1132 (2014/15 – 2016/7) by Jan van Niekerk (CRI), Charl Kotze (CRI), Tertia van Wyk (USPP) and P.H. Fourie (CRI)

Summary

Spray application forms the backbone of pre-harvest pest and disease management strategies in South African citrus production. Due to zero tolerance status of Citrus black spot (CBS; caused by *Phyllosticta citricarpa*) for export to the European Union, growers tend to use high application fungicide volumes ranging from 6000 to 12000 l ha^{-1}. However, high spray volumes are costly in terms of off-target losses (run-off and drift) and environmental pollution, amount and cost of water, fuel and plant protection product (PPP), the strain on equipment, and it is more labour intensive and therefore ultimately less efficient than reduced volume applications. The potential of reduced volume application for the control of citrus black spot have been investigated in various previous studies. However, the potential must be proven through seasonal bio-efficacy trials. In the past season, two trials were done where different spray machines applying different volumes (750 L/ha – 8900 L/ha) were evaluated in terms of different spray deposition parameters and also bio-efficacy in terms of disease (including CBS) and pest control on fruit. The results in both trials were similar. These indicated that the lower volume applications often performed better with regards to spray deposition quantity on leaves and fruit. However, deposition uniformity and quality of the low volume applications were often the same or poorer than the high volume applications. This was then also shown in the bio-efficacy control data. The high volume applications consistently gave the best results in controlling mealy bug and red scale on fruit.

This showed that at least for these two pests higher volumes are giving better control results. The CBS rating will be conducted at fruit maturity.

Opsomming

Spuit toediening vorm die ruggraat van voor-oes pes en plaag beheer strategieë in Suid-Afrikaanse sitrus produksie. A.g.v. die zero toleransie status van sitrus swartvlek (CBS; veroorsaak deur *Phyllosticta citricarpa*) vir uitvoer na die Europese Unie, is produsente geneig om hoë swamdoder spuit volumes te gebruik wat strek tussen 6000 tot 12 000 l ha^{-1}. Hoë spuit volumes is duur in terme van produk verloor a.g.v. afloop en drif, omgewings besoedeling, die hoeveelheid water en koste daarvan, brandstof en plant beskermingsproduk wat gebruik word, die slytasie op masjinerie. Dit is ook baie arbeidsintensief en daarom op die einde minder effektief as verlaagde volume toediening. Die potensiaal van verlaagde volume toediening vir die beheer van sitrus swartvlek is al ondersoek in vorige studies. Maar, of dit in praktyk sal werk kan slegs getoets word deur seisoenale bio-effektiwiteitsproewe. In die afgelope seisoen is twee proewe gedoen waar verskillende spuitmasjiene, teen verskillende toedieningsvolumes (750 L/ha – 8900 L/ha), getoets is ten opsigte van spuitdeposisie parameters en ook beheer van siektes (inlsuitend CBS) en insekplae op vrugte. Die resultate was in beide proewe ooreenstemmend. Dit het aangedui dat die laer volume toedienings dikwels beter vertoon het in terme van die verskillende spuit deposisie hoeveelheid op blare en vrugte. In terme van deposisie uniformiteit en kwaliteit het die laer volume toedienings dikwels dieselfde of swakker resultate gegee as die hoër volume toedienings. Hierdie verskille was ook duidelik uit die beheer van bogenoemde insekte. Die hoëvolume toedienings het konstant beter beheer van witluis en rooi dopluis op vrugte gegee. Dit het dus getoon dat minstens vir die beheer van hierdie twee insekplae, hoër volume toedienings beter is. Die CBS vlakke sal later op volwasse vrugte bepaal word.

3.4.7 PROGRESS REPORT: Epidemiology, inoculum potential and infection parameters of Citrus Black Spot

Project RCE-6 (Apr 2015 – Jan 2018) by P.H. Fourie and M. Kellerman (CRI)

Summary

Citrus Black Spot (CBS) is the most important fungal disease of citrus in South Africa, because of its quarantine status in major export countries. Epidemiology of CBS is not fully understood, since there is currently no method to distinguish between its ascospores compared to the non-pathogenic *P. capitalensis* ascospores, and also because generic infection models are available, but actual infection has not been measured yet. Knowledge gaps also still exist with regards to fruiting body maturation and spore germination requirements, for both asexual and sexual proliferation cycles. Germination studies were done with pycnidiospores, where the effect of spore age, generation, temperature, germination medium and wetness interruption was investigated. Better germination results were obtained with spores older than 1 day (>80% vs. 40 – 70% for spores suspended in orange juice) and from the second and subsequent generations of spores oozing from pycnidia (>50% vs. 40%). For *in vivo* trials, 1.5% Valencia juice is not a requirement for germination, as was the case for *in vitro* trials on microscope slides. A big difference was seen between germination on glass slides (0 – 80%) vs. leaves (0 – 60%) at different temperatures. Whether leaves were sterilised or not also had an effect (0- 46% vs. 0 – 92% for unsterilised and sterilised leaves, respectively). Great differences were observed among trials, which indicate that some unknown factor is playing a role in the germination ability of pycnidiospores. Wetness interruption trials showed that pycnidiospores are able to germinate and survive a 3 h period of dryness, even as early as 4 h after inoculation. Less than 1000 spores could not reliably be quantified by the qPCR protocol, but spores ranging between 1×10^3 and 5×10^6 spores mL^{-1} could be quantified. An average ratio of 1.137 for *P. citricarpa : P. capitalensis* pycnidiospores were observed from 1:1 spiked samples. Only 3 orchard samples had enough spores to be detected by qPCR. In future, an active spore sampling method will be used. The confocal laser microscopy images indicated very similar trends for pycnidiospore germination and appressorium formation on both lemon and lime leaves, which indicate that lime trees' tolerance to CBS is not connected to the germination process.

Opsomming

Sitrusswartvlek (SSV) is die belangrikste swamsiekte op sitrus in Suid-Afrika, vanweë die kwarantynstatus wat dit geniet in uitvoerlande. Die epidemiologie van SSV word nie ten volle verstaan nie, omdat daar nie tans ŉ metode is om tussen askospore van die patogeen en nie-patogeniese *P. capitalensis* te onderskei nie, en ook omdat daar slegs generiese infeksiemodelle bestaan, en werklike infeksie nie gemeet is nie. Daar is steeds gapings in die kennis oor vrugstruktuur rypwording en spoorontkieming se vereistes, vir beide die seksuele en nie-seksuele lewensiklus. Spoorontkiemingstudies is gedoen met piknidiospore, en die effek van spoorouderdom, -generasie, temperatuur, ontkiemingsmedium, en onderbreking van natheidsperiode is ondersoek. Beter ontkieming is gesien met spore ouer as 1 dag (>80% vs. 40 – 70% vir spore in lemoensap),

asook van die tweede en opvolgende generasies spore wat deur piknidia geproduseer word (>50% vs. 40%). Vir *in vivo* proewe, is gevind dat 1.5% Valenciasap nie nodig is vir ontkieming, soos die geval was vir *in vitro* proewe op mikroskoopglasplaatjies nie. ʼn Groot verskil is gesien in ontkieming tussen spore op glasplaatjies (0-80%) en op blare by verskillende temperature (0 – 60%). Die sterilisasie van blare het ook ʼn effek gehad (0 – 46 vs. 0 – 92% vir ongesteriliseer en gesteriliseerde blare, onderskeidelik). Groot verskille is tussen proewe gesien, wat dui daarop dat ʼn ander onbekende faktor ontkieming beinvloed. Natheidsperiode onderbrekingsproewe het gewys dat piknidiospore ʼn 3h-droë periode kan oorleef, selfs so vroeg as 4 h na inokulasie. Minder as 1000 spore kon nie betroubaar gekwantifiseer word deur qPCR nie, maar spoorkonsentrasies tussen 1×10^3 en 5×10^6 spore.mL^{-1} kon. ʼn Gemiddelde verhouding van 1.137 vir *P. citricarpa : P. capitalensis* piknidiospore is van 1:1 steekproefmonsters gekry. Slegs 3 boordmonsters het genoeg spore gehad om deur qPCR opgetel te word. In die toekoms sal ʼn aktiewe spoorvang metode gebruik word. Die konfokale laser mikroskopie beelde het gewys dat soortgelyke patrone vir piknidiospoor ontkieming en appressorium formasie op beide suurlemoen en lemmetjie blare gebeur. Dit wys dat lemmetjie bome se toleransie tot SSV nie iets met die ontkiemingsproses te doen het nie.

3.4.8 PROGRESS REPORT: Improved Citrus Black Spot management through web-based information systems

Project RCE-7 (1129) (December 2014 – December 2018) by M Kellerman, J.G. van Zyl and P.H. Fourie (CRI)

Summary

Citrus Black Spot (CBS) is the most important fungal disease of citrus in South Africa. Using proper spore dispersal and maturation models together with accurate weather data, integrated into easy to use web-based software, better decision making in terms of timing of spray application and choice of fungicides used can be made. CBS risk can also be determined more accurately from season to season. Various CBS disease prediction models have been identified and in order to determine which model works best, a model shootout have been proposed in a new CRI funded project. An IT company have been contracted for development of the web-based prediction system. Shapefiles have been created identifying citrus regions and weather reading points. A web-based weather service has been identified with weather data recording starting in July 2016. A CBS model, including sub-models for CBS ascospore and pycnidiospore development, dispersal and infection, were formulated and presented to the IT company for programming. A good-to-spray model has also been developed and handed over to the development contractors. Alpha/Beta roll-out happened in August 2016. Up till now, 88 users viewed the website 602 times, and we received feedback that led to improvements in models as well as user friendliness of website. Validation led to some changes in the algorithm for better accuracy, and is ongoing together with other CBS models.

Opsomming

Sitrus swartvlek is die belangrikste swamsiekte in sitrus in Suid-Afrika. Deur behoorlike spoor vrystelling en rypwording modelle te gebruik saam met akurate weer data, geïntegreer in verbruikersvriendelike web-gebaseerde sagteware, kan dit die produsent help om beter besluite op plaasvlak te neem in terme van tydsberekening van spuit toediening en keuse van swamdoder. CBS risiko kan ook van seisoen tot seisoen bepaal word. Verskeie siekte voorspellingsmodelle is geïdentifiseer en ʼn evaluasie van beskikbare modelle is voorgestel in ʼn nuwe CRI-befondsde projek. ʼn Sagteware ontwikkelings maatskappy is gekontrakteur vir die ontwikkeling van die web-gebaseerde siekte voorspellingsisteem. “Shapefiles” wat sitrus produksie-areas sowel as weerpunte bevat is geskep. ʼn Web-gebaseerde weerdiens is geïdentifiseer om weerdata te bekom vanaf Julie 2016. ʼn CBS voorspellingsmodel, met sub-modelle vir askospoor en pyknidiospoor ontwikkeling, vrystelling en infeksie, is geformuleer en aan die programeerders verduidelik. ʼn “Good-to-spray” model is ook ontwikkel en oorgehandig aan die web ontwikkelaars. Die Alfa/Beta bekendstelling van die webtuiste is gedoen in Augustus 2016. Tot op hede, het 88 gebruikers die webtuiste 602 keer besoek, en terugvoer gegee wat tot verbetering van modelle en gebruikersvriendelikheid gelei het. Validasie het gelei tot veranderinge in die webtuiste se algoritme vir beter akkuraatheid, en dit word voorgesit tesame met ander swartvlek modelle.

3.4.9 PROGRESS REPORT: Epidemiology of CBS in different geographic areas and development of a risk management system for Citrus Black Spot

Project RCE-8 (2014/12-2018/01) by Susan du Raan (QMS Laboratories)

Summary

Ascospore release data for Citrus Black Spot (CBS) for geographically different citrus production areas in South Africa was generated. Data was generated for areas including Letsitele, Hoedspruit, Noordgrens, Tshipise, Origstad, Burgersfort, Nelspruit and Eastern Cape (Kirkwood/Addo). The data will be used to validate

and improve current models for CBS. No data was, however, generated for further objectives of the study (objectives 2-5), due to different reasons including failure of proposed methods to yield positive results.

Opsomming

Askospoorvrystellingsdata vir sitrus swartvlek (SSV) is in verskillende sitrusproduserende areas in Suid-Afrika gegenereer. Data is vir die areas, insluitend Letsitele, Hoedspruit, Noordgrens, Tshipise, Orighstad, Burgersfort, Nelspruit en Oos-Kaap (Kirkwood/Addo), gegenereer. Die data gaan gebruik word vir die bevestiging en verbetering van bestaande SSV voorspellingsmodelle. Vir die verdere doelwitte (doelwitte 2-5) van die projek is geen data verkry nie as gevolg van verskillende redes, insluitend die oorspronklike metodes wat nie gewerk het nie en dus nie resultate opgelewer het nie.

3.4.10 PROGRESS REPORT: Detection and spread of Citrus Black Spot pathogens

RCE9 (10/12/2014 – 31/03/2018) by Pedro Crous, (Westerdik Fungal Biodiversity Institute - Netherlands)

Summary

The genus *Phyllosticta* occurs worldwide, and contains numerous plant pathogenic, endophytic and saprobic species. *Phyllosticta citricarpa* is the causal agent of Citrus Black Spot (CBS). This disease is widespread in citrus-growing regions, but is absent within countries of the European Union (EU), where it is subject to phytosanitary legislation. *Phyllosticta citricarpa* is frequently confused with *P. capitalensis*, which is a non-pathogenic endophyte, commonly isolated from citrus leaves and fruits and a wide range of other hosts. Three additional *Phyllosticta* species are associated with disease symptoms of *Citrus* spp. in Asia: *P. citriasiana, P. citrichinaensis* and *P. citrimaxima*, while *P. citribraziliensis* occurs as an endophyte on citrus in South America. European citrus plantings were originally established from plant material imported from the CBS endemic Asia since the 4th century AD. To investigate the putative incursion and persistence of *Phyllosticta* species on infected plant material, we explored the occurrence and the diversity of *Phyllosticta* spp. associated with *Citrus* spp. in European orchards, nurseries and gardens. Once several *Phyllosticta* strains were isolated, phylogeny, morphology, genotypes, mating types and pathogenicity were studied. We collected 64 isolates of which 52 were used in a multi-locus DNA dataset consisting of the ITS, *actA*, *tef1*, *gapdh*, LSU and *rpb2* gene. Two isolates from Florida, USA, three isolates from China and several reference strains from Australia, South Africa and South America were included in the phylogeny of 99 isolates of *Phyllosticta*. Based on the data generated here, we recovered four *Phyllosticta* species associated with citrus plants in EU countries. In the EU, they were not found to be widespread, and were never associated with infections. Symptoms of CBS were not observed during the surveys, or follow-up surveys in 2017, indicating that the fungi persisted but did not cause disease. The isolates of these species were isolated from dead leaves as endophytes, but not associated with disease.

Opsomming

Die genus *Phyllosticta* kom wêreldwyd voor, en verteenwoordig etlike plantpatogene, endofitiese en saprofitiese spesies. *Phyllosticta citricarpa* is die organisme wat Swartvlek (CBS) veroorsaak. Alhoewel die siekte wyd verspreid voorkom in lande waar sitrus geplant word, is dit afwesig in Europa, waar streng fitosanitêre maatreëls toegepas word. *Phyllosticta citricarpa* word maklik verwar met *P. capitalensis*, ŉ nie-patogeniese endofiet wat algemeen in blare en vrugte voorkom (ook op ander gewasse). Drie addisionele *Phyllosticta* spesies word geassosieer met siekte simptome van *Citrus* spp. in Asië: *P. citriasiana, P. citrichinaensis* en *P. citrimaxima*, terwyl *P. citribraziliensis* voorkom as endofiet op citrus in Brazil. Europese citrus is oorspronklik gevestig uit materiaal afkomstig uit Asië ongeveer in die 4de eeu na Christus. Een doel van die projek was om die moontlike voorkoms (en oorlewing) van *Phyllosticta* spesies op citrus plantmateriaal in Europa te ondersoek. Om dit vas te stel is verskeie opnames in verskillende lande, kwekerye en tuine in Europa uitgevoer. Alle *Phyllosticta* isolate wat geïsoleer is, is daarna onderwerp aan ondersoek (morfologie, filogenie, genotipe en paringstipe bepaling, patogenisiteit en genoom data). ŉ Multi-lokus DNA datastel van ITS, *actA, tef1, gapdh*, LSU en *rpb2* is gegenereer van 99 *Phyllosticta* isolate, waarvan 64 afkomstig is uit Europese lande (opnames in 2015 en 2016), twee uit Florida (VSA), en drie uit China. Gebaseer op die data was vier *Phyllosticta* soorte geïdentifiseer op sitrus in Europese lande. Die *Phyllosticta* spesies was egter heel beperk in hul verspreiding, en was nooit geassosieer met siekte simptome in die veld nie. Geen siekte simptome is waargeneem nie, selfs nie gedurende ŉ opvolg opname wat in 2017 uitgevoer is in dieselfde gebiede nie. Die isolate was geisoleer uit dooie blare as endofiete, maar was nie geassosieer met siekte nie.

3.5 PROGRAMME: POSTHARVEST DISEASES

Programme coordinator: Wilma du Plooy (CRI)

3.5.1 Programme summary

Postharvest fungicides can be applied to fruit using several methods, namely pre-packline drench application, dip, flooder, in-line aqueous spray, wax and bin drench treatments. Imazalil (IMZ) and thiabendazole are the most widely used remedies in citrus postharvest decay, providing effective curative control and sporulation inhibition of *Penicillium*, but provides practically no control against sour rot (3.5.4). As part of a resistance management strategy, it is not recommended to include IMZ during drenching. Pyrimethanil (PYR) and thiabendazole is therefore regarded as the main components of this application. The ability of two different sanitisers (Chlorine and HPPA) to reduce sour rot inoculum in solution, while maintaining fungicide concentration and residue levels for effective green mould control, were compared. The compatibility between sanitisers and fungicides was not observed in this study, with addition of both sanitisers contributing to sour rot control. HPPA is effective at a short exposure time (1 – 3 min) at the high pH used in this study (> 10), which indicates it can be incorporated with commercial drenching when pH is not regulated. Differential PYR residue loading was seen between the top and bottom bin levels during commercial packhouse trials with the upper level loading higher residue levels compared to the bottom level. The physical parameters of pH, temperature, exposure time, and their effect on chemicals used in the dip tank were optimised, and an industry recommendation will be completed in 2017 (project 1126 finalisation).

More recent protocols and fungicides used in packhouses, as well as alternative fungicides, were evaluated for their effect against latent citrus black spot (CBS) infections as well as the reproductive potential of the lesions (3.5.2). Fruit with CBS lesions, as well as asymptomatic fruit with latent infections were subjected to standard packhouse sanitation and fungicide treatments, cold storage (singularly and combined) and incubated at conditions to enable expression of latent infections. The full packhouse treatment along with the storage period gave significant control of latent infections. The overall reproductive ability of lesions was very low, with less than 2.1% of all lesion that formed on both Valencia's and Eureka lemons developing pycnidia. With the finalisation of project 1127 it was found that the combined epidemiological requirements for pycnidiospore release, along with results from trials, indicate that harvested citrus fruit is not an epidemiologically significant pathway for the spread of CBS.

Essential oils (EO) are known to protect against various postharvest fungal decay pathogens. The EO materials are being tested in various forms including coatings, aqueous environments and in headspace, with the primary focus encapsulting the EO in a slow-release nano- or micro particle (PHI 66 – section 1 – M.Tech.). For headspace analysis, an accurate and reliable method are being developed to measure the extent of volatilisation and therefore the degree of protection offered by active metabolites in the EO under storage conditions for fruit. A customised gas chromatograph was designed and constructed for the sampling of headspace (PHI section 1 – M.Tech). Chemometric analysis of an extensive dataset generated by gas chromatography-mass spectrometry is currently underway (PHI 66 – D.Tech.).

In project 123 (3.5.3) several alternative and potentially new products were evaluated, with varying results – the most successful were an azoxystrobin formulation against *Penicillium digitatum*. Products combining hydrogen peroxide and acetic acid were once again investigated as alternative sanitizers in the fungicide bath. The sensitivity of pyrimethanil and stability of propiconzole in the presence of the active was confirmed. Phytochemical reactions to some liquid formulations is a danger. A powder formulation of PAA was successfully evaluated. Water sanitation is still very prominent, and several products with regards to this aspect was offered for testing, with variable results.

Plants produce an extensive diversity of compounds known as phytochemicals, all of which are functional in the plant in some or other way. The role of phytochemicals in either eliciting or inhibiting the ability of *Phyllosticta citricarpa* (citrus black spot, CBS) to infect citrus fruit is being investigated (3.5.5). Cultivars with varying susceptibility to CBS are being investigated, looking at the apolar (waxes, lipids, oils) and polar (flavonoids, anthocyanidins, alkaloids, glycocides) fractions in the rind phytochemistry. Currently used cultivars include Bitter Seville, which is accepted have low susceptibility to CBS infection, Valencia orange with medium susceptibility, and highly susceptible lemon. In addition, kumquats are included as a resistant type.

A new fungicide to control sour rot, caused by the ascomycete *Galactomyces citri-aurantii* is needed since guazatine (a guanidine fungicide) is being phased-out for citrus fruit exported to the EU. Propiconazole is a newly introduced postharvest fungicide that is available to the South African citrus industry. This demethylation-inhibitor (DMI) triazole fungicide is being registered for postharvest use on sour rot decay on citrus fruit in South Africa. Baseline sensitivity to propiconazole was determined for single spored South African isolates of *G. citri-aurantii* causing sour rot and *Penicillium digitatum* causing green mould (3.5.6).

Recent fungal contamination on wooden pallet bases used for export highlighted a problem of which the source is apparently unknown (3.5.7). This degradation of pallet bases when the consignment reaches its market has raised questions on the quality of the wood used in the manufacturing of these bases. Further concerns were

the contribution of packhouse storage methods to the fungal contamination of the bases, and the possible role of environmental factors (for instance moisture, UV degradation, and insect infestation). However, it is fungal decay that is currently the hazard with the highest priority. Heavily contaminated pallets are a point source of fungal spore dissemination that poses a deterioration risk to the boxes stacked on these pallets, increases the spore load in cold rooms, and presents a phytosanitory concern (project 1165, phase 1). This study looks at the microbiome on contaminated pallet bases, manufacturing aspects of the bases, the role of storage at packhouses, options for wood treatment, as well as possible contribution from the shipping containers to the degradation of wooden pallet bases.

Programopsomming

Naoes swamdoders kan met behulp van verskeie metodes aangewend word, naamlik voor-pakhuis stortbehandeling, dip, in-lyn spuit en stortbehandelings en in die waks (3.5.4). Die mees gebruikte chemikalieë vir na-oes siektebeheer is tans imazalil en thiabendasool, met goeie kuratiewe werking teen en inhibisie van *Penicillium* sporulasie, maar dit bied geen beskerming teen suurvrot nie. As deel van weerstandsbestuur word imazalil egter nie in die voorpakhuisstort aanbeveel nie. Pirimetaniel en thiabendasool word dus as die hoofkomponente van hierdie behandeling beskou. Die vermoë van twee saniteerders (Chloor en HPPA) om suurvrot inokulum in oplossing te verminder, tesame met die fungisiedkonsentrasie en residuvlakke wat nodig is vir effektiewe groenskimmel beheer, is vergelyk. Onverenigbaarheid tussen saniteermiddels en swamdoders is nie waargeneem nie, terwyl byvoeging van beide saniteermiddels bydra tot suurvrot beheer. HPPA was effektief binne ŉ kort blootstellingstyd (1 – 3 min) teen hoë pH in hierdie studie (> 10), wat aandui dat dit ingesluit kan word in kommersiële stortbaddens waar die pH nie gereguleer word nie. Die PYR residue lading in die plukkratte het gewissel van bo na onder, met die boonste kratte wat beter residue laai in vergelyking met die onderstes. Optimisering van die fisiese parameters (pH, temperatuur en blootstellingstyd) en hul effek op die chemikalieë vir die dompeltenk is voltooi, en industrie aanbevelings sal later in 2017 voltooi wees (projek 1126 gefinaliseer).

Meer onlangse pakhuis protokolle en swamdoders is herevalueer, asook alternatiewe swamdoders, vir hul uitwerking teen latente sitrus swartvlek (SSV) infeksies asook hul effek op die reproduktiewe potensiaal van letsels (3.5.2). Vrugte met SSV letsels, asook asimptomatiese vrugte met latente infeksies is aan standaard pakhuis sanitasie en swamdoder behandelinge, koelkamer (enkel en gekombineer) blootgestel en geïnkubeer onder omstandighede wat simptoomuitdrukking aanhelp. Die volledige pakhuisbehandeling, tesame met die kouestoor tydperk, het aansienlike beheer van latente infeksies gegee. Oor die algemeen was die voortplantingsvermoë van letsels baie swak, met minder as 2,1% van alle letsels, gevorm op beide Valencia en Eureka suurlemoene, wat piknidia ontwikkel het. Met die finalisering van projek 1127 is bevind dat die gekombineerde epidemiologiese vereistes vir piknidiospoor vrystelling, saam met die resultate van hierdie proewe, daarop dui dat ge-oesde sitrusvrugte nie 'n epidemiologies pad vir die verspreiding van SSV is nie.

Dit is bekend dat sommige essensieële olies (EO) beskerming teen nadelige na-oes swampatogene bied. Die EOs word getoets in verskeie vorms wat insluit bedekkings, waterige matrikse en in bodamp vorm, met spesifieke fokus op die enkapsulering van die olie in ‘stadig-vrystellende nano-of mikropartikels (PHI 66 – M.Tech studie). ŉ Akkurate en betroubare bodamp metode is ontwikkel om die mate van vervlugtiging en dus die graad van beskerming te bepaal wat deur aktiewe metaboliete in die essensiële olie gebied word. ŉ Doelgemaakte gaschromatograaf is ontwerp en gebou vir bodamp analiese. Chemometriese analise van die uitgebreide datastel wat met behulp van gas chromatografie-massa spectrometrie bekom is, word tans uitgevoer (PHI 66 - D.Tech. studie).

In projek 123 (3.5.3) was verskeie produkte getoets, waarvan die resultate baie variërend was - die mees suksesvolle proeflopie was met ŉ azoksiestrobien verbinding teen *Penicillium digitatum*. Produkte wat waterstofperoksied met asynsuur kombineer (PAA) is weereens ondersoek as alternatiewe sanitasie agente en vir die verenigbaarheid met die belangrikste swamdoders in die fungisiede bad. PAA het geen uitwerking op die konsentrasie van propikonasool gehad nie, maar was nadelig vir pyrimethaniel. Fitochemiese reaksies is ŉ gevaar by sommige vloeibare formulasies. ŉ Poeier formulasie van PAA is suksesvol geëvalueer. Watersanitasie is steeds prominent en verskeie produkte was aangebied en getoets ten opsigte van hierdie aspek.

Plante produseer ŉ baie uitgebreide diversiteit van verbindings bekend as fitochemikalieë, wat almal op een of ander wyse funksioneel is in die plant. Die rol wat fitochemie speel om die infeksie deur *Phyllosticta citricarpa* (sitrus swartvlek, SSV) òf aan te moedig, òf te inhibeer, word in hierdie studie ondersoek (3.5.5). Kultivars met verskillende vatbaarhede vir SSV word ondersoek deur bestudering van die apolêre fraksies (was, olie, lipiede) en polêre fraksies (flavonoïede, antosianiene, alkaloïede, glikosiede) in die vrugskil. Huidiglik word Bitter Seville, as ŉ lae vatbare tipe, medium vatbare navel en hoogsvatbare suurlemoen tipes gebruik, terwyl kumkwat as n nie-vatbare tipe bygevoeg is.

Sitrusvrugte wat uitgevoer word na die EU benodig 'n nuwe swamdoder vir die heheer van suurvrot, wat veroorsaak word deur die askomiseet *Galactomyces citri – aurantii.* Dit is belangrik sedert guazatine, 'n guanidine swamdoder uitgefaseer is. Propikonasool is 'n nuwe ingevoerde na oes-swamdoder wat beskikbaar is in die Suid-Afrikaanse sitrusbedryf. Die demetilasie-inhibeerder (DMI) triasool swamdoder word geregistreer vir na-oes gebruik op suurvrot op sitrusvrugte in Suid-Afrika. Basislyn sensitiwiteit vir propikonasool is getoets vir Suid-Afrikaanse isolate van *G. citri-aurantii*, wat suurvrot veroorsaak, of *Penicillium digitatum*, wat groenskimmel veroorsaak (3.5.6).

Onlangse swamkontaminasie op die houtbasisse van sitruspallette het ŉ problem uitgelig waar die oorsaak daarvan onbekend is (3.5.7). Hierdie degradering van die basisse wanneer besendings op die oorsese market aankom het vrae laat ontstaan rondom die kwaliteit van die hout wat gebruik word. Daar het bekommernis geheers oor die bydrae van die pakhuis tot swak palette as gevolg van opberging, asook watter omgewingsfaktore ŉ rol sou speel (vog, UV afbreking en insekbesmetting). Dis egter die swamverrotting wat tans die hoogste prioriteit geniet. Swaar besmette basisse dra die risiko dat dit kartonne in die pallet ook mag besmet, bydra tot die verhoging van die spoorlading in opbergingskamers en dit is ŉ fitosanitêre risiko. Hierdie studie fokus dus op die mikrobioom van besmette houtbasisse, vervaardigingsaspekte van die houtbasisse, die rol wat pakhuisopberging speel, verskillende opsies vir houtbehandeling, asook die moontlike bydrae van behoueringseenhede tot die degradering van die houtbasisse.

3.5.2 FINAL REPORT: Singular and combined effects of postharvest treatments on viability and reproductive ability of *Phyllosticta citricarpa* infections

Project 1127 (2016/17 – 2017/18) by Wouter Schreuder Jnr (USPP), Wilma du Plooy (CRI), Catherine Savage (CRI), Cheryl Lennox (USPP), Tian Schutte (CRI), and P.H. Fourie (CRI)

Summary

Although citrus black spot is mostly regarded as a disease that should be dealt with pre-harvest, postharvest treatments and storage have a large effect and implications on symptom expression. This project was co-funded by the Postharvest Innovation Fund (PHI) to investigate the effect of postharvest treatments on symptom expression and lesion viability. Only more recent protocols and fungicides used in packhouses, as well as alternative fungicides, were evaluated for their effect against latent CBS infections as well as the reproductive potential of the lesions. Fruit with CBS lesions, as well as asymptomatic fruit with latent infections were subjected to standard packhouse sanitation and fungicide treatments, cold storage (singularly and combined) and incubated at conditions to enable expression of latent infections. The full packhouse treatment along with storage period gave significant control of latent infections. The overall reproductive ability of lesions were very low, with less than 2.1% of all lesions that formed on both Valencia's and Eureka lemons developing pycnidia. Three alternative single treatments showed potential to control latent infections: fludioxonil, potassium sorbate and Propirly 270 EC (Propiconazole + Pyrimethanil). Fludioxonil and Propirly 270 EC led to moderate to significant control of latent infections on both Valencia oranges and Eureka lemons. Potassium sorbate moderately controlled latent CBS infections in both Valencia oranges and Eureka lemon trials. The combined epidemiological requirements for pycnidiospore release along with results from trials indicate that harvested fruit is not an epidemiologically significant pathway for the spread of CBS.

Opsomming

Hoewel sitrus swartvlek meestal beskou word as 'n siekte wat voor-oes behandel moet word, het na-oes behandelings ŉ groot effek op simptoom uitdrukking. Hierdie projek was mede-befonds deur die Postharvest Innovation Fund (PHI) om die effek van na-oes behandelings op simptoom uitdrukking en letsel lewensvatbaarheid te ondersoek. Slegs meer onlangse pakhuis protokolle en swamdoders is herevalueer, asook alternatiewe swamdoders, vir hul uitwerking teen latente swartvlek infeksies asook hul effek op die reproduktiewe potensiaal van letsels. Vrugte met swartvlek letsels, asook asimptomatiese vrugte met latente infeksies is aan standaard pakhuis sanitasie en swamdoder behandelinge, koelkamer (enkel en gekombineer) blootgestel en geïnkubeer onder omstandighede wat simptoomuitdrukking aanhelp. Die volledige pakhuisbehandeling, tesame met die kouestoor tydperk, het aansienlike beheer van latente infeksies gegee. Oor die algemeen was die voortplantingsvermoë van letsels baie swak, met minder as 2,1% van alle letsels gevorm op beide Valencia en Eureka suurlemoene wat piknidia ontwikkel het. Drie alternatiewe, enkele behandelings het potensiaal gewys om latente infeksies te beheer: fludioxonil, potassium sorbate and Propirly 270 EC (propiconazole + pyrimethanil). Fludioxonil en Propirly 270 EC het matige tot aansienlike beheer van latente infeksies op beide Valencia lemoene en Eureka suurlemoene getoon. Kaliumsorbaat het matig beheer van latente swartvlek infeksies op beide Valencia lemoene en Eureka suurlemoen getoon. Die gekombineerde epidemiologiese vereistes vir piknidiospoor vrystelling saam met die resultate van hierdie proewe, dui daarop dat ge-oesde vrugte nie 'n epidemiologies pad vir die verspreiding van swartvlek is nie.

Introduction

Citrus black spot is currently the major factor threatening market access to the EU. The EU market is the main market for South African export citrus. Although this disease is best managed in the orchard, some of the infections do escape the orchard treatments and are intercepted by EU inspectors. Postharvest treatments may at least assist in reducing these interceptions by inhibiting the development of these latent infections. Very little is known in the postharvest field with regards to CBS and a thorough investigation is necessary.

Citrus black spot (CBS; caused by *Phyllosticta citricarpa*) is a very relevant and controversial disease for the South African citrus export industry. Although this disease is only of cosmetic concern, EU market regulators are constantly threatening to close citrus imports from SA due to the A1-quarantine status of the pathogen. It is therefore important that every possible aspect of citrus production research should focus on methods and treatments to reduce the development of CBS.

From a postharvest perspective it is relevant to assess normal packhouse treatments for the control or inhibition of CBS. Korf (1998) conducted an *in vitro* study where it was shown that postharvest fungicides imazalil (IMZ), thiabendazole (TBZ), prochloraz, guazatine (GZT) and sodium ortho-phenylphenate (SOPP) significantly reduced conidial germination and appressorium formation of *P. citricarpa*. Results from previous work testing the effect of postharvest fruit treatments on the viability of CBS lesions indicated that efficacy varies (Agostini *et al*., 2006; Yan *et al*., 2014), but Korf *et al.* (2001) demonstrated a 3- to 7-fold reduction in CBS lesion viability. However, none of these studies investigated the effects of treatments on the reproductive ability of CBS lesions (latent or visible), neither has modern packhouse treatments been evaluated. *In vitro* work however, does not correlate directly with *in vivo* results, as the contact effect of the fungicides are highly variable when on the fruit surface. This study is therefore important in terms of new and alternative management strategies of CBS.

The effect of typical South African postharvest treatment protocols need to be tested, as well as new and alternative fungicides together with non-fungicidal compounds such as GRAS chemicals. This assessment will elucidate their effect on the viability and reproductive ability of CBS lesions. The study hinges on the framework of a master's degree aiming to determine the singular and combined effects of postharvest treatments on viability and reproductive ability of *Phyllosticta citricarpa* infections. Apart from updated treatment protocols, the gaps for future research will be identified.

<u>Objective 1</u>: A thorough literature search on all publications related to:

1. Overall introduction to CBS and its economic impact
2. Isolation methods of *P. citricarpa* from fruit
3. Postharvest control methods for *P. citricarpa*
4. PCR protocols for *P. citricarpa*
5. Artificial inoculation of *P. citricarpa* on citrus fruit

<u>Objective 2:</u> Investigate the singular and combined effects of standard fungicide postharvest treatments and cold storage regimes on the viability and reproductive ability of citrus black spot lesions.

1. Fruit
    a. Infected citrus fruit will be sourced from unsprayed and/or neglected orchards. Fruit will be sorted into batches of asymptomatic (but putatively latently infected fruit) and symptomatic fruit. On symptomatic fruit, existing lesions will be individually characterised according to the described CBS symptom types and a photo-record of each fruit are to be kept as reference.
2. Treatments
    a. Postharvest treatment regimes
    Each active will be applied singly and in all practically possible combinations on symptomatic and asymptomatic fruit through the CRI Nelspruit laboratory drench, wash, dip, flooder or wax applicators.
        i. Drench
            1. TBZ, PYR, GZT and 2.4-D
            2. TBZ, Philabuster, GZT and 2.4-D
        ii. Washing system
            1. Chlorine
            2. HPPA
        iii. IMZ treatment
            1. Flooder (25, 35 and 45°C)

            2. Dip (25, 35 and 45°C)
        iv. Wax (TBZ, IMZ and 2.4-D)
    b. Cold storage regime
        i. Cold sterilisation (-0.5°C)
        ii. Normal shipping (3.5°C)
        iii. Ambient (22.0°C)
3. Evaluation
   Existing and new lesions will be characterised and recorded by means of a photo-record of each fruit. These lesions will be subjected to the following assessments:
    a. Reproductive ability
        i. Fruit will be incubated at 23°C under high humidity for 2 weeks. At regular intervals, lesions will be inspected for the formation of pycnidia in the lesions; the number of pycnidia developing in each lesion will be recorded.
        ii. Additionally, oozing of pycnidiospores will be recorded and the germination ability of the spores microscopically quantified after incubation in 0.3% citric acid droplets on microscope slides.
    b. Viability of lesions
        i. Lesions in which pycnidia developed, or lesions that expanded, will be regarded as alive, but the presence of DNA of *P. citricarpa* will be determined by means of DNA extraction from the lesion and species-specific PCR.
        ii. Lesions that failed to produce pycnidia or failed to expand will be subjected to viability and identification tests. These will include isolation onto growth medium and PCR identification from each lesion.

Objective 3: Investigate the singular and combined effects of new and alternative fungicides as well as non-fungicide compounds applied in heated or ambient aqueous applications on the viability and reproductive ability of citrus black spot lesions. Fruit selection, symptom characterisation and recording as well as evaluation will be similar to that described above. The following treatments are being evaluated:

1. Fungicides and alternatives:
    a. Propiconazole + Pyrimethanil
    b. Propiconazole
    c. Fludioxonil
    d. Pyrimethanil
    e. Sodium carbonate
    f. Potassium sorbate
    g. Calcium carbonate
    h. Essential oil
    i. Postharvest wax
2. Temperatures: 25, 35 and 45°C.
3. Exposure time: 15, 45, 90 and 180 s.

Materials and methods

Single and combination treatments were applied to fruit. Single treatments consisted of a drench (pyrimethanil, thiabendazole, guazatine and 2,4-D), chlorine wash, imazalil dip treatments at 25, 35, 45 and 55°C, and wax (imazalil, thiabendazole and 2,4-D). Exposure times were 15, 45, 90 and 180 seconds. The full packhouse treatment consisted of a drench, chlorine wash, imazalil dip at 35^{0}C and finally a wax application. In the full packhouse treatment the drench application was applied 24 hours before any of the other treatments to try and simulate what happens in a real packhouse. After the treatments were applied, fruit were packed into cartons with pulp trays and the cartons placed inside plastic bags to ensure a high relative humidity in the carton. The combination treatment consisted of drench, chlorine, dip (35°C) and wax. The combination treatment was split for incubation at ambient (22°C) and either 7°C for lemon fruit or 4°C for Valencia orange fruit.

Fruit were stored at either cold storage (Lemons = 7^{0}C; Valencia = 4^{0}C) or ambient conditions (22^{0}C, 79-85 % Relative Humidity) for 5 weeks, upon which it was moved to an incubation room. The incubation room was specifically designed to promote lesion expression of latent CBS infections. The room was at a constant 27^{0}C with 24-hour light and a Relative Humidity > 85%. The fruit were kept in these conditions for three weeks while being evaluated. Hereafter, the reproductive potential of the lesions was determined.

Results and discussion

Objective / Milestone	Achievement
1. Literature review	The student completed the literature review that was included in an MSc Thesis.
2.Plan trials	Trials were planned with the input of all role players. The planned trials were all completed as planned.
3.Conduct trials	Including the trials for 2016, 17 trials were completed successfully. The fruit in all the trials were fruit from various orchards with potential CBS infection, were treated with individual packhouse treatments, or with the full packhouse combination. Fruit were evaluated for suppression of lesion formation, spore release and productive viability.

All the objectives were met in their entirety. However, the 2017 technology transfer on conventional packhouse treatments and alternative products and applications (SASPP congress and CRI Packhouse Workshops) were not done, as the candidate (W Schreuder) has accepted a full-time position in the industry and could not be made available for these events. As these topics were dealt with extensively in 2016 during the workshops and at the 9th CRI Symposium, it was not considered a shortcoming.

Full results and discussion is attached as appendix 1 (MSc Thesis)

Conclusion

The individual treatments showed variable results but the combination treatments gave consistent significant control of latent infections. Lesion viability studies showed that treating fruit with postharvest fungicides that have visible lesions did not affect lesion viability. These fruits should be removed during sorting for product quality reasons. The IMZ dip controlled latent infection, with variable results. The full packhouse treatment exerted significant control on new lesion development and pycnidia formation. The wax application retards spore release by acting as a barrier and has moderate to significant control of latent infections.

Some individual treatments showed consistent results (fludioxonil, Propirly, potassium sorbate), others showed variable results (commercial coating wax, propiconazole, sodium bicarbonate). Single treatments that gave consistent control on both fruit types have the potential to be used for the control of latent CBS infections. Due to the low infection levels, present on the fruit, for more representative results trials should be repeated on orchards with high disease pressure. The formulation that contains both pyrimethanil and propiconazole (Propirly) seem to have a synergistic effect, improving control of latent infections. There is no significant difference in control efficacy between combinations where guazatine was substituted with propiconazole. Combination treatments (1 and 2) showed consistent and moderate to significant control of latent infections. The full packhouse treatment resulted in significant control of pycnidia formation on newly formed lesions.

The lesions that develop on harvested and packhouse treated fruit from latent infections have very poor reproductive capability. Both the 2015 and 2016 combination trials showed that the percentages of lesions that developed pycnidia were extremely low.

Technology transfer

Schreuder, W., Savage, C., Erasmus, A., Lennox, C. and Fourie, P. 2016. The effect of postharvest treatments on CBS development. CRI Packhouse Workshops.

Schreuder, W., Erasmus, A., Schutte, T., Du Plooy W., Lennox, C. and Fourie, P. 2016. Effect of postharvest treatments on development of latent CBS infections. Poster presented at the 9th CRI Symposium, Champagne Castle Sports Resort.

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APPENDIX 1
CHAPTER 2

Investigating the singular and combined effects of standard postharvest fungicide treatments and cold storage regimes on the viability and reproductive capability of citrus black spot lesions

ABSTRACT

Phyllosticta citricarpa is the causal agent of citrus black spot, and is regarded as a quarantine organism. Some South African export markets, such as the European Union, have imposed very limiting trade restrictions for fresh fruit exported from production areas where this organism occurs. CBS is effectively controlled using timely applied preharvest fungicide sprays. However, fruit infections that were not prevented often remain latent until after the asymptomatic fruit is harvested, treated and packed in packhouses and exported. The objective of this research chapter was to evaluate the efficacy of postharvest treatments for the control of latent CBS infections, and determine the reproductive ability of lesions that formed after these treatments. Trials were conducted on naturally infected fruit, harvested from four Eureka lemon and four Valencia orange orchards. Fruit with CBS lesions, as well as asymptomatic fruit with latent infections were subjected to standard packhouse sanitation and fungicide treatments, cold storage (singularly and combined), subjected to a 5-week cold or ambient storage and incubated for 2 weeks at conditions conducive to expression of latent infections. The pre-packhouse drench and chlorine wash had no significant effect on control of latent infections. On the other hand, imazalil dip treatments at 25 to 55°C mostly resulted in moderate to significant control of latent infection. Although there was no direct correlation to temperature of application, IMZ dips appear to be more effective on Valencia oranges. Wax application to fruit also resulted in moderate to significant control of latent infections, but more importantly, during viability studies it was observed that lesions on fruit treated with wax did not release pycnidiospores from pycnidia that formed in new lesions. The full packhouse treatment, along with a cold storage period (Valencia oranges 4°C, Eureka lemons 7°C), consistently effected significant control. Viability trials conducted on newly formed lesions proved that fruit lesions have a very low reproductive capability with 0.001-2.09% new lesions on Eureka lemons, and 0-0.35% on Valencia oranges forming pycnidia. Trials conducted on fruit with visible lesions indicated that post-harvest treatments had no consistent effect on lesion viability. The combined epidemiological requirements for pycnidiospore release along with results from trials indicate that harvested fruit is not an epidemiologically significant pathway for the spread of CBS.

INTRODUCTION

South Africa is the second largest exporter of fresh citrus fruit worldwide, with exports accounting for approximately a quarter of the global market (CGA, 2016). In 2015, 40% of citrus exports went to European markets (CGA, 2016). Maintaining access to these export markets requires adherence to the specific quality standards that have been imposed (European Commission, 2000).

Citrus black spot caused by *Phyllosticta citricarpa* (McAlpine) van der Aa, is a cosmetic disease of citrus fruit. The disease remains mostly latent in infected fruit and can express a variety of black spot lesions on rinds of maturing fruit. These lesions are not progressive postharvest decays, but makes fruit aesthetically unfit for the fresh fruit market (Kotzé, 1981). In severe cases, and in highly suitable climates, heavy fruit infection can also lead to premature fruit drop and crop loss (Araújo *et al.*, 2013). In South Africa, CBS rarely causes crop loss, but it is an economically important disease. *Phyllosticta citricarpa* is regarded as a quarantine pathogen in certain countries and the presence of CBS in production regions (Carstens *et al.*, 2012) or the presence of CBS lesions on fruit limit access to those markets. Current EU regulations, for example, allow citrus fruit to be exported only if produced in pest-free zones of production, or orchards that were managed free from the disease. Whole consignments will be rejected if one or more CBS lesions are observed upon inspection of fruit (Anonymous, 2000). The EU's zero tolerance for CBS has been challenged by South Africa (South African CBS PRA, 2000-2009) and the EU's recent pest risk assessment for *P. citricarpa* (EFSA, 2014) was criticised by an international panel of CBS experts (CBS Expert Panel, 2013; 2014; 2015). The CBS Expert Panel (2013, 2014, 2015) agreed with earlier pest risk assessments, conducted by South Africa and USA (USDA-APHIS, 2010), in which it was concluded that fruit is not a realistic pathway for CBS to enter, establish, or spread and have significant economic impact within the EU.

Citrus black spot is primarily controlled in the orchard through the application of curative and/or protective fungicidal sprays to protect young, susceptible fruit during the fruit susceptibility period (the first 4 to 5 months after petal drop) (Kiely, 1948; Schutte, 2002; Schutte *et al.*, 2003; Miles *et al.*, 2004). Such a preharvest spray program generally consists of 3 to 4 applications, combining and alternating between mancozeb, benzimidazoles, strobilurins and copper (Kellerman and Kotzé, 1977; Schutte *et al.,* 2003; Miles *et al.,* 2004). Whilst very high levels of control can be achieved (Makowski *et al*., 2014), even timeously applied fungicides can result in variable protection due to climatic conditions, coverage of the fruit surface achieved by the spray, and cultivar susceptibility (Calavan, 1960; Kiely, 1969, 1970, 1971; Schutte, 2002; Schutte *et al.*, 2003).

CBS disease control measures are very effective, but fruit with latent infections can remain asymptomatic during picking and packing, and might subsequently display lesion expression during shipping (Kiely, 1948; Loest, 1958; Brodrick, 1969). The export of fresh citrus from affected production areas is therefore becoming increasingly difficult, particularly to the EU markets with a zero tolerance for CBS. Furthermore, unacceptable levels of non-compliance might lead to closure of the market (WTO, 1993; Anonymous, 2000).

South African fresh fruit has some of the longest export routes: from the time of harvest until the fruit reaches the consumer, can take between 6 to 10 weeks depending on the export country (Pelser, 1977). Due to these long periods, producers and packhouses are required to apply effective control measures, such as postharvest fungicides, wax coatings and sanitation practices to maintain product quality and limit postharvest decay. Asymptomatic fruit with latent *P. citricarpa* infections, as well as fruit with CBS lesions will be subjected to these standard packhouse handling and treatment processes. It is therefore important to evaluate the effects of these treatments on *P. citricarpa* infection and expression on the harvested fruit.

The first published studies evaluating postharvest fungicides were conducted by Korf in 1998. During these trials, Korf (1998) conducted *in vitro* studies which showed postharvest fungicides imazalil (IMZ), thiabendazole (TBZ), prochloraz, guazatine (GZT) and sodium ortho-phenylphenate (SOPP) significantly reduced conidial germination and appressorium formation of *P. citricarpa*. Korf also conducted *in vivo* trials using chlorine, and found that a chlorine treatment had no significant effect on CBS infections. Results from previous work testing the effect of postharvest fruit treatments on the viability of CBS lesions varies (Korf *et al*., 2001; Agostini *et al*., 2006), but Korf *et al*. (2001) has demonstrated a 3- to 7-fold reduction in CBS lesion viability. However, none of these studies investigated the effects of treatments on the reproductive ability of CBS lesions (latent or visible), neither has modern packhouse treatments been evaluated. Agostini *et al.* (2006) conducted fruit trials using different postharvest fungicides, and reported that none of the fungicides had a significant effect on lowering postharvest disease incidence. Instead, storing fruit at 8°C significantly reduced postharvest incidence of CBS. Seberry *et al.* (1967) reported that applying a wax coating to Valencia fruit significantly reduced the expression of CBS lesions of stored fruit.

The aim of this study was to evaluate the effect of current postharvest fungicides and application methods being used in South African citrus packhouses on latent CBS infection, focussing on viability and reproductive capability.

MATERIALS AND METHODS

Fruit used in trials

Eureka lemons and Valencia oranges were used in all the trials, and were chosen for their high susceptibility to *P. citricarpa* (Kiely, 1948). Fruit used in the trials were harvested from farms located in Gauteng, Mpumalanga and Eastern Cape provinces. Lesion-free fruit were collected from unsprayed or abandoned orchards that were verified to have CBS infection following prior inspection. Fruit were harvested into plastic fruit crates (325 x 505 x 245 mm) and transported to CRI facility. Eight different orchards were used: four Eureka lemon and four Valencia orange orchards.

Postharvest treatments

Individual treatments of the pre-packhouse drench, chlorine wash, wax and imazalil dips (applied at 25°C, 35°C, 45°C and 55°C) were evaluated as single treatments. The full packhouse treatment consisted of the pre-packhouse drench, chlorine wash, imazalil dip at 35°C, and a wax application, and was evaluated as a combination treatment.

Pre-packhouse drench

Fruit are drenched before degreening to minimize postharvest decay, primarily caused by *Penicillium* spp. and *Galactomyces* spp. (Ladaniya, 2010). The drench mixture of thiabendazole (1000 $mg.L^{-1}$), pyrimethanil (1000

mg.L^{-1}), guazatine (1000 mg.L^{-1}) and 2,4-D (250 mg.L^{-1}) was prepared by filling a container with 50 L of tap water and adding fungicides in the order that follows, while constantly stirring the mixture to keep all the fungicides in suspension: 100 mL thiabendazole (Thiabendazole, 500 g.L^{-1} SC, ICA International Chemicals, Stellenbosch, South Africa), 125 mL pyrimethanil (Protector, 400 g.L^{-1} SC, ICA International Chemicals, Stellenbosch, South Africa), 240 mL guazatine (CitriCure, 210 g.L^{-1} SL, ICA International Chemicals, Stellenbosch, South Africa) and 500mL 2,4-D (2,4-dichlorophenoxy acetic acid) (Deccomone, 25 g.L^{-1} SL, Citrashine, Johannesburg, South Africa).
In these trials, a simulated dip-drench system was used (Christie, 2016). Fruit were packed into plastic crates (dimensions: 325 x 505 x 245 mm) which were used to simulate the standard 800 L orchard bin used by commercial citrus packhouses. The drench solution was applied at a rate of 12.5 L/min per crate (Christie, 2016) at an exposure time of 1 min (a dosage similar to commercial drench applicators), and then left to dry for 24 hours before other treatments were applied, before incubation.

Chlorine wash/dip

The chlorine wash system is generally a wet dump flume system where the fruit first enters the packhouse after degreening, or alternatively (if not degreened) directly from the orchard, depending on the producing unit's operations. The main purpose of the wash system is to remove spores, organic material, and dirt that may have collected on the fruit in the orchard, and during picking and transport (Lesar, n.d.). Chlorine acts as a sanitiser to reduce the build-up of fungal and bacterial spores in the wash water, preventing it from becoming an inoculum source.

The wash solution was prepared by adding 15 g of calcium hypochlorite (HTH Pool Chlorine, 680 g.kg $^{-1}$ WP) to 50 L of tap water to give a 200 mg.L^{-1} total chlorine solution (CRI recommendation sheets, 2015). Hydrochloric acid was used to adjust the pH to 6.5-7.5 and confirmed by pH meter (Jenway Model 3310; Bibby Scientific Limited, Staffordshire, UK). To simulate the wet dump sanitation system, fruit were submerged in this solution for 90 seconds and then left to dry.

Fungicide bath

The specific purpose of the imazalil dip tank is control of green mould (*Penicillium digitatum*) (Harding, 1976), with the commonly used operating temperature of 35°C (Erasmus *et al.,* 2011, 2013). In this study treatments were applied at 25°C, 35°C, 45°C and 55°C to evaluate how temperature would affect treatment efficacy.

An IMZ solution was prepared by dissolving 80.4 g of imazalil sulphate (Imazacure, 750 g.kg^{-1} SG, ICA International Chemicals, Stellenbosch, South Africa) in 120 L of tap water to give a 500 mg.L^{-1} solution. The pH was constantly measured with a pH meter (Jenway Model 3310; Bibby Scientific Limited, Staffordshire, UK) and kept between 3.5 – 4 by adding hydrochloric acid. This was done to keep residue loading constant at different application temperatures (Erasmus *et al.,* 2011, 2013). Citrus fruit were submerged in the solution for 60 seconds and then put over revolving brushes, as is the common practice in packhouses. The equipment used for the dip application was an experimental packline developed and custom built to simulate commercial packlines.

Wax application

A smaller version of a commercial waxing unit (Decco Citrashine (Pty) Ltd, South Africa, Johannesburg) was used for application of a polyethylene-based wax (PolyOrange, 18% solids, Decco Citrashine (Pty) Ltd, Johannesburg, South Africa). The wax suspension consisting of imazalil (2000 mg.L^{-1}), thiabendazole (4000 mg.L^{-1}) and 2,4-D (250 mg.L^{-1}) was prepared by pouring 25 L of wax into the mixing tank and adding the treatment chemicals to the wax while the pump was constantly mixing the solution. The individual fungicides were first dissolved in 100 mL of warm water (40°C), before adding them to the mixing tank in the following order: 100 mL of imazalil (Imazacure, 500 g.L^{-1} EC, ICA International Chemicals, Stellenbosch, South Africa), 200 mL of thiabendazole (Thiabendazole, 500 g.L^{-1} SC, ICA International Chemicals, Stellenbosch, South Africa) and 250 mL 2,4-D (2,4-dichlorophenoxy acetic acid) (Deccomone, 25 g.L^{-1} SL, Decco Citrashine (Pty) Ltd, Johannesburg, South Africa).

The wax applicator tank was filled and left to agitate for 15 min before treatments commenced. The wax brushes were optimally wetted (damp to the touch) before use, and the wax applicator calibrated to administer 1 - 1.2 L of wax per ton of fruit.

Cold storage

Valencia oranges and Eureka lemons were placed into cold storage as a single treatment, and as part of the combination treatment. Eureka lemons are more prone to cold damage at low temperatures, therefore lemons

were stored at 7°C and Valencias, less prone to cold damage, were stored at 4°C for 5 weeks. Valencias used in cold sterilization trials were stored at -0.5°C for 24 days, then moved to cold storage (4°C) for remaining 11 days, as per industry recommendations.

Residue analysis

Six fruit were sampled from replication one and replication four, after each treatment application. The sampled fruit were placed in residue bags and deep frozen (-20°C), then later used to prepare pulp for residue analysis. The fruit inside residue bags were defrosted, measured and weighed. The fruit were then chopped and macerated to a fine pulp using a blender (Salton Elite, Amalgamated Appliance Holdings Limited, Reuven, South Africa) and the pulp was then re-frozen. Sub-samples of the macerated fruit were submitted for IMZ (chloramizole), PYR (pyrimethanil), TBZ (thiabendazole) and 2,4-D (Free acid) residue analyses by Hearshaw and Kinnes Analytical Laboratory (Cape Town, South Africa). The samples were extracted using acetonitrile followed by a matrix solid phase dispersion extraction. The extracts were analysed using liquid chromatography mass spectrometry (LCMS/MS; Agilent 6410, Agilent Technologies Inc., Santa Clara, CA, USA). The data collected were and the range at which fungicide residues were loaded were determined to confirm that no maximum residue limits (MRL) were exceeded (SAS Institute Inc. Cary, NC, USA).

Trials and evaluations

The trials that were conducted per treatment consisted of four replicates, with each replicate consisting of 12 fruit. For residue analysis, six extra fruit was included in the first and fourth replicates of each treatment.

Effects of treatments on latent infections

Latent infection trials were conducted four times each on Eureka lemons and Valencia oranges. Treatment protocols were as follows:

- Individual applications of the pre-packhouse drench, chlorine wash, wax and imazalil dips (applied at 25°C, 35°C, 45°C and 55°C) and cold storage (5 weeks at 7°C for lemons or 4°C for oranges).
- The full packhouse treatment consisted of the pre-packhouse drench, chlorine wash, imazalil dip at 35°C, and a wax application, and evaluated as the combination treatment. In this case, the pre-packhouse drench application was applied 24 hours before any of the other treatments to simulate the practices followed in a commercial packhouse. The full packhouse treatment was done in duplicate, with and without cold storage.

After treatments were applied, fruit were packed into lock back table grape cartons (APL cartons, Worcester, South Africa) on count SFT13 nectarine trays (Huhtamaki South Africa (Pty) Ltd, Atlantis, South Africa). Each carton was covered with a transparent polyethylene bag and sealed with a cable tie to ensure that a high relative humidity atmosphere was maintained.

The single treatments and one of the full packhouse treatments were stored at room temperature (22°C, 79-85% RH) for 5 weeks, along with an untreated control. A duplicate of the full packhouse treatment was placed into cold storage for 5 weeks, also along with individual cold storage treatment.

Effects of different cold storage regimes on latent infections

The effect of the full packhouse treatment, followed by storing CBS infected fruit at different cold storage regimes, was evaluated. The trials were conducted three times on Valencia oranges. The full packhouse treatment was done in triplicate. One full packhouse treatment was stored at ambient conditions along with an untreated control for 5 weeks, another was placed into cold storage (4°C) for 5 weeks along with an untreated control, and the third full packhouse treatment went into cold sterilization (-0.5°C) for 24 days along with an untreated control, where after the fruit were moved to cold storage (4°C) for another 11 days.

After 5 weeks, fruit were moved from the storage areas into the incubation room, for incubation and rating as described below.

Effects of treatments on symptomatic fruit

The effects of postharvest fungicide treatments on fruit with CBS lesions were also evaluated. In these trials, Eureka lemon fruit with visible hard spot lesions were used. Single and combination treatments were applied to fruit with lesions, as described above, except for combinations treatments with cold storage.

After 2 weeks, six fruit from each replicate were used to determine reproductive potential of treated lesions. From the remaining six fruit in each replicate, four random fruit were chosen and then four random lesions per fruit were isolated, to determine viability. A total of 96 lesions were isolated per treatment. The selected

lesions were marked and the fruit submerged in 70% ethanol for 1 min, then placed on sterilized tissue paper inside the laminar flow to dry. Once the fruit had dried the marked lesions were isolated onto Potato-Malt-Yeast agar (PMY agar) plates (Korf, 1998). PMY agar is consists of 24 g potato-dextrose agar (PDA; DifcoTM; Becton, Dickinson and company; Sparks; MD; USA), 1 g yeast extract (YE, Biolab, Merck, Gauteng, RSA), 1 g malt extract (ME, Biolab, Merck, Gauteng, RSA) and 8 g agar (DifcoTM; Becton, Dickinson and company; Sparks; MD; USA), hydrated and sterilised in 1 L of deionised water. Lesion viability was evaluated as described below.

Lesions were isolated by first cutting a small square (1 cm^2) around the lesion. Using the scalpel, a thin layer consisting of the albedo and bottom of the lesion was removed and discarded. With the bulk of the lesion and some flavedo being exposed, an approximately 250 µm cross-section of the lesion and surrounding tissue was removed. This cross-section was then cut into four equal quarters, with the lesion in the centre. The four quarters were then plated out onto PMY agar and the rest of the lesion was removed and placed into 1.5 mL Eppendorf tube to be used for PCR identification. The plates were placed in an incubator (24-hour light, 25°C and RH> 85%), left for 10 to 14 days and then inspected.
Each plate represented a single lesion, therefore if there was mycelial growth from any of the four cross section pieces, the lesion was counted as still being viable. The data was used to determine the percentage lesions still alive, and how the different postharvest treatments affected lesion viability.

Evaluation

Lesion expression and reproductive ability

After 5 weeks, fruit stored at cold or ambient conditions were moved to an incubation room. The incubation room was optimised to enhance lesion expression, with the temperature of 25-27°C, 24-hour lighting and RH > 85%, whilst RH inside the bags were near-100%. The option to apply ethepon to accelerate lesion expression (Schutte and Beeton, 1999; Baldassari et al., 2007) was not considered as it was imperative that conditions in a commercial supply chain be simulated. The fruit were incubated for 2 weeks and evaluated for lesion expression.

The CBS lesions on each fruit were counted on day 0 (day when removed from cold or ambient storage), day 7 and day 14, after having been placed in the incubation room. The number of lesions that developed pycnidia was also recorded. After the last count was completed, all fruit with lesions that did not form pycnidia were discarded. Fruit with lesions that formed pycnidia were used in the further determination of the reproductive potential of lesions.

Pycnidiospore release and spore viability

Pycnidiospore release was induced using a solution made of Valencia juice and citric acid. This solution was prepared by adding 50 mL of a 5% citric acid solution to 100mL of 100% freshly squeezed Valencia orange juice. Using a pipet (Pipetman®, Gilson, S.A.S, Villiers, France-le-Bel) this solution was pipetted into 2 mL Eppendorf tubes and centrifuged for 5 min at 16 000 rpm the supernatant pipetted into 5 mL glass vials and autoclaved for 15 min at 121°C.

Within each treatment, 5 pieces of fruit with lesions with visible pycnidia were selected. On each fruit one lesion was marked and cleaned superficially using an earbud dipped in 70% ethanol, and then left to dry in the laminar flow. Once dry, a 5 µL drop of the prepared Valencia juice and citric acid solution was placed on the lesion, using a 20 µL pipette (Pipetman®, Gilson, S.A.S, Villiers, France-le-Bel). The droplet was left on the lesion for 30 minutes, allowing enough time for spores to be released into the droplet (Korf, 1998), without the droplet drying out. Using a pipette, the droplet was collected and the lesion was washed 5 times, every time using a new 5 µL droplet of sterilized water, to collect as many suspended pycnidiospores as possible. All the liquid collected from the lesion and plated onto a water-agar plate (WA; DifcoTM; Becton, Dickinson and company; Sparks; MD; USA) and incubated in a laminar flow for 24 hours at 22°C, allowing the droplets on the agar surface to dry. After the drying, the plate was examined under a stereo microscope (Zeiss Stemi DV4, Carl Zeiss (Pty) Ltd, Germany) and 5 pycnidiospores were single-spored onto oatmeal agar plates (OA, DifcoTM; Becton, Dickinson and company; Sparks; MD; USA). The OA plates were placed in an incubator (24-hour light, 25°C and RH>85%) and left for 10 to 14 days. Oatmeal agar is a selective medium for *P. citricarpa* and *Phyllosticta* mycelium growth with a yellow halo around the colony will indicate that the harvested pycnidiospores were viable, and positively identify it as *P. citricarpa* (Truter, 2010; Anonymous, 2014). If *Phyllosticta* mycelium growth is present but no halo has formed, it is indicative of the non-pathogenic endophyte *P. capitalensis*.

Verification of lesion diagnosis

As per the methods described by Hu *et al.* (2013), real-time polymerase chain reaction (qPCR) was used to verify the morphological identification *P. citricarpa* from the subsamples for each trial.

DNA was extracted from the citrus black spot lesions using the PROMEGA Wizard ® Genomic DNA Purification Kit (Promega, Madison, WI, USA). The manufacturer's instructions were slightly modified as lesion size was typically small. Pellets were re-suspended in 25 µl DNA rehydration solution. Control samples were included in the DNA extraction, including a positive fruit control (lesions from fruit that previously tested positive for *P. citricarpa*) and negative fruit control (fruit with no lesions obtained from a disease-free orchard). The elution volume was reduced to 1 × 75µL (Meyer, 2006). The extractions were placed in a 1.0% agarose gel with TBE buffer.

Species-specific real-time primers were used to amplify the ITS-region of *P. citricarpa*. The primer pair, GCITSF (5'- CCT GAA AGG TGA TGG AAG GG - 3') and GCITSR (5'- CGC CAA AGC AAC ATG GTA GAT A- 3') (Hu *et al*. 2013) was used in a 20 µl reaction to amplify the region. The qPCR reaction consisted of 2x KAPA Probe Fast qPCR master mix (KAPA Biosystems, Wilmington, MA, USA), 400 nm forward and reverse primer, 200 nm probe and 6 µl of PCR grade water. The thermal cycle consisted of an initial activation step of 95°C for 3 min, then 40 cycles of 95°C for 15 s and 59°C for 30 s. Control samples were a previously positive PCR sample as positive control as well as a no-template control.

Statistical analysis

The counts of the newly formed lesions were used to calculate the percentage control of individual replicates for each treatment relative to the mean lesion expression in the untreated control of each trial. One iteration of the Grubbs lower-tailed test at 5% significance level was performed on percentage control data to identify outliers, which were removed. The counts of lesions containing pycnidia were expressed as mean percentage pycnidia-forming lesions for each replicate in a treatment, within each trial. Data were subjected to appropriate analyses of variance (ANOVA). Fisher's LSD was calculated to identify significant differences between treatments, using a confidence interval of 90%. All statistical analyses were done using statistical analysis software Addinsoft XLSTAT Version 2015.8.18 (www.xlstat.com).

RESULTS

Effects of postharvest treatments on latent infections present on Eureka lemons

Ratings were conducted over a period of 14 days; during this period an increase in average lesions expressed per Eureka lemon fruit was observed (day 0 after treatment storage and start of incubation period = 5.9; day 7 = 13.8; day 14 = 31.03 average lesions per fruit). Not only was the climatic conditions in the incubation room perfect for lesion expression, but also for the development of postharvest fruit rotting fungi. The results from day 7 and day 14, shown in Table 1, have the same trend in terms of percentage control of latent infection The day 7 data were regarded as more reliable and discussed below; by day 14 many fruits had rotten due to postharvest decay (mostly green mould and Phytophthora brown rot) and resulted in missing data points making data set less reliable. Trial one (3.74 lesions per fruit) and two (7.10) had significant lower average number of lesions expressed than trial three (38.97) and four (78.59).

ANOVA of percentage control data indicated significant effects for trials ($P = 0.001$), for treatments ($P < 0.0001$) and no significant interaction between these factors ($P = 0.045$). The lower disease expression in the control fruit of trial two led to negative percentage values in some treatments, and the mean percentage control in this trial (-35.7%) differed significantly from the others three trials (20.8-53.8%). Table 1 shows the mean percentage control observed following various treatments in the four trials conducted on Eureka lemons. The treatment efficacy can be summarised as follows: no significant control with control levels ranging from -5 to 19% (Pre-packhouse drench, Chlorine wash, IMZ Dip at 35); low levels of control (20-39%; Cold storage, IMZ Dip at 45°C); moderate levels of control (40-59%; Wax, IMZ Dip at 25 and 55°C, Combination Ambient); and significant levels of control (60-100%; Combination with cold storage (7°C)). Individual treatments generally gave variable levels of control, whilst control by the combination treatments was more consistent, as is evident from the standard deviance.

ANOVA of percentages of newly formed lesions that developed pycnidia on Eureka lemons indicated significant differences between trials ($P < 0.0001$), but not between treatments ($P = 0.180$). Trial 1 had significantly more reproductive lesions (1.87%) compared with the other trials (0.05-0.08%). Less than 2.1% of newly formed lesions developed pycnidia. Except for the IMZ dip at 25°C (2.1%), most individual (0.034-0.567%) and combination (0.001-0.320%) treatments had markedly lower percentages of reproductive lesions than the control treatment (1.055%) (Table 1). The counts for lesions with pycnidia on day 14 was generally lower than that on day 7 due to rotten fruit that had to be discarded; data were not analysed further).

Effects of postharvest treatments on latent infections present on Valencia oranges

Ratings were conducted over a period of 14 days; during this period an increase in average lesions expressed per Valencia orange fruit was observed (day 0 = 0.146; day 7 = 0.348; day 14 = 0.542 average lesions per fruit). It was evident from these results that the disease pressure on Valencia oranges was markedly lower than that observed on Eureka lemons. Mean number of lesions in the control treatments ranged from 0.44 to 1.29. The climatic conditions in the incubation room accelerated the development of postharvest fruit rotting fungi. The results from day 7 and day 14, shown in Table 2, have the same trend in terms of percentage control of latent infection. The raw data collected on day 7 were used for statistical analysis because by day 14 too many fruits had rotten and resulted in large quantities of missing data points.

ANOVA of percentage control data indicated significant effects for trials ($P < 0.0001$), for treatments ($P < 0.0001$) and a significant interaction between these factors ($P = 0.0002$). The interaction was ascribed to variable results of individual treatments, and the combined results for the trials are presented. Significantly higher levels of control were observed in trials 3 and 4 (means of 75.2 and 70.0%, respectively), than trial 2 (47.1%) and trial 1 (24.5%). Table 2 shows the mean percentage control observed following various treatments in the four trials conducted on Valencia oranges. The treatment efficacy can be summarised as follows: no significant control with levels ranging from 0 to19% (Drench, Cold storage); moderate levels of control (40-59%; Chlorine wash, Combination 1 (Ambient)); and significant levels of control (60-100%; Combination 1 with cold storage (at 4°C), Wax, IMZ Dip at all temperatures).

The standard deviance indicated the variability in control for some individual treatments (cold storage, drench) and more consistent control for IMZ dip treatments, wax and combination treatments. No significant difference was observed between IMZ dips at the different temperatures. Both the combination treatments (at ambient or cold storage) consistently resulted in significant levels of CBS control.

ANOVA of percentage data of reproductive lesions indicated no significant effects between trials ($P = 0.388$), a significant treatment effect ($P = 0.051$) and a significant interaction between these factors ($P = 0.028$). The trial effect was attributed to variable results for the individual treatments and the mean treatment effects are reported. Table 2 shows the mean percentage of newly formed lesions that developed pycnidia on Valencia oranges as rated on day 7; similar to lemons, the numbers of pycnidia rated on day 14 was similar or reduced, due to the rotten fruit not being rated. Less than 0.17% of newly formed lesions developed pycnidia, and were in most cases similar to the untreated control (0.06%). Pycnidia formation completely inhibited on fruit that received the combination treatments (at ambient or cold storage).

Effect of postharvest treatments on symptomatic fruit

ANOVA of the mean percentage viable preformed hard spot lesions indicated that no significant difference between treatments ($P = 0.412$) existed. Lesion viability ranged from 79.2 to 100% and none of the treatments differed significantly ($P = 0.412$) from the control treatment (79.2%) (results not shown). Results were variable, which was attributed to the manual nature of the isolation technique, contamination of plates by co-isolated saprophytes, and age differences of isolated lesions. Lesions treated with wax proved to be harder to isolate. Hard spot lesions were used in the isolations; however, these lesions act as the perfect point of entry for saprophytes and secondary pathogens, which was evident on the PMY agar plates. Treating lesions with ethanol did not kill the mycelium of saprophytes present inside the lesion.

Effects of different cold storage regimes on latent infections

Due to postharvest rotting observed in previous trials data for the day 7 rating were used for statistical analysis. Mean number of lesions in the control treatments ranged from 1.02 to 8.05.
Table 3 shows results for percentage control of latent infection present on fruit treated with combination treatment and different storage regimes. ANOVA indicated a significant difference between treatments ($P = 0.005$), but no significant effect for trials ($P = 0.283$) or the interaction between treatments of different trials ($P = 0,507$). No significant difference in lesion control between fruit stored at 4°C (52.6%) and -0.5°C (54.5%) (Table 3). However, cold storage in combination with the packhouse treatments showed significantly higher levels of control, with no difference between 4°C (96.3%) and -0.5°C (95.5%). The packhouse treatments without any additional cold treatment resulted in 84.2% control of latent infections. The standard deviance indicated consistent levels of control from combination treatments and more variation of the cold storage treatments alone.
Less than 0.35% of lesions, as observed in the control treatment, developed pycnidia (Table 3). The full packhouse treatment combined with -0.5°C cold storage reduced this significantly to 0.16%, whilst the other treatments had intermediate levels of reproductive lesions (0.195-0.292%).

Pycnidiospore release and spore viability

Successful pycnidiospore release from pycnidia was achieved by using the method described above. Spore release initiation from pycnidia present in virulent spot lesions ranged from 1 min to 5 mins. Spore release initiation from pycnidia present in hard spot and cracked spot lesions only started 7 min after a droplet was placed on a lesion, taking up to 20 mins with older lesions. An example of pycnidia releasing pycnidiospores is shown in Figure 1.
Spores were harvested from oozing pycnidia, but due to contamination in >60% of the oatmeal agar plates, results from these spore viability tests could not be analysed. Further investigation indicated that the lesions themselves were the source of contamination. After 2 weeks' incubation at high humidity and 25°C, many saprophytes and other opportunistic pathogens established in the dead tissue that forms part of the CBS lesions. Different chemicals were used to sanitize the lesion (3% sodium hypochlorite solution, 2 min dip in 70% ethanol, chlorine wash, ozone wash), but none were successful. The method to test pycnidiospore viability therefore needs to be refined and developed further.
During oozing trials, it was observed that lesions covered with an intact wax coating did not release spores: the wax appeared to be physically restricting spore release. This applied to the individual wax treatment and combination treatments.

Verification of lesion diagnosis

In all the PCR cycles the positive control tested positive and the negative control tested negative, showing both the extraction and PCR run was executed correctly. In summary, 93.7% of all the samples tested positive for *P. citricarpa*. A single lesion from lemons tested negative, while the remainder (n = 8) were lesions from oranges. The negative samples collected from Valencia oranges were subsequently identified as *Colletotrichum gloeosporioides*, a fungus that creates very similar lesions to that of *P. citricarpa* and which is commonly co-isolated from CBS lesions.

Residue analysis

The range of residue levels for the various fungicides that were used in the different treatments is given in Table 4. These residue results indicated that all treatment applications yielded expected residue results when following industry-simulated applications, and showed that MRL's were not exceeded for any of the fungicides used in these trials. Low levels of pyrimethanil contamination were observed in a few wax treatment samples. The source of this contamination was confirmed to be the rollers that form part of the forced air drying tunnel.

DISCUSSION

In these trials, we made use of natural infection, which could not be quantified before treatment application. The trial setup needed to account for the unknown infection levels present on the fruit so that results could be interpreted in terms of control. Since it could not be quantified accurately prior to each trial, possible variation was addressed by increasing the sample size of fruit used in each treatment. We also tripled the sample size of the control fruit to determine a more accurate level of infection present on fruit. Whilst latent infection levels in the control treatments varied markedly between trials, and between Eureka lemons and Valencia oranges, similar trends in control of latent infections following the various treatments were observed. In the PCR analysis of the subsample of each trial, > 93% tested positive for *P. citricarpa,* showing that visual symptom diagnosis was accurate.

Incubation conditions employed presented optimal conditions for development of latent CBS infections as well as pycnidium formation, *i.e.* warm temperature and high humidity (Kiely, 1948; Baldassari *et al.*, 2007; Wang and Dewdney, unpublished results). Evaluations were conducted after 7 and 14 days' incubation, and whilst markedly higher numbers of lesions were recorded on day 14, this assessment was often problematic due to development of postharvest decay. However, results based on the 7- and 14-day assessment correlated and similar levels of control were recorded.

The cold storage treatment showed low and variable levels of control of latent infections on lemons (32.5%) and oranges (12.1%) in the initial trials. However, in the cold sterilization trials treatments showed moderate control of latent infections (52.6-54.5%), albeit at variable levels. Previous studies conducted by Agostini *et al*. (2006) indicated that cold storage (8°C) reduced the postharvest incidence of CBS lesions on fruit. Agostini hypothesised that the lower temperatures retard fungal growth, and in turn, symptom development.

The pre-packhouse drench results from both lemons and oranges showed the treatment had no significant control of latent infections. Korf *et al.* (2001) demonstrated a high level of *in vitro* inhibition of *P. citricarpa* by thiabendazole and guazatine, both used in the preharvest drench in this study, but also found no significant

inhibition when evaluating these fungicides in *in vivo* trials. This discrepancy shows that *in vitro* studies can only be used as an indicator of a chemical's potential use against a pathogen, and are not necessarily indicative of *in vivo* efficacy.

The chlorine wash showed low and variable control of latent infections on lemons (16.8%), but showed moderate and variable levels of control when applied to oranges (46.0%). Korf *et al*. (2001) showed that chlorine had no effect on CBS infection (visible lesions) on fruit.

The efficacy of IMZ dip treatments in controlling latent infections when applied to Eureka lemons ranged between -4.6 to 53.5%, with high variability, depending on the temperature of application. However, on Valencia oranges IMZ dip treatments showed significant and more consistent control of latent infections, with no significant difference between application temperatures (69.4-81.5%). Unpublished results (M.D. Laing) indicated the effect of brief hot water dips (68°C for 20 s) demonstrated some level control of latent infections. Whilst the IMZ dips in this study was conducted a lower temperatures, a temperature effect was not observed.

The wax treatment showed moderate to good (46.4% on Eureka lemons and 61.7% on Valencia oranges) control of latent infections. This supports the study by Seberry *et al.* (1967) which found that by applying a wax coating to Valencia fruit significantly reduced symptom development on stored fruit. *Phyllosticta citricarpa* is essentially an endophyte, expressing lesions only as the fruit becomes weaker (Kiely, 1948; Kotze, 1981). By delaying fruit ripening and aging using wax and cold storage, the pathogen development can be suppressed. Furthermore, it was also observed that the wax coating restricted the release pycnidiospores, which is an important observation considering that most fresh fruit exported is treated with a wax coating. The wax coating restricts moisture loss and restricts ageing (Deetlefs, 1959; Long and Leggo, 1959).
The combination of packhouse treatments consistently showed significant control of latent CBS infections on Valencia oranges (56.1%) and Eureka lemons (58.4%). Placing fruit into cold storage after the application of the full packhouse treatment further improved the levels of control (71.4 - 73%). This observation was confirmed when comparing the combination of treatments alone (84.2%) and with standard cold storage (4°C) with cold-sterilisation cold storage (-0.5°C) (95.6-96.3% control). Agostini *et al.* (2006) showed the controlling effects of cold storage, but found no consistent control following packhouse treatments. However, our findings confirm those of Korf *et al.* (2001) where a 3- to 7-fold reduction in lesion viability was demonstrated following the combined packhouse treatments. However, isolations from hard spot lesions in our study did not indicate any control relative to untreated fruit.

It is evident from all the trials conducted that postharvest CBS lesions expressed on fruit have a very low reproductive potential. Regardless of the trial and type of treatment applied to fruit, the percentages of lesions that developed pycnidia were extremely low: 0.001 – 2.09% on Eureka lemons and 0 – 0.35% on Valencia oranges. Korf *et al.* (2001) showed variable effects of the individual postharvest treatments on the viability of pycnidiospores harvested from hard spot lesions on treated fruit, viable spores could not be harvested from fruit treated in the combined treatments. Due to high levels of contamination, pycnidiospore viability testing could not be performed in our study.

Results from our study and those presented by Korf *et al.* (2001) showed variable levels of control by individual packhouse treatments, but more consistent and higher levels of control following the combined treatments, especially when combined with cold storage. This indicates cumulative or synergistic effects of these treatments against latent and visible CBS infection. The combination treatment with cold storage is the standard packhouse treatment for fresh citrus fruit, and it could therefore be accepted that CBS lesion that were present in the packhouse, and particularly those that develop postharvest will have very poor reproductive ability, even when incubated as highly suitable conditions.

CONCLUSION

The individual treatments showed variable results but the combination treatments had consistently significant control of latent infections. Lesion viability studies showed that treating fruit with postharvest fungicides that have visible lesions did not affect lesion viability. These fruits should be removed during sorting for product quality reasons. The IMZ dip controlled latent infection, at variable results. The full packhouse treatment exerted significant control on new lesion development and pycnidia formation. The wax application retards spore release by acting as a barrier and has moderate to significant control of latent infections.
The lesions that develop on harvested and packhouse treated fruit from latent infections have very poor reproductive capability. The trials showed that the percentages of lesions that developed pycnidia were extremely low. The combined epidemiological requirements for pycnidiospore release, the fact that pycnidia requires the presence of water for pycnidiospore release, and pycnidiospores rely on water to move over short distances (<1m) and long wetness periods on susceptible host tissue for infection (Kiely, 1948; McOnie, 1965; Spósito et al., 2008, 2011) indicate that harvested fruit is not a epidemiologically significant pathway for spread

of CBS, as was also concluded by previous CBS pest risk assessments (South African CBS PRA, 2000-2009; USDA-APHIS, 2010).

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TABLES AND FIGURES

Table 1: Mean percentage control (with standard deviation) of latent *P. citricarpa* infections on Eureka lemons and percentage of newly formed lesions that produced pycnidia, as observed 6 weeks (including a 1-week incubation period) after treatment with various standard individual and combination postharvest treatments.

Treatment [a]	Percentage control of latent infections (standard deviance) [b] Day 7		Percentage control of latent infections (standard deviance) [b] Day 14		Percentage reproductive lesions [b] Day 7		Percentage reproductive lesions [b] Day 14	
Control Ambient	-		-	-	1.06	ab	0.10	bcd
Cold storage (7°C)	32.5 (49.76)	bcd	47.34 (31.27)	bc	0.39	b	0.20	a
Pre-packhouse drench	9.51 (50.62)	de	26.43 (47.67)	cd	0.41	b	0.07	de
Chlorine wash	16.77 (68.82)	de	26.68 (63.91)	cd	0.04	b	0.07	de
IMZ Dip at 25^{0}C	53.54 (49.30)	abc	59.55 (38.86)	ab	2.10	a	0.20	ab
IMZ Dip at 35^{0}C	-4.56 (60.45)	e	6.67 (56.53)	d	0.57	b	0.17	abc
IMZ Dip at 45^{0}C	27.85 (58.35)	cd	29.97 (58.46)	c	0.03	b	0.08	cde
IMZ Dip at 55^{0}C	51.89 (39.16)	abc	62.76 (26.26)	ab	0.40	b	0.06	de
Wax	46.43 (43.70)	abc	56.85 (28.08)	ab	0.36	b	0.15	abcd
Combination (Ambient)	58.36 (30.56)	ab	68.43 (28.36)	ab	0.32	b	0.02	e
Combination (Cold 7°C)	71.43 (24.67)	a	76.91 (12.49)	a	0.00	b	0.01	e

[a]Combination is the full packhouse treatment, IMZ dip refer to the fungicide bath, control ambient was used to calculate percentage control hence no value,

[b] Mean percentages followed by the same letter in each column do not differ significantly per Fisher's least significant difference test ($P < 0.01$; LSD= 27.054 and 1.184, respectively)

Table 2: Mean percentage control (with standard deviation) of latent *P. citricarpa* infections on Valencia oranges and percentage of newly formed lesions that produced pycnidia, as observed 6 weeks (including a 1-week incubation period) after treatment with various standard individual and combination postharvest treatments.

Treatment [a]	Percentage control of latent infections (standard deviance) [b] Day 7		Percentage control of latent infections (standard deviance) [b] Day 14		Percentage reproductive lesions [b] Day 7		Percentage reproductive lesions [b] Day 14	
Control Ambient	-	-	-	-	0.06	bc	0.03	cd
Cold storage (7°C)	12.10 (58.70)	d	8.26 (59.22)	e	0.15	ab	0.13	bc
Pre-packhouse drench	2.04 (66.49)	d	-6.14 (58.64)	e	0.06	bc	0.01	cd
Chlorine wash	46.04 (41.57)	c	31.55 (52.40)	d	0.05	bc	0.34	a
IMZ Dip at 25^{0}C	69.36 (27.42)	ab	62.89 (34.39)	bc	0.10	abc	0.16	b
IMZ Dip at 35^{0}C	74.36 (17.74)	ab	73.24 (21.87)	bc	0.17	a	0.10	bcd
IMZ Dip at 45^{0}C	81.54 (13.47)	a	78.32 (12.62)	ab	0.02	c	0.05	bcd
IMZ Dip at 55^{0}C	76.83 (13.89)	ab	67.96 (19.27)	bc	0.03	c	0.06	bcd
Wax	61.66 (30.57)	abc	62.18 (31.95)	bc	0.01	c	0.01	cd
Combination (Ambient)	56.10 (49.15)	bc	54.10 (43.38)	c	0.00	d	0.02	d
Combination (Cold 7°C)	72.98 (65.60)	ab	97.31 (3.86)	a	0.00	d	0.01	d

[a]Combination is the full packhouse treatment, IMZ dip refer to the fungicide bath, control ambient was used to calculate percentage control hence no value.

[b] Mean percentages followed by the same letter in each column do not differ significantly per Fisher's least significant difference test ($P < 0.01$; LSD= 21,008 and 0,099, respectively)

Table 3: Mean percentage control (with standard deviation) of latent *P. citricarpa* infections on Valencia oranges and percentage of newly formed lesions that produced pycnidia, as observed 6 weeks (including a 1-week incubation period) after treatment of full packhouse treatment and 5-week cold treatment at various temperatures.

Treatment [a]	Percentage control of new lesions (standard deviance) [b]		Percentage reproductive lesions [c]	
Control Ambient	-	-	0.35	a
Combination (Ambient)[1]	84.2 (18.12)	ab	0.29	ab
Cold Storage (4°C)	52.57 (58.05)	c	0.23	ab
Combination (4°C)	96.34 (7.47)	a	0.20	ab
Cold Storage (-0,5°C)	54.45 (47.46)	bc	0.28	ab
Combination (-0,5°C)	95.55 (8.85)	a	0.16	b

[a] Combination is the full packhouse treatment, control ambient was used to calculate percentage control hence missing value.

[b] Showing the combined results of three trials conducted on Valencia oranges. Mean percentages followed by the same letter in columns do not differ significantly per Fisher's protected t-test least significant difference (P ≤0.05; LSD= 29,966).

[c] Showing the combined results of three trials conducted on Valencia oranges. There is no significant difference between treatments per Fisher's protected t-test least significant difference (P = 0,18 LSD= 0,156).

Table 4: Range of residue levels detected on Eureka lemons and Valencia oranges following various standard individual and combination postharvest treatments.

Trial	Treatments	Residue mg/kg [a]			
		Pyrimethanil [1]	**Thiabendazole [2]**	**2,4-D [3]**	**Imazalil [4]**
Eureka lemons / Valencia oranges					
	Control	0	0	0	0
	Pre-packhouse drench	0.63 - 4.03	0.19 - 2.24	0.12 - 0.67	0
	IMZ Dip 25°C	0	0	0	0.17 - 1.82
	IMZ Dip 35°C	0	0	0	0.21 - 1.77
	IMZ Dip 45°C	0	0	0	0.25 - 2,46
	IMZ Dip 55°C	0	0	0	0.69 - 4,62
	Wax	0 - 0,81	0.01 - 4,93	0,1 - 0.61	1,98 - 4,25
	Combination 1 (Ambient)	0.69 - 3,17	0.14 - 4.91	0.15 - 0,60	2.21 - 4.96
	Combination 1 (7°C/ 4°C)	0.85 - 5,0	0.13 - 5.00	0.12 - 0.77	1.27 - 4,87
Cold Storage (Valencia oranges)					
	Control	0	0	0	0
	Combination 1 (Ambient)	1.02 - 1.38	2.20 - 2.59	0.1 - 0.12	0.20 - 2.94
	Combination 1 (4°C)	1.16 - 2.11	1.89 - 3.39	0.09 - 0.14	2.47 - 3.01
	Combination 1 (-0.5°C)	1.19 - 2,14	2.03 - 2.80	0.10 - 1.0	2,59 - 2.88

[a] The maximum residue range shown here includes, the lowest MRL found out of all the trials to the highest MRL found for a specific treatment.

[1] Pyrimethanil has a maximum residue limit of 8 mg/kg, when exporting to the EU.
[2] Thiabendazole has a maximum residue limit of 5 mg/kg, when exporting to the EU.
[3] 2,4-D has a maximum residue limit of 1 mg/kg, when exporting to the EU.
[4] Imazalil has a maximum residue limit of 5 mg/kg, when exporting to the EU.

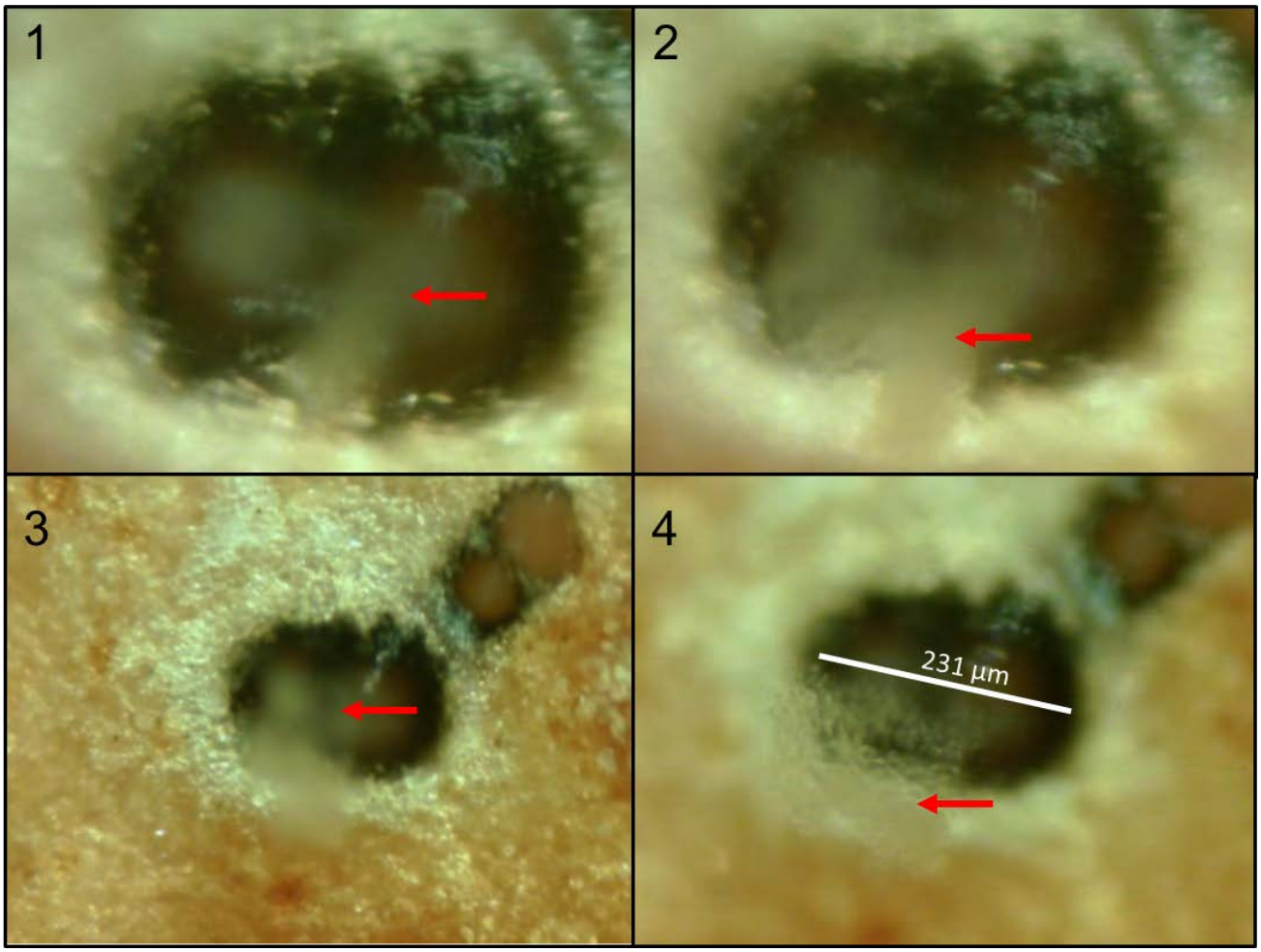

Figure 1: Photo plates depicting the progressive release of hyaline pycnidiospores from two adjacent pycnidia in two virulent spot lesions (photos 1 and 2, and 3 and 4, respectively). The spores are released in a gelatinous mass oozing from the pycnidia, visible as an increasingly blurred area (red arrows). In photo 4 individual pycnidiospores can be discerned, with the combined diameter of the two pycnidia indicated as 231 µm.

APPENDIX 2
CHAPTER 3

Effects of packhouse treatments and alternative compounds on the viability and reproductive ability of citrus black spot lesions

ABSTRACT

Phyllosticta citricarpa, causal agent of citrus black spot, a leaf and rind-borne disease with serious phytosanitary ramifications for citrus exporters. This fungal disease is especially important when exporting to specific markets, such as the European Union where phytosanitary trade restrictions against fruit from production areas where this organism occurs have been imposed. Following the trials conducted in 2015, and after the loss of guazatine MRL for postharvest use on all citrus exported to EU markets, research was continued to find substitutes that could be incorporated into the already established postharvest treatment regimes. Alternatives with MRL's accepted in the EU was selected and the full packhouse treatment, as applied in 2015, was repeated in 2016. Offered as a suitable alternative for guazatine, propiconazole was introduced in the South African postharvest industry recommendations and the active was therefore used as substitute for guazatine. There was no significant difference in terms of control between fruit that received the 2015 or 2016 full packhouse treatment. As in 2015 trials, fruit that received the full packhouse treatment, along with a cold storage period (Valencia oranges 4°C, Eureka lemons 7°C), showed significant control of both lesions and pycnidia formation. Three alternative single treatments showed potential to control latent infections: fludioxonil, potassium sorbate and Propirly 270 EC

(Propiconazole + Pyrimethanil). Fludioxonil and Propirly 270 EC had moderate to significant control of latent infections in both Valencia oranges and Eureka lemons. Potassium sorbate moderately controlled latent CBS infections in both Valencia oranges and Eureka lemon trials. Due to climatic conditions, disease incidence for the 2016 production season was extremely low. Although some of the fungicides used in this trial can potentially control latent CBS infections, further studies over more production seasons are required before recommendations can be made.

INTRODUCTION

Citrus black spot (CBS) is not a postharvest fruit rotting disease but causes superficial lesions on fruit rind. However, under highly suitable conditions, high levels of infection can sometimes lead to premature fruit drop (Whiteside, 1993). The perceived economic threat posed by CBS had led to it being declared as an A1-quarantine organism by the European Union (Directive 98/2/EC) despite numerous reports to the contrary (Truter, 2010; CBS Expert Panel, 2013; 2014; 2015).

CBS is a monocyclic disease (Agrios, 2005). Furthermore, *P. citricarpa* has two distinct life cycles: a teleomorph stage, which acts as the main source of inoculum in the orchard, producing ascospores from pseudothecia that develop and mature in leaf litter (McOnie,1965; Fourie *et al.*, 2013). The second stage is anamorphic and the secondary inoculum source, producing pycnidiospores from pycnidia on certain fruit, leaf or twig lesions. Whilst the epidemiological contribution of the anamorphic or asexual stage is regarded as minor, it is more important in lemon that has overlapping fruit sets (Kiely, 1948b; Kotzé 1963, 1981; Sutton and Waterson, 1966) and in countries with highly suitable climates (Spósito *et al.*, 2007, 2011).

Most commercially grown citrus varieties are susceptible to infection, with Eureka lemons and Valencia oranges being the most susceptible (Kiely, 1948a, 1970; Brodick, 1969). As per packhouse regulations, fruit expressing any lesions are removed from the packline during sorting. However, the biggest threat to export is the presence of latent infections that can remain asymptomatic for a long period and only be expressed after packaging and shipping. Should any fruit expressing lesions be found in a consignment of fruit by countries that has imposed CBS phytosanitary restriction, the whole consignment may be rejected, resulting in financial losses (CGA, 2016).

In 2015 trials were conducted to evaluate the efficacy of the standard packhouse fungicidal, sanitation and cold storage treatments, as singular and combined treatments, in suppressing latent infections. These studies included guazatine, a highly active fungicide against sour rot, caused by *Galactomyces citri-auranti*. However, early in the 2016 season the use of this compound in citrus packhouses was constrained due to the European Union lowering the maximum residue level (MRL) to 0.05 $mg.kg^{-1}$ (European Commission, 2016). Propiconazole was subsequently deployed to control postharvest sour rot infections (McKay *et al.,* 2012). As a postharvest fungicide against sour rot, propiconazole is a relatively new registration in South Africa for use on citrus. The full packhouse treatment of 2015 needed to be repeated, and had to include alternative chemicals that could be incorporated into the existing packhouse regime, as well as other actives that had suitable MRL on citrus to allow postharvest use. Propiconazole, as the replacement for guazatine, was added to the list of new alternatives to be tested.

Propiconazole was first registered in 1988 for use on seed grass and later on cereals for control of preharvest diseases (Worthing, 1983; Thomson, 1997; European Protection Agency, 2006), and has mainly been used as a preharvest application. It is a triazole regarded as a medium risk fungicide group (FRAC, 2016), and functional as a systemic fungicide that has both protective and curative activity (Worthing 1983; Thomson, 1997). No previous studies have been conducted to determine whether propiconazole is effective against CBS infection.

Fludioxonil, another chemical that has not previously been widely used in the South African citrus industry, is a synthetic analogue of the bacterial metabolite pyrrolnitrin. It was first evaluated for postharvest use as an alternative to imazalil (Schirra *et al.,* 2005; Zhang and Timmer, 2007; Förster *et al*., 2007). The active causes inhibition of mycelium growth, lower osmotic signal transduction leading to inhibition of spore germination and germ tube elongation (Rosslenbroich and Stuebler, 2000). As a broad spectrum, non-systemic fungicide with long residual activity (PMEP, 2016), and, because it has been considered as an alternative to imazalil it was prudent to include this fungicide in the trials. Fludioxonil has been used in black spot trials conducted by Agostini *et al*. (2006), but these studies showed that no single fungicide application had any measurable effect on inhibiting lesion formation.

Sodium carbonate (Na_2CO_3) is a GRAS (generally regarded as safe) chemical acceptable to use in all food products (Lakhanisky, 2012) and has been used as an antimicrobial agent. The compound can be used on all

fruit exported to the EU, with no MRL limits (European Commission, 2016). Prior to the trials conducted in this research chapter there was no available literature indicating that sodium carbonate has previously been used to control citrus black spot infection.

Another GRAS chemical used in citrus packhouses as a postharvest decay remedy is potassium sorbate. Unfortunately, it's not allowed to be used on any food exported to the EU (European Commission, 2016), but is known to inhibit the growth of a broad spectrum of microorganisms (Mendonca, 1992). No previous studies have been conducted to evaluate the effect of potassium sorbate on latent citrus black spot infections.

A new product recently introduced for postharvest use, is Fortisol CA Plus (formulation, manufacturer, address), which contains water-soluble salts calcium, potassium and phosphorus. The developers of this product claim that it stimulates the fruit's natural defences against active pathogens. This was the incentive for including Fortisol in the trials against latent CBS infection. Other than the marketing material, there was limited literature available regarding the claims and purported efficacy (Citrosol, 2016).

The objective of this research chapter was to evaluate alternative fungicides and GRAS chemicals that can possibly be incorporated into a control programme against latent CBS infection.

MATERIALS AND METHODS

Fruit used in trials

Eureka lemons and Valencia oranges were used in all the trials. These cultivars were chosen for their high susceptibility to infection by *P. citricarpa* (Kiely, 1948a). Fruit used in the trials were harvested from farms located in Gauteng and Mpumalanga provinces. Lesion-free fruit were collected from unsprayed or abandoned orchards that were verified to have CBS infection following prior inspection. Fruit were harvested into plastic fruit crates (325 x 505 x 245 mm) and transported to the laboratory. Fruit from seven different orchards were used: three Eureka lemon and four Valencia orange orchards.

Postharvest treatments

The full packhouse treatment consisted of the pre-packhouse drench (mixture 1 or 2), chlorine wash, imazalil dip at 35°C, and a wax application, and evaluated as the combination 1 (with guazatine) and combination 2 (with propiconazole instead of guazatine).

Pre-packhouse drench (mixture 1 and 2)

Fruit are drenched before degreening to minimize postharvest decay, primarily caused by *Penicillium* spp. and *Galactomyces* spp. (Ladaniya, 2010). Two drench mixtures were prepared, one containing guazatine and the other containing propiconazole. The drench mixture 1 contained thiabendazole (1000 mg.L^{-1}), pyrimethanil (1000 mg.L^{-1}), guazatine (1000 mg.L^{-1}) and 2,4-D (250 mg.L^{-1}) was prepared by filling a container with 50 L of tap water and adding fungicides in the order that follows, while constantly stirring the mixture to keep all the fungicides in suspension: 100 mL thiabendazole (Thiabendazole, 500 g.L^{-1} SC, ICA International Chemicals, Stellenbosch, South Africa), 125 mL pyrimethanil (Protector, 400 g.L^{-1} SC, ICA International Chemicals, Stellenbosch, South Africa), 240 mL guazatine (CitriCure, 210 g.L^{-1} SL, ICA International Chemicals, Stellenbosch, South Africa) and 500mL 2,4-D (2,4-dichlorophenoxy acetic acid) (Deccomone, 25 g.L^{-1} SL, Citrashine, Johannesburg, South Africa).

Drench mixture 2 contained the same compounds, but: propiconazole (600 mg.L^{-1}) was used as a replacement for guazatine: 250 mL of a formulated product containing both pyrimethanil and propiconazole (Propirly 270 EC, PYR: 150 g.L^{-1}, PPC 120 g.L^{-1}, ICA International Chemicals, Stellenbosch, South Africa) was used, and resulted in a 750 mg.L^{-1} PYR and 600 mg.L^{-1} PPC solution.

A simulated dip-drench system was used (Christie, 2016). Fruit were packed into plastic crates (dimensions: 325 x 505 x 245 mm) which were used to simulate the standard 800 L orchard bin used by commercial citrus packhouses. The drench solution was applied at a rate of 12.5 L/min per crate (Christie, 2016) at an exposure time of 1 min (a dosage similar to commercial drench applicators), and then left to dry for 24 hours before other treatments were applied, before incubation.

Chlorine wash/dip

The wash solution was prepared by adding 15 g of calcium hypochlorite (HTH Pool Chlorine, 680 mg.kg^{-1}, WP) to 50 L of tap water to give a 200 mg.L^{-1} solution. Hydrochloric acid was used to adjust the pH to 6.5 -7.5 and

confirmed by pH meter (Jenway Model 3310; Bibby Scientific Limited, Staffordshire, UK). To simulate the wet dump sanitation system, fruit were submerged in this solution for 90 seconds and then left to dry.

Fungicide bath

An IMZ solution was prepared by dissolving 80.4 g of imazalil sulphate (Imazacure, 750 $g.kg^{-1}$ SG, ICA International Chemicals, Stellenbosch, South Africa) in 120 L of tap water to give a 500 mg/L solution. The pH was constantly measured with a pH meter (Jenway Model 3310; Bibby Scientific Limited, Staffordshire, UK) and kept between 3.5 – 4 by adding hydrochloric acid. This was done to keep residue loading constant at different application temperatures (Erasmus *et al.,* 2011). Citrus fruit were submerged in the solution for 60 seconds and then put over revolving brushes, as is the common practice in packhouses. The equipment used for the dip application was an experimental packline developed and custom built to simulate commercial packlines.

Wax application

A smaller version of a commercial waxing unit (Decco Citrashine (Pty) Ltd, South Africa, Johannesburg) was used for application of a polyethylene-based wax (PolyOrange, 18% solids, Decco Citrashine (Pty) Ltd, Johannesburg, South Africa). The wax suspension consisting of imazalil (2000 mg. L^{-1}), thiabendazole (4000 mg. L^{-1}) and 2,4-D (250 mg. L^{-1}) was prepared by pouring 25 L of wax into the mixing tank and adding the treatment chemicals to the wax while the pump was constantly mixing the solution. The individual fungicides were first dissolved in 100 mL of warm water (40°C), before adding them to the mixing tank in the following order: 100 mL of imazalil (Imazacure, 500 $g.L^{-1}$ EC, ICA International Chemicals, Stellenbosch, South Africa), 200 mL of thiabendazole (Thiabendazole, 500 $g.L^{-1}$ SC, ICA International Chemicals, Stellenbosch, South Africa) and 250 mL 2,4-D (2,4-dichlorophenoxy acetic acid) (Deccomone, 25 $g.L^{-1}$ SL, Decco Citrashine (Pty) Ltd, Johannesburg, South Africa).

The wax applicator tank was filled and left to agitate for 15 min before treatments commenced. The wax brushes were optimally wetted (damp to the touch) before use, and the wax applicator calibrated to administer 1 - 1.2 L of wax per ton of fruit.

Cold storage

Valencia oranges and Eureka lemons were placed into cold storage as a single treatment, and as part of the combination treatment. Eureka lemons are more prone to cold damage at low temperatures, therefore lemons were stored at 7°C and Valencias, less prone to cold damage, were stored at 4°C for 5 weeks.

Alternative fungicides

All alternatives fungicides were applied at recommended dosages as per their registered labels or commercial recommendations. Fruit were submerged in 5-L solutions containing:

- 12 mL of propiconazole (Propicure 250 EC, ICA International Chemicals, Stellenbosch, South Africa) (600 mg. L^{-1}),
- 13 mL of fludioxonil (Tutor 500 SC, ICA International Chemicals, Stellenbosch, South Africa) (600 mg. L^{-1}),
- 12 mL propiconazole + pyrimethanil (Propirly 270 EC, ICA International Chemicals, Stellenbosch, South Africa) (750 mg. L^{-1} pyrimethanil, 600 mg. L^{-1} propiconazole),
- 100 mL water-soluble salts of Ca, K and P (Fortisol CA Plus, Citrosol, Partida Alamed, Spain) (2% solution, as per Citrosol recommendation),
- 50 g potassium sorbate (1% solution, Smilanick *et al.,* 2008), or
- 100 g sodium bicarbonate (2% solution, Palou *et al.*, 2016).

The solutions were prepared by adding the individual chemicals to a bucket filled with 5 L of tap water at 35°C. Fruit were submerged for 90 seconds before placing them in plastic fruit crates (325 x 505 x 245 mm) and allowing them to dry off. Since most propiconazole is removed through brushing (REFREF), no fruit was subjected to drying by brushing, in order to keep all the treatment implementation, the same.

Residue analysis

Six fruit were sampled from replication one and replication four, after each treatment application. The sampled fruit were placed in residue bags and deep frozen (-20°C), then later used to prepare pulp for residue analysis. The fruit inside residue bags were defrosted, measured and weighed. The fruit were then chopped and macerated

to a fine pulp using a blender (Salton Elite, Amalgamated Appliance Holdings Limited, Reuven, South Africa) and the pulp was then re-frozen. Sub-samples of the macerated fruit were submitted for IMZ (chloramizole), PYR (pyrimethanil) PPC (propiconazole) GZT (guazatine acetate), FLU (fludioxonil), TBZ (thiabendazole) and 2,4-D (Free acid) residue analyses by Hearshaw and Kinnes Analytical Laboratory (Cape Town, South Africa). The samples were extracted using acetonitrile followed by a matrix solid phase dispersion extraction. The extracts were analysed using liquid chromatography mass spectrometry (LCMS/MS; Agilent 6410, Agilent Technologies Inc., Santa Clara, CA, USA). The data collected were and the range at which fungicide residues were loaded were determined to confirm that no maximum residue limits (MRL) were exceeded (SAS Institute Inc. Cary, NC, USA).

Trials and evaluations

Each treatment had four replicates, with each replicate consisting of 12 fruit. For residue analysis, extra fruit was included in the first and fourth replicates of each treatment.

Effects of combination and alternative treatments on latent infections

The trials were repeated three times on Eureka lemons and four times on Valencia oranges. The alternative compounds were applied as single treatments of propiconazole, Propirly (propiconazole and pyrimethanil), fludioxonil, Fortisol CA PLUS (water-soluble salts of Ca, K and P), potassium sorbate, sodium bicarbonate and wax only (polyethylene wax) and evaluated.

The full packhouse treatment consisted of the pre-packhouse drench (mixture one or two), chlorine wash, imazalil dip at 35°C, and a wax application, and evaluated as combination 1 (with guazatine) or combination 2 (with propiconazole) treatment. In this case, the pre-packhouse drench application was applied 24 hours before any of the other treatments to simulate the practices followed in a commercial packhouse. Both combination treatments were done in duplicate, with and without cold storage.

After treatments were applied, fruit were packed into lock back table grape cartons (APL cartons, Worcester, South Africa) on count SFT13 nectarine trays (Huhtamaki South Africa (Pty) Ltd, Atlantis, South Africa). Each carton was covered with a transparent polyethylene bag and sealed with a cable tie to ensure that a near-100% RH was maintained.

The single treatments (alternatives) and one of each of the combination treatments were stored at room temperature (22°C, 79-85% RH) for 5 weeks, along with an untreated control. A duplicate of the full packhouse treatment was placed into cold storage for 5 weeks, also along with individual cold storage treatment.

Evaluation

Lesion expression and reproductive ability

After 5 weeks, fruit stored at cold or ambient conditions were moved to an incubation room. The incubation room was optimised to enhance lesion expression, with the temperature of 25-27°C, 24-hour lighting and RH > 85%. The fruit were incubated for 2 weeks while being evaluated.

The CBS lesions on each fruit were counted on day 0 (day when removed from cold or ambient storage) and days 7 after having been placed in the incubation room. The number of lesions that developed pycnidia was also recorded.

Verification of lesion diagnosis

As per the methods described by Hu *et al.* (2013), real-time polymerase chain reaction (qPCR) was used to verify the morphological identification *P. citricarpa* from the subsamples for each trial.

DNA was extracted from the citrus black spot lesions using the PROMEGA Wizard ® Genomic DNA Purification Kit (Promega, Madison, WI, USA). The manufacturer's instructions were slightly modified as lesion size was typically small. Pellets were re-suspended in 25 µl DNA rehydration solution. Control samples were included in the DNA extraction, including a positive fruit control (lesions from fruit that previously tested positive for *P. citricarpa*) and negative fruit control (fruit with no lesions obtained from a disease-free orchard). The elution volume was reduced to 1 × 75µL (Meyer, 2006). The extractions were placed in a 1.0% agarose gel with TBE buffer. Species-specific real-time primers were used to amplify the ITS-region of *P. citricarpa*. The primer pair, GCITSF (5'- CCT GAA AGG TGA TGG AAG GG - 3') and GCITSR (5'- CGC CAA AGC AAC ATG GTA GAT A- 3') (Hu *et al*. 2013)

was used in a 20 µl reaction to amplify the region. The qPCR reaction consisted of 2x KAPA Probe Fast qPCR master mix (KAPA Biosystems, Wilmington, MA, USA), 400 nm forward and reverse primer, 200 nm probe and 6 µl of PCR grade water. The thermal cycle consisted of an initial activation step of 95°C for 3 min, then 40 cycles of 95°C for 15 s and 59°C for 30 s. Control samples were a previously positive PCR sample as positive control as well as a no-template control.

Statistical analysis

The counts of the newly formed lesions were used to calculate the percentage control of individual replicates for each treatment relative to the mean lesion expression in the untreated control within each trial. The counts of lesions containing pycnidia was expressed as mean percentage pycnidia-forming lesions for each replicate in a treatment, within each trial. The data from all the trials conducted on Eureka lemons and all the trials conducted on Valencia oranges were thereafter grouped and used in appropriate analyses of variance (ANOVA). Fisher's LSD was calculated to identify significant differences between treatments, using a confidence interval of 95%. All statistical analyses were done using statistical analysis software Addinsoft XLSTAT Version 2015.8.18 (www.xlstat.com).

RESULTS

Effects of combination and alternative treatments on latent infections present on Eureka lemons

The incubation conditions were ideal for CBS symptom and pycnidium development, but also promoted the development of postharvest fruit rotting fungi. Due to postharvest rot, one trial had to be discarded and the 14-day rating data could not be used. The data collected on day 7 was used for statistical analysis. Mean number of lesions in the control treatments ranged from 4.65 to 5.48. ANOVA of percentage control data indicated a significant trial x treatment interaction ($P = 0.041$), significant effects for treatments ($P = 0.001$), but no significant difference between trials ($P = 0.917$). The interaction was attributed to variable results of some individual treatments (propiconazole, sodium bicarbonate, wax only and Fortisol CA PLUS). Means for the two trials were considered and results are presented in Table 1. The treatment efficacy can be summarised as follows: no significant control with levels ranging from 0 to 25% (wax only), low levels of control (26 to 50%; propiconazole, sodium bicarbonate, Fortisol CA PLUS); moderate levels of control (51 to 75%; cold storage, fludioxonil, potassium sorbate, Combination 1 (ambient), Combination 2 (ambient)); and significant levels of control (76 to 100%; Propirly, Combination 1 (7°C), Combination 2 (7°C)). Standard deviance values (Table 1) indicate that control was mostly consistent, but variable for propiconazole, sodium bicarbonate, wax only and Fortisol CA PLUS. There was no significant difference between Combination 1 (with guazatine) and Combination 2 (with propiconazole). The combination treatments (1 and 2), along with a cold storage (7°C) consistently resulted in significant levels of CBS control (80%). From the single treatments, Propirly (Propiconazole + Pyrimethanil) had the highest efficacy in control of latent CBS infections (78.8%). ANOVA of percentage data of newly formed lesions that developed pycnidia on Eureka lemons indicated significant effects for trials ($P = 0.001$), treatments ($P < 0.0001$) and a significant interaction between trials and treatments ($P = 0.004$). The interaction was attributed to differences in disease incidence and variable results between the two trials, but the data were combined in the analysis. Less than 0.25% of newly formed lesions developed pycnidia (Table 1). The combination 1 (ambient) (0.21%), wax (0.18%), potassium sorbate (0.18%), sodium bicarbonate (0.25%) and Fortisol CA PLUS (0.22%) formed more reproductive lesions than the control (0.17%), but did not differ significantly. The most of the individual (0.05 – 0.14%) treatments had markedly lower percentages of reproductive lesions than the control treatment (0.17%), Whilst the combination treatments with cold storage (0.002%) had significantly fewer reproductive lesions.

Effects of combination and alternative treatments on latent infections present on Valencia oranges

As was experienced with the lemon trials, one trial had to be discarded due to postharvest decay, and data collected on day 7 was used for statistical analysis. Due to generally unsuitable climatic conditions during the fruit susceptibility period the latent infection levels on Valencia fruit were very low. Mean number of lesions in the control treatments ranged from 0.025 to 1.13. ANOVA of percentage control data indicated significant effects for treatments and trials ($P < 0.0001$), but no significant interaction between trials and treatments ($P = 0.088$). The significant difference between trials may be ascribed to differences in disease pressure with mean control observed in Trial 2 (87.2%), significantly higher than in Trials 1 and 3 (62.6-69.3%). Table 2 shows the mean percentage control observed following various treatments in the three trials conducted on Valencia oranges. The treatment efficacy can be summarised as follows: low level control with levels ranging from 26 to 50% (Fortisol CA PLUS); moderate levels of control (51 to 75%; Cold storage, sodium bicarbonate, potassium sorbate, Wax only, Combination 1 (Ambient)) and significant levels of control (76 to 100%; Combination 2 (Ambient), Fludioxonil,

Propiconazole, Propirly, Combination 1 (4°C), Combination 2 (4°C)). Standard deviance values (Table 2) indicate that control was mostly consistent, but variable for Fortisol CA PLUS. There was no significant difference between Combination 1 (with guazatine) and Combination 2 (with propiconazole). The combination treatments (1 and 2), along with a cold storage (4°C) consistently resulted in significant levels of CBS control (84.3 to 92.9%). From the single treatments, Propirly (Propiconazole + Pyrimethanil) had the highest efficacy in control of latent CBS infections (100%). ANOVA of percentage data of newly formed lesions that developed pycnidia on Eureka lemons indicated no significant effects for trials (P = 0.44), treatments (P = 0.07) or the interaction between trials and treatments (P = 0.87). Due to the low levels of pycnidia formation there was no significant difference between treatments. Less than 0.11% of newly formed lesions developed pycnidia (Table 2). The combination 1 (ambient) (0.03%), wax (0.1%) and Fortisol CA PLUS (0.04%) formed more reproductive lesions than the control (0.01%), but did not differ significantly. The most of the individual (0 – 0.008%) treatments had markedly lower percentages of reproductive lesions than the control treatment (0.01%), whilst the combination treatments with cold storage (0%) developed no reproductive lesions.

Verification of lesion diagnosis

PCR verification of lesion diagnosis was conducted on 77 samples. In all the PCR cycles both the positive control and negative control tested true, showing both the extraction and PCR run was executed correctly. In summary, 98.7% of all the samples tested positive for *Phyllosticta citricarpa*. One lesion from lemons tested negative, but was not identified further.

Residue analysis

The range of residue levels for the various fungicides that were used in the different treatments is given in Table 3. These residue results indicated that all treatment applications yielded expected residue results when following industry-simulated applications, and showed that MRL's were not exceeded for any of the fungicides used in these trials. In these trials Guazatine MRL's did not exceed, but are not of any consequence due to the loss of the MRL.

DISCUSSION

Previous studies by Korf et al. (2001) looked at conventional chemistry use at the time, showing the usefulness of such postharvest applications. New actives, GRAS chemicals and alternative combinations implemented in recent times were included in this study, and were similarly proven to be valuable in the control of CBS.

Due to the extremely low likelihood of artificially inoculating P. citricarpa, only natural infection was used in these trials. This implies that infection rates could therefore not be quantified before treatment application, thus the trial protocol accounts for the unknown infection levels present on the fruit so that results could be interpreted in terms of control. Possible variation was addressed by increasing the sample size of fruit used in each treatment. Whilst latent infection levels in the control treatments varied markedly between Eureka lemons and Valencia oranges, similar trends in control of latent infections following the various treatments were observed. In the PCR analysis of the subsample of each trial, > 98% tested positive for P. citricarpa, showing that visual symptom diagnosis was accurate.

The cold storage treatment on Valencia (58.4%) and Eureka (52%) fruit resulted in similar percentage control of latent infections, resulting in moderate control. Agostini et al (2006) postulated that the lower temperatures create an unsuitable environment, slowing fungal growth and controlling latent infections.

Single treatments with fludioxonil (70.2 to 82.2%), Propirly (78.8 to 100%) and potassium sorbate (68.8 to 70.1%) showed consistent levels of control on both Eureka lemons and Valencia oranges. No one of the other GRAS chemicals resulted in any significant control of lesion expression. This concurs with studies by Palou et al. (2016) which indicated that alternative GRAS chemicals, though effective, still only offer limited postharvest efficacy, and that combination of such chemicals with conventional chemistry is still necessary in the foreseeable future. The wax only treatment showed variable results on Valencias (60%) and Eureka (21.4%), with inconsistent levels of control, as shown by standard deviance. Seberry et al., (1967) showed that treating fruit with wax coating gave significant control of latent infections, which is shown by results from trials conducted on Valencia oranges.

Propiconazole gave better and more consistent control on the Valencia fruit (91.7%) than on Eureka lemons (30.5%). Treatment using Propirly 270 EC (a pyrimethanil blend with propiconazole at a lower concentration) consistently resulted in significant levels of control that would suggest that the added pyrimethanil has a synergistic effect, resulting in better control of latent infections. The sodium bicarbonate treatment resulted in low and variable

of latent infections on Eureka lemons (35.6%), and moderate and more consistent control on Valencia oranges (65.9%). The inconsistent treatment efficacy between cultivars can be attributed to differences in infection levels between trials and cultivars. Agostini et al., (2006) reported that no single treatment had any significant effect on control of latent infections.

There was no significant difference between combination 1 (with guazatine) and combination 2 (with propiconazole). This lead to the conclusion that the substitution of guazatine with propiconazole did not affect the efficacy of the full packhouse treatment. Comparable with previous results from 2015 trials, the combination (1 + 2) with a cold treatment (Valencia 84.3 to 92.7%, Eureka 80%) showed higher levels of control than treated fruit stored at ambient (Valencia 63.3 to 81.4%; Eureka 66.3 to 72.4%). Control fruit had an overall low level of reproductive lesions (lemons 0.17%; oranges 0.01%). The combination treatments (1 and 2) along with a cold storage treatment lowered the formation of reproductive lesions (0 – 0.002%). This study confirms that fruit lesions have a very low reproductive potential, and by applying a postharvest packhouse treatment the low reproductive potential is lowered even further. New advances and improvements in postharvest fruit sanitation may offer additional control of latent infections and pycnidial development, and should be included in future research (Feliziani et al., 2016).

CONCLUSION

Some individual treatments showed consistent results (fludioxonil, Propirly, potassium sorbate), others showed variable results (commercial coating wax, propiconazole, sodium bicarbonate). Single treatments that gave consistent control on both fruit types have the potential to be used for the control of latent CBS infections. Due to the low infection levels, present on the fruit, for more representative results trials should be repeated on orchards with high disease pressure. The formulation that contains both pyrimethanil and propiconazole (Propirly) seem to have a synergistic effect, improving control of latent infections. There is no significant difference in control efficacy between combinations where guazatine was substituted with propiconazole. Combination treatments (1 and 2) showed consistent and moderate to significant control of latent infections. The full packhouse treatment resulted in significant control of pycnidia formation on newly formed lesions. The results from combination treatments in 2015 trials directly correlates with results from 2016 trials. The lesions that develop on harvested and packhouse treated fruit from latent infections have very poor reproductive capability. The trials showed that the percentages of lesions that developed pycnidia were extremely low, comparable to results from 2015 trials.

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TABLES AND FIGURES

Table 1: Mean percentage control (with standard deviation) of latent *P. citricarpa* infections on Eureka lemons and percentage of newly formed lesions that produced pycnidia, as observed 6 weeks (including a 1-week incubation period) after treatment with various alternative individual and standard combination postharvest treatments.

Treatments [a]	**Percentage control of latent infections (Standard deviance)** [b]		**Percentage reproductive lesions** [c]	
Control Ambient	-	-	0.17	abc
Cold storage (7^{0}C)	51.98 (21.66)	abc	0.14	bcd
Fludioxonil	70.20 (9.86)	ab	0.10	cde
Propiconazole	30.49 (61.15)	c	0.09	cde
Propirly	78.83 (11.26)	a	0.05	de
Potassium sorbate	70.08 (30.94)	ab	0.18	abc
Sodium bicarbonate	35.55 (58.82)	bc	0.25	a
Fortisol CA plus	31.15 (47.79)	c	0.22	ab
Wax Only	21.38 (42.79)	c	0.18	abc
Combination 1 (Ambient)	72.39 (19.29)	a	0.21	ab
Combination 1 (7°C)	80.00 (19.24)	a	0.002	e
Combination 2 (Ambient)	66.27 (21.01)	ab	0.09	cde
Combination 2 (7°C)	80.00 (15.87)	a	0.002	e

[a] Combination 1 (with guazatine) and combination 2 (with propiconazole) is the full packhouse treatment, control ambient was used to calculate percentage control hence no value.

[b, c] Mean percentages followed by the same letter in each column do not differ significantly per Fisher's least significant difference test (P ≤0.05; LSD = 34.811 and 0.109, respectively).

Table 2: Mean percentage control (with standard deviation) of latent *P. citricarpa* infections on Valencia oranges and percentage of newly formed lesions that produced pycnidia, as observed 6 weeks (including a 1-week incubation period) after treatment with various alternative individual and standard combination postharvest treatments.

Treatments [a]	**Percentage control of latent infections (Standard deviance)**[b]		**Percentage reproductive lesions** [c]	
Control Ambient	-	-	0,010	b
Cold storage (4°C)	58.36 (30)	d	0,008	b
Fludioxonil	82.15 (17.33)	abc	0,00	b
Propiconazole	91.69 (11.29)	a	0,00	b
Propirly	100.00 (0)	a	0,00	b
Potassium Sorbate	61.84 (33.85)	cd	0,00	b
Sodium Bicarbonate	65.88 (34.67)	bcd	0,007	b
Fortisol CA plus	34.63 (52.17)	e	0,04	b
Wax Only	59.96 (22.71)	d	0,10	a
Combination 1 (Ambient)	63.30 (36.40)	cd	0,03	b
Combination 1 (4°C)	84.34 (15.18)	ab	0,00	b
Combination 2 (Ambient)	81.40 (17.78)	abc	0,00	b
Combination 2 (4°C)	92.87 (9.78)	a	0,00	b

[a] Combination 1 (with guazatine) and combination 2 (with propiconazole) is the full packhouse treatment, control ambient was used to calculate percentage control hence no value.

[b, c] Mean percentages followed by the same letter in each column do not differ significantly per Fisher's least significant difference test (P ≤0.05; LSD= 20.98 and LSD= 0,109 respectively).

Table 3: Range of residue levels detected on Eureka lemons and Valencia oranges following various alternative individual and standard combination postharvest treatments.

Treatments[a]	Residue mg/kg [b]						
	Pyrimethanil[1]	**Propiconazole[2]**	**Fludioxonil[3]**	**Guazatine[4]**	**Thiabendazole[5]**	**2,4 D[6]**	**Imazalil[7]**
Control	0	0	0	0	0	0	0
Propiconazole	0	1.98 - 6.05	0	0	0	0	0
Fludioxonil	0	0	0.31 - 2.76	0	0	0	0
Propirly	2.12 - 5.67	1.11 - 3.69	0	0	0	0	0
Potassium sorbate	0	0	0	0	0	0	0
Sodium bicarbonate	0	0	0	0	0	0	0
Fortisol CA plus	0	0	0	0	0	0	0
Wax Only	0	0	0	0	0	0	0
Combination 1	0.49 - 1.6	0	0	0.23 - 3.89	1.03 - 4.13	0.11 - 0.32	0.84 - 4.36
Combination 2	0.68 - 2.05	0.38 - 1.16	0	0	0.35 - 3.90	0.04 - 0.54	1.95 - 5.0

[a] Combination 1 (with guazatine) and combination 2 (with propiconazole) is the full packhouse treatment, control ambient was used to calculate percentage control hence no value.

[b] The maximum residue range shown here includes, the lowest MRL found out of all the trials to the highest MRL found for a specific treatment.

[1] Pyrimethanil has a maximum residue level of 8 mg/kg, when exporting to the EU.
[2] Propiconazole has a maximum residue level of 9 mg/kg, when exporting to the EU.
[3] Fludioxonil has a maximum residue level of 10 mg/kg, when exporting to the EU.
[4] Guazatine no longer has a MRL in the EU (0.05)
[5] Thiabendazole has a maximum residue level of 5 mg/kg, when exporting to the EU.
[6] 2,4-D has a maximum residue level of 1 mg/kg, when exporting to the EU.
[7] Imazalil has a maximum residue level of 5 mg/kg, when exporting to the EU.

3.5.3 **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 Wilma du Plooy, Catherine Savage and P. H. Fourie (CRI)

Summary

Several products were evaluated, with varying results – the most successful was an azoxystrobin formulation against *Penicillium digitatum*. Products combining hydrogen peroxide and acetic acid were once again investigated as alternative sanitizers in the fungicide bath. The sensitivity of pyrimethanil and stability of propiconzole in the presence of the active was confirmed. Phytochemical reactions to some liquid formulations is a danger. A powder formulation of PAA was successfully evaluated, and as a result of the CRI contribution to the development of this product, the CRI will receive royalties from the sale of this product. Water sanitation is still very prominent, and several products with regards to this aspect was offered for testing.

Opsomming

Verskeie produkte is getoets, waarvan die resultate baie variërend was - die mees suksesvolle proeflopie was met ŉ azoksiestrobien verbinding teen *Penicillium digitatum*. Produkte wat waterstofperoksied met asynsuur kombineer (PAA) is weereens ondersoek as alternatiewe sanitasie agente en vir die verenigbaarheid met die belangrikste swamdoders in die fungisiedbad. PAA het geen uitwerking op die konsentrasie van propikonasool gehad nie, maar was nadelig vir pyrimethaniel. Fitochemiese reaksies is ŉ gevaar by sommige vloeibare formulasies. ŉ Poeier formulasie van PAA is suksesvol geëvalueer en as gevolg van die CRI se bydrae in die ontwikkeling in die produk, is die verkope daarvan onderworpe aan tantieme oorbetalings soos onderhandel. Watersanitasie is steeds prominent en verskeie produkte was aangebied ten opsigte van hierdie aspek.

Introduction

This project offers an ongoing industry service to evaluate potential new postharvest disease control products or options, as well as to conduct *ad hoc* experimentation. Products are mostly submitted from private companies, or projects/products are selected by the researchers involved. Given limited time and resources, requests are screened based on industry priorities. Below are brief reports of the activities in the project during the 2016/17 report year.

Objective / Milestone	Achievement
1. New potential products will be tested as sanitation agents and/or fungicides, this specifically include seeking actives for the control of *Phytophthora* brown rot and sour rot	None of products tested were effective against the pathogens. *Phytophthora* brown rot is a time and resource intensive pathogen in terms of inoculation and current resources does not allow for further testing of products against this pathogen. More feasible solutions against this pathogen will be investigated in project 1030 (preharvest).
2. Introduce and implement the application of GRAS chemicals into the citrus postharvest industry	Several trials were conducted. No viable alternatives have been found as yet.
3. Assist CRI DC with packhouse resistance testing	Swabs are either collected by extensionists visiting packhouses, or sent to the DC. Most do not indicate shifts in sensitivity, however, there were detections. The implicated packhouses were consulted on remedial action.
4. Analytical lab focus – ring test with the aim to reduce variability	Four analytical laboratories partook in the ring test – three had acceptable results, however, the PPECB laboratory failed to detect correct levels of the spiked fungicide they were presented with.

Alternative products

A number of GRAS chemicals and alternative postharvest products were evaluated, in particular focussing on sour rot and green mould. The results were highly variable (Appendices 1-5)

Packhouse sanitation

Products offered as water sanitation options in citrus packhouses were evaluated. PAA is an effective sanitiser, but several reports of phytotoxicity or “burn” was received from soft citrus packers in particular. A powder formulation that negate this problem has been registered and is being introduced as a commercial option. We did several tests with this product (Appendix 6-8).

Ring tests
Hearshaw and Kinnes. Microchem and Hortec all managed to detect the spiked fungicides within an acceptable range of the concentrations presented to them. The PPECB laboratory, however, failed the test. A further test will be done early in the 2017 season, which will be presented without the laboratories knowing that it is part of a ringtest. This will be done to prevent a "best-foot-forward" effect whereby non-standard levels of precision are achieved.

Resistance monitoring
Swabs from actively working packhouses are tested regularly throughout the season. This service will be continued and expanded in 2017. A few incidences of sensitivity shifts in pathogen resistance was detected and the packhouses concerned consulted about the issue. Suitable measures to curb this problem was suggested in cooperation with the extensionists.

Technology transfer

Information on grower talks and presentations at conferences where results from CRI-funded research were presented.

Further objectives (milestones) and work plan

1. New potential products will be tested as sanitation agents and/or fungicides; this specifically include seeking actives for the control of sour rot
2. Introduce and implement the application of GRAS chemicals into the citrus postharvest industry
3. Seek effective products and technologies for water sanitation in citrus packhouses
4. Analytical lab focus – ring test with the aim to reduce variability
5. Assist CRI DC with packhouse resistance testing

<u>Quarterly milestones for Jan-Mar, Apr-Jun, Jul-Sep, Oct-Dec 2017 and Jan-Mar 2018</u>

Apr-June 2017
- Objective 1 – New potential products: Acquire and test products
- Objective 2 – GRAS chemicals: Conduct pilot trials
- Objective 3 – Water sanitation: Conduct pilot trials
- Objective 4 – Residue ring test: Prepare for ring test
- Objective 5 – Packhouse resistance testing: Assist DC when needed

Jul-Sep 2016
- Objective 1 – New potential products: Acquire and test products
- Objective 2 – GRAS chemicals: Conduct pilot trials
- Objective 3 – Water sanitation: Conduct pilot trials
- Objective 4 – Residue ring test: Conduct ring test
- Objective 5 – Packhouse resistance testing: Assist DC when needed

Oct-Dec 2016
- Objective 1 – New potential products: Write reports and give feedback
- Objective 2 – GRAS chemicals: Compile and analyse data

- Objective 3 – Water sanitation: Compile and analyse data
- Objective 4 – Residue ring test: Compile data and give feedback
- Objective 5 – Packhouse resistance testing: Assist DC when needed

Jan-Mar 2017

- Objective 1 – New potential products: Present interesting results at workshops
  Write progress report
- Objective 2 – GRAS chemicals: Present interesting results at workshops
  Write progress report
- Objective 3 – Water sanitation: Present interesting results at workshops
  Write progress report
- Objective 4– Residue ring test: Present interesting results at workshops
  Write progress report
- Objective 5 – Packhouse resistance testing: Write progress report

3.5.4 PROGRESS REPORT: Precision fungicide application for the control of postharvest diseases on citrus

Project 1126 (2015/16-2016/17) by Catherine Savage (CRI), Wilma du Plooy (CRI), Cheryl Lennox (USPP) and P. H. Fourie (CRI)

Summary

Postharvest fungicides can be applied to fruit using several methods, namely pre-packline drench application, dip, flooder, in-line aqueous spray, wax and bin drench treatment. Imazalil (IMZ) and thiabendazole are the most widely used remedies in citrus postharvest decay, providing effective curative control and sporulation inhibition of *Penicillium*, but provides practically no control against sour rot. As part of a resistance management strategy, it is not recommended to include IMZ during drenching. Pyrimethanil (PYR) and thiabendazole is therefore regarded as the main components of this application. Part one of this study was conducted on drench application in terms of the influence of infection age, fruit orientation (pole), treatment exposure time and the addition of adjuvants and sanitisers on disease control. The ability of two different sanitisers (Chlorine and HPPA) to reduce sour rot inoculum in solution while maintaining fungicide concentration and residue levels for effective green mould control were compared. Incompatibility between sanitisers and fungicides was not observed in this study, with addition of both sanitisers providing effective sour rot control. HPPA is effective at a short exposure time (1 – 3 min) at the high pH used in this study (> 10), which indicates it can be incorporated with commercial drenching when pH is not regulated. Differential PYR residue loading was seen between the top and bottom bin levels during commercial packhouse trials with the upper level loading higher residue levels compared to the bottom level. This could be due to poor solution coverage associated with drenching stacked fruit bins. Part two of this study focusses on optimising the physical parameters of pH, temperature, exposure time, and their effect on chemicals used in the dip tank, and will only be completed in 2017.

Opsomming

Na-oes swamdoders kan met behulp van verskeie metodes aangewend word, naamlik voor-pakhuis stortbehandeling, dip, in-lyn spuit en stortbehandelings en in die waks. Die mees gebruikte chemikalieë vir na-oes siektebeheer is tans imazalil en thiabendasool, met goeie kuratiewe werking teen en inhibisie van *Penicillium* sporulasie, maar dit bied geen beskerming teen suurvrot nie. As deel van weerstandsbestuur word imazalil egter nie in die voorpakhuisstort aanbeveel nie. Pirimetaniel en thiabendasool word dus as die hoofkomponente van hierdie behandeling beskou. In deel een van hierdie studie is gefokus op die voorpakhuis stort behandeling in terme van infeksie ouderdom, vrug oriëntasie, blootstellingstyd en die byvoeging van benatters en saniteermiddels ten opsigte van siektebeheer. Die vermoë van twee saniteerders (Chloor en HPPA) om suurvrot inokulum in oplossing te verminder, tesame met die fungisiedkonsentrasie en residuvlakke wat nodig is vir effektiewe groenskimmel beheer, is vergelyk. Onverenigbaarheid tussen saniteermiddels en swamdoders is nie waargeneem nie, terwyl byvoeging van beide saniteermiddels suurvrot effektief beheer het. HPPA was effektief binne ŉ kort blootstellingstyd (1 – 3 min) teen hoë pH in hierdie studie (> 10), wat aandui dat dit ingesluit kan word in kommersiële stortbaddens waar die pH nie gereguleer word nie. Die PYR residue

lading in die plukkratte het gewissel van bo na onder, met die boonste kratte wat beter laai in vergelyking met die onderstes. Hierdie is waarskynlik as gevolg van swak bedekking met die oplossing in die gestapelde kratte. Deel twee van die studie fokus op optimisering van die fisiese parameters (pH, temperatuur en blootstellingstyd) en hul effek op die chemikalieë vir die dompeltenk, en sal teen 2017 voltooi wees.

3.5.5 PROGRESS REPORT: Epicuticular wax composition of CBS resistant and susceptible citrus cultivars

Project 1135 (2016/17 – 2018/19) by Wilma du Plooy (CRI), Wilma Augustyn (TUT), Catherine Savage (CRI), Charl Kotze (CRI), Providence Moyo (CRI)

Summary

Plants produce an extensive diversity of compounds known as phytochemicals, all of which are functional in the plant in some or other way. The role of phytochemicals in either eliciting or inhibiting the ability of *Phyllosticta citricarpa* (citrus black spot, CBS) to infect citrus fruit is being investigated. Cultivars with varying susceptibility to CBS are being investigated, looking at the apolar (waxes, lipids, oils) and polar (flavonoids, anthocyanidins, alkaloids, glycocides) fractions in the rind phytochemistry. Currently used cultivars include Bitter Seville, which is accepted to have low susceptibility to CBS infection, Valencia orange with medium susceptibility, and highly susceptible lemon. In addition, kumquats are included as a resistant type. The composition of the epicuticular wax layer of the different citrus cultivars over time (from fruit set until harvest) will be determined. Comparison over similar developmental periods will be attempted amongst all citrus cultivars studied. Bitter Seville is believed to contain the favourable hard C_{28} – C_{33} chains from an earlier stage of fruit development, rendering it more resistant towards citrus black spot infections. Alternatively, the non-susceptible nature of kumquats may reside in the phytochemical composition of the peel of this unique citrus.

Opsomming

Plante produseer ŉ baie uitgebreide diversiteit van verbindings bekend as fitochemikalieë, wat almal op een of ander wyse funksioneel is in die plant. Die rol wat fitochemie speel om die infeksie deur *Phyllosticta citricarpa* (sitrus swartvlek, SSV) òf aan te moedig, òf te inhibeer, word in hierdie studie ondersoek. Kultivars met verskillende vatbaarhede vir SSV word ondersoek deur bestudering van die apolêre fraksies (was, olie, lipiede) en polêre fraksies (flavonoïede, antosianiene, alkaloïede, glikosiede) in die vrugskil. Huidiglik word Bitter Seville as ŉ lae vatbare tipe, medium vatbare navel en hoogsvatbare suurlemoen tipes gebruik, terwyl kumkwat as n nie-vatbare tipe bygevoeg is. Die samestelling van die epikutikulêre waslaag van die verskillende sitrustipes tydens die verskillende ontwikkelingfases (vrugset tot volwasenheid) sal ondersoek word. Daar sal gepoog word om die vergelykings oor die ontwikkelingstydperke te maak tydens die bestudering van die verskillende kultivars. Bitter Seville het skynbaar gunstige harde C_{28} – C_{33} waskettings tydens vroeë ontwikkelingstadiums, wat dit meer bestand maak teen SSV infeksie. Alternatiewelik mag die nie-vatbare aard van kumkwatvrugte wees as gevolg van die fitochemiese samestelling van die skil van hierdie unieke sitrus.

3.5.6 PROGRESS REPORT: Studies on the management of sour rot and green mould with propiconazole

Project 1141 (2016/17 – 2018/19) by Lindokuhle C. Mamba (SU), Charles Stevens (SU), Cheryl Lennox (SU), Julia Meitz-Hopkins (SU), Wilma du Plooy (CRI), P. H. Fourie (CRI)

Summary

A new fungicide to control sour rot, caused by the ascomycete *Galactomyces citri-aurantii* is needed since guazatine, a guanidine fungicide, is being phased-out for citrus fruit exported to the EU. Propiconazole is a newly introduced postharvest fungicide that is available to the South African citrus industry. This demethylation-inhibitor (DMI) triazole fungicide is being registered for postharvest use on sour rot decay on citrus fruit in South Africa. Baseline sensitivity to propiconazole was determined for single spored South African isolates of *G. citri-aurantii* causing sour rot and *Penicillium digitatum* causing green mould. The mean effective concentration for 50% reduction of mycelial growth (EC_{50} value) was 0.313 µg/ml (range 0.004-1.087 µg/ml) for fifty-two isolates of *G. citri-aurantii*, and 0.149 µg/ml (range 0.025-0.405 µg/ml) for forty-nine *P. digitatum* isolates, respectively. Drench application of propiconazole was studied to determine the time frame (6h, 14 h, 18 h, 24 h) in which the fungicide has to be applied when fruit arrives from the orchard in the packhouse. Additionally, the exposure time (1 min, 2 min, 3 min) required to obtain adequate coverage of the fungicide applied to the fruit was evaluated. Results from inoculations of lemons with a *G. citri-aurantii* isolate indicated that propiconazole can effectively control sour rot infection when treatment is applied at 600 µg/ml within 14 h of inoculation at 1 min exposure time (flow rate approximately 500 L/min). Clementine treatments had to be applied within 6 hours of infection, on the other hand to reach full effectivity.

Opsomming

Sitrusvrugte wat uitgevoer woord na die EU benodig 'n nuwe swamdoder om suurvrot, wat veroorsaak word deur die ascomycete *Galactomyces citri – aurantii* te beheer. Dit is belangrik sedert guazatine, 'n guanidine swamdoder uitgefaseer word. Propiconasool is 'n nuwe ingevoerde, na oes-swamdoder wat beskikbaar is in die Suid-Afrikaanse sitrusbedryf. Die demetilasie-inhibeerder (DMI) triasool swamdoder word geregistreer vir na-oes gebruik op suurvrot van sitrusvrugte in Suid-Afrika. Basislyn sensitiwiteit vir propiconasool is getoets vir Suid-Afrikaanse isolate van *G. citri-aurantii*, wat suurvrot veroorsaak, of *Penicillium digitatum*, wat groenskimmel veroorsaak. Die gemiddelde effektiewe konsentrasie vir 50% vermindering van miseliumgroei (EC_{50}-waarde) was 0,313 mg / ml (reeks 0,004-1,087 mg / ml) vir *G. citri-aurantii*, en 0,149 mg / ml (reeks 0,025-0,405 mg / ml) van *P. digitatum*. Dompel toepassing van propiconasool was getoets om die tyd te bepaal (6h, 14 h, 18 h, 24 h) waarin die swamdoder moet toegedien word wanneer vrugte uit die boord in die pakhuis kom. Verder is die blootstellingtyd (1 min, 2 min, 3 min) wat nodig is, om goeie bedekking van die swamdoder op die vrugte te kry, getoets. Toetse in suurlemoene met 'n *G. citri-aurantii* isolaat, het aangedui dat propiconasool suurvrot infeksie effektief kan beheer, wanneer behandeling toegepas word by 600 mg / ml vir 1 min binne 14 uur na inokulering (vloeitempo ongeveer 500 L / min). Toetse in clementines het aangedui dat behandeling binne 6 ure toegepas word.

3.5.7 PROGRESS REPORT: Fungal degradation of wood pallets used in export of citrus fruit

Project 1165 (2016/17 – 2018/19) by Wilma du Plooy (CRI), Catherine Savage (CRI) and Jan van Niekerk (CRI)

Summary

Recent fungal contamination on wooden pallet bases used for export highlighted a problem of which the source is apparently unknown. This degradation of pallet bases when the consignment reaches its market has raised questions on the quality of the wood used in the manufacturing of these bases. Further concerns were the contribution of packhouse storage methods to the fungal contamination of the bases, and the possible role of environmental factors (for instance moisture, UV degradation, and insect infestation). However, it is fungal decay that is currently the hazard with the highest priority. Heavily contaminated pallets are a point source of fungal spore dissemination that poses a deterioration risk to the boxes stacked on these pallets, will increase the spore load in cold rooms, and presents a phytosanitory concern. This study looks at the microbiome on contaminated pallet bases, manufacturing aspects of the bases, the role of storage at packhouses, options for wood treatment, as well as possible contribution from the shipping containers to the degradation of wooden pallet bases.

Opsomming

Onlangse swamkontaminasie op die houtbasisse van sitruspallette het ŉ probleem uitgelig waar die oorsaak daarvan onbekend is. Hierdie degradering van die basisse wanneer besendings op die oorsese market aankom het vrae laat ontstaan rondom die kwaliteit van die hout wat gebruik word. Daar het bekommernis geheers oor die bydrae van die pakhuis tot swak palette as gevolg van opberging, asook watter omgewingsfaktore ŉ rol sou speel (vog, UV afbreking en insekbesmetting). Dis egter die swamverrotting wat tans die hoogste prioriteit geniet. Swaar besmette basisse dra die risiko dat dit kartonne in die pallet ook mag besmet, dra by tot die verhoging van die spoorlading in opbergingskamers en is ŉ fitosanitêre risiko. Hierdie studie fokus dus op die mikrobioom van besmette houtbasisse, vervaardigingsaspekte van die houtbasisse, die rol wat pakhuisopberging speel, verskillende opsies vir houtbehandeling, asook die moontlike bydrae van behoueringseenhede tot die degradering van die houtbasisse.

3.5.8 PROGRESS REPORT: Application of nanotechnology to decrease the volatility of effective essential oils in different applications against citrus postharvest fungi

Project PHI 66 (1 January 2015 to 30 December 2017) by Sandra Combrinck (TUT), Thierry Regnier (TUT), Wilma Augustyn (TUT), and Wilma Du Plooy (CRI)

Summary

This project is aimed at the development of slow-release essential oil (EO) encapsulated in nanoparticles. Essential oils are known to protect against various postharvest fungal decay pathogens. The EO materials will be tested in various forms including in coatings, aqueous environments and in headspace. For headspace analysis, an accurate and reliable method must be developed to measure the extent of volatilisation and therefore the degree of protection offered by active metabolites in the EO under storage conditions for fruit. A gas chromatograph was designed for the sampling of headspace and has since been constructed. Two particle

types have been synthesized and partially characterized and *in vitro* trials confirming the efficacy of some oils, have been completed. Although an *in vivo* trial at CRI to confirm the inhibitory effects of two commercial essential oils was unsuccessful due to the inoculation technique selected, a reduction in the size of lesions was observed compared to the controls. Good progress was made with a study aimed at identifying other essential oils with good activity against green and blue moulds, as well as against sour rot. Chemometric analysis of an extensive dataset generated by gas chromatography-mass spectrometry is currently underway.

Opsomming

Hierdie studie is op die ontwikkeling van nanopartikels wat essensiële olies enkapsuleer en stadig vrystel gemik. Dit is reeds bekend dat sommige van hierdie olies beskerming teen nadelige na-oes swampatogene bied. Die EOs sal getoets word in verskeie vorms wat insluit bedekkings, waterige matrikse en in bodamp vorm. ʼn Akkurate en betroubare bodamp metode moet ontwikkel word om die mate van vervlugtiging en dus die graad van beskerming te bepaal wat deur aktiewe metaboliete in die essensiële olie gebied word. Hierdie metode sal toegepas word onder toestande waaronder vrugte geberg word. ʼn Gas chromatograaf is ontwerp vir bodamp analise en is reeds gebou. Twee tipes partikels is sinteties berei en gedeeltelik gekarakteriseer. *In vitro* studies wat die effektiwiteit van die olies bevestig is reeds afgehandel. Hoewel ʼn *in vivo* studie by CRI, wat gedoen is om die swaminhibisie aktiwiteit van twee kommersiele EOs te bepaal, groetendeels onsuksesvol was as gevolg van die inokulasietegniek wat gebruik is, was die grootte van die letsels tog kleiner teenoor die van die negatiewe kontroles. Goeie vordering is gemaak metʼn studie wat daarop gemik is om ander essensiele olies met goeie aktiwiteit teen groen en blou skimmel en suurvrot te identifiseer. Chemometriese analise van die uitgebreide datastel wat met behulp van gas chromatografie-massa spectrometrie bekom is, word tans uitgevoer.

3.6 **CRI DIAGNOSTIC CENTRE** (Elaine Basson, Charmaine Olivier, Aubrey Metane, Samuel Ndlovu, and Jan van Niekerk)

Analysis	Citrus nurseries	Commercial samples	Other crops	Research samples
Nematode:Roots	13	286	13	1417
Nematode:Soil	5	6	15	1653
Phytophthora	5485[1]	354	66	1662
Water spore trap	155	2	4	0
Black spot identification (PCR)	0	132	0	9
Black spot benzimidazole resistance	0	23	0	8
Citrus greening (PCR)	0	3	0	0
Post Harvest Resistance	0	47	0	64
Fruit & Foliar identification	0	22	5	16
Soil dilution plating	0	2	4	0
Internal Fruit Quality	0	1	0	0
SUB-TOTALS	**5658**	**878**	**107**	**4829**

[1] Total samples received for citrus nurseries – includes quarterly samples, re-tests and non-certified nurseries

Citrus Certified 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, 4356[2] nursery samples were received by the diagnostic centre for *Phytophthora* analyses. Of these samples, 4.82% 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% tested positive.

Commercial samples

Samples were received from the following citrus growing areas: Eastern Cape, Gauteng, Limpopo, Mpumalanga, Northern Cape, North West, and Western Cape. Most of the samples received from citrus growers were analysed for *Phytophthora nicotianae* and the citrus nematode, *T. semipenetrans*. Thirty-two percent of the 286 samples analysed for citrus nematode had counts above the threshold value of 1000 females per 10g of roots, and nematicide treatments were recommended. Fifty-three percent of the 354 samples analysed for *Phytophthora* tested positive.

Other crops

Nematode counts were done on soil or root samples of Banana, Butternut, Dragon Fruit, Granadilla, Kiwi, Macadamia, Maize, Stevia and Stonefruit. Nematodes found present on these crops included: *Scutellonema, Meloidogyne, Pratylenchus, Tylenchus, Criconema, Aphelenchus, Xiphinema, Hoplolaimus, Helicotylenchulus,* and *Hemicycliophora. Phytophthora* and *Pythium* analyses were done on Butternut, Compost, Dragon Fruit, Granadilla, Kiwi and Macadamia. The diagnostic centre analysed 26 soil samples from macadamia nurseries for the presence of *Phytophthora cinnamomi*.

Research samples

Nematode and *Phytophthora* analysis were done on 4732 samples from experimental trials.

Footnote:
[2] Sample number and the percentage positive are only for certified nurseries and only for the quarterly samples received.

CRI DIAGNOSTIESE SENTRUM (Elaine Basson, Charmaine Olivier, Aubrey Metane, Samuel Ndlovu, and Jan van Niekerk)

Ontleding	**Sitrus kwekerye**	**Kommersiële monsters**	**Ander gewasse**	**Navorsings-monsters**
Aalwurms: Wortels	13	286	13	1417
Aalwurms: Grond	5	6	15	1653
Phytophthora	5485[1]	354	66	1662
Water spoorlokval	155	2	4	0
Swartvlek (PKR)	0	132	0	9
Swartvlek benzimidazole bestandheid	0	23	0	8
Sitrusvergroeningsiekte (PKR)	0	3	0	0
Na-oes bestandheid (Imazalil)	0	47	0	64
Vrug- en blaar identifikasie	0	22	5	16
Grondverdunningsplate	0	2	4	0
Interne vrugkwaliteit	0	1	0	0
TOTAAL	**5658**	**878**	**107**	**4829**

[1] Totale hoeveelheid monsters ontvang van gesertifiseerde kwekerye – sluit in kwartaal monsters, hertoets monsters en nie-gesertifiseerde kwekerye

Sitrus Gesertifiseerde Kwekerye

Dit is verpligtend vir al die sitruskwekerye wat aan die Sitrus Verbeteringskema deelneem om kwartaalikse monsters vir *Phytophthora* te laat ontleed. Die besproeiingswater moet ook deur middel van die spoorlokval metode vir *Phytophthora* getoets word. In totaal 4356[2] monsters is deur die diagnostiese sentrum vir *Phytophthora* ontleding ontvang, waarvan 4.82% positief getoets het. Benewens die water en grondmonsters, moet kwekerye een keer per jaar ŉ wortelmonster instuur om vir die teenwoordigheid van *Tylenchulus semipenetrans* te toets. Van die wortelmonsters wat ontvang is, het 0.0% positief vir die teenwoordigheid van *T. penetrans* getoets en van die grondmonsters het 0.0% positief getoets.

Kommersiële monsters

Monsters is uit die volgende sitrusverbouingsareas ontvang: Oos-Kaap, Gauteng, Limpopo, Mpumalanga, Noord-Kaap, Noord-Wes, en Wes-Kaap. Die meeste van die monsters wat van sitrusprodusente ontvang is, is vir *Phytophthora nicotianae* en die sitrusaalwurm, *Tylenchulus semipenetrans*, ontleed. Twee-en-dertig persent van die 286 aalwurmmonsters wat ontleed is, het tellings hoër as die drempelwaarde van 1000 wyfies per 10g wortels gehad. Aalwurmdoderbehandelings is in daardie gevalle aanbeveel. Drie-en-vyftig persent van die 354 monsters wat vir *Phytophthora* ontleed is het positief getoets.

Ander Gewasse

Aalwurmtellings is op grond- of wortelmonsters van Piesangs, Butternut, Dragon Fruit, Granadilla, Kiwi, Macadamia, Mielies, Stevia en Steenvrugte gedoen. Aalwurms teenwoordig gevind op hierdie gewasse sluit in: *Scutellonema, Meloidogyne, Pratylenchus, Tylenchus, Criconema, Aphelenchus, Xiphinema, Hoplolaimus, Helicotylenchulus,* en *Hemicycliophora.* Butternut, Kompos, Dragon Fruit, Granadillas, Kiwi en Makadamias monsters is vir *Phytophthora* en *Pythium* ontleed. Die diagnostiese sentrum het 26 monsters vanaf makadamia kwekerye ontvang om vir *Phytophthora cinnamomi* te ontleed.

Navorsingsmonsters

Aalwurm en *Phytophthora* ontledings is op 4732 monsters afkomstig uit navorsingsprojekte gedoen.

Voetnota:
[2] Monster hoeveelheid en die persentasie positief is net vir gesertifiseerde kwekerye en slegs vir die kwartaal monsters ontvang.

4 PORTFOLIO: CITRICULTURE

4.1 PORTFOLIO SUMMARY

By Paul Cronjé (CRI-SU)

The overall aim of the portfolio is to ensure the sustainable and profitable production and export of citrus fruit and all projects should support this. Research in the Citriculture portfolio included ongoing culitvar and rootstock evaluation, evaluation of strategies to improve yield and fruit quality with water, nutrition and tree management actions. In addition, aspects that can impact on the fruit quality during the postharvest handling and cold chain was addressed. During the previous season, the culitvar team continued to evaluate promising cultivars per production area in order to serve as support for growers' decisions on orchard establishment or replacement and comprehensive details are supplied in the report. In the research project on alternative bearing significant progress has been made in understanding the underlying control of this physiological phenomena, which can reduce suitable production. By altering management actions in the orchards such as pruning or thinning or employing technologies such as covering the trees with shade netting, fruit quality and yield could be improved. Research into irrigation in collaboration with the Water Research Commission (WRC) will draw to a close during the 2017 and a full conclusive report will be available in 2018 as well as projects onto the contribution of various organic materials to soil N and K. The results from the first season of the shade netting experiments indicate a positive impact on fruit size and yield and no negative affect on rind colour development resulting in a higher export volume. These results will be followed up with more information form the second season in (2017). The negative impact of delaying the period between harvest and packing, and therefore rind dehydration, has been illustrated in Valencia orange and late mandarin fruit. In addition, the efficacy of thiabendazole applied as soon as possible after harvest has been illustrated. The application of late N (Mar/Apr) has been found to not increase rind disorders or delay in colour development if the orchard is not over irrigated. In order to identify susceptibility to rind disorder VIS/NIR technology could potentially be used as it can identify fruit according to canopy position, know to influence susceptibility to disorders. The cold chain of the SA citrus industry is complex and all stages in this process must receive adequate in-depth research attention such as precooling in order to ensure export of high quality fruit to cold sterilisation and conventional markets.

PORTEFEULJE OPSOMMING

Die doelwit in hierdie portefeulje in aspekte wat betrekking het op sitrusproduksie, wat ondersteun moet word in alle projekte, is om tot konstante en finansieel sinvolle opbrengs en uitvoer by te dra. Die navorsing in die portefeulje sluit deurlopende cultivar en onderstam evaluasies asook navorsing om die vrug opbrengs en kwaliteit te verhoog deur water, voeding en boom bestuur in. Daarby word aspekte wat negatief op die vrugkwaliteit kan inwerk in die na-oes koueketting aangesprek. Gedurende die voringe seisoen het die culitvar evaluasie span voortgegaan om belowende cultivars te evalueer per area om inligting beskikbaar te stel vir produsente vir gebruik in besluite tydens boord vestiging. In die navorsing projek op alternatiewe drag is betekenisvolle vordering gemaak in die ontrafeling van die onderliggende meganisme wat die komplekse fisiologiese proses beheer en wat konstante opbrengste nadelig beïnvloed. Deur bestuuraksies in die boord te verander soos snoei en uitdun of deur die gebruik van nuwe tegnologie soos skadunette kan vrug kwaliteit en opberging verbeter word. Die navorsing wat gedoen was in samewerking met die Water Navorsing Kommissie (WNK) kom tot ŉ einde in 2017 en die volle verslae sal in 2018 beskikbaar wees soos ook die resultate in die projekte wat gefokus het op die invloed van verskillende organies materiale op grond N en K. Daar is aangetoon dat daar n negatiewe impak is indien daar ŉ lang periode tussen pluk en pak voorkom wat die vrugte dehidreer en wat lei tot meer gepokte skil in Valencia en mandaryn vrugte. Om daarby aan te sluit is die positiewe impak van thiabendazole wat so spoedig moontlik na oes toegedien moet word bevestig. Laat N toeding (Mrt/Apr) het oor die twee jaar projek nie enige negatiewe impak op skilkleur of voorkoms van defekte gemaak nie, maar waar oor besproeiing voorgekom het was die skilkleur nadelig beïnvloed. ŉ Langtermyn doelwit in die portefeulje bly om vrugte wat sensitief is vir skildefekte te identifiseer in die pakhuis en deur die gebruik van VIS/NIR blyk dit of posisie in die blaardak voorspel kan word wat ŉ verband toon met sensitiwiteit. Die kompleksiteit van die SA sitrus koueketting vereis in diepte navorsing oor aspekte soos voorverkoeling om

te verseker vrugte van hoe kwaliteit kan uitgevoer word na konvensioneel asook koue sterilisasie protokol markte.

4.2 PROGRAMME: RIND CONDITION

Programme coordinator: Paul Cronjé (CRI-SU)

4.2.1 Programme summary

The research focus in this program remain two-fold: firstly, to identify factors influence the rind sensitivity to a disorder and secondly postharvest strategies and technologies to reduce the impact of disorders. In research on non-chilling pitting of 'Valencia' orange significant diffidence in susceptibility between cultivars, production units and season was found (4.2.3). Canopy position and over maturity seems to be less influential in determining sensitivity. However, a delay between harvest and packing of fruit as well as excluding TBZ significantly increase pitting incidence. Pitting in 'nadorcott' mandarin was also linked to postharvest dehydration however additional application of nitrogen during phase II did not result in a reduction in colour development or increase in susceptibility to pitting. Culitvar choice significantly influence susceptibly to chilling injury of mandarin fruit and M37 as well as I22 was identified as highly chilling susceptible (4.2.5). By using Vis/NIR non-destructive methods was tested to predict the quality of citrus fruit. The results obtained in this study confirmed the role that pre-harvest conditions, notably canopy position, plays in maintaining the rind and internal quality of mandarin, orange and grapefruit. The lack of sunlight lead to higher chilling injury incidence of inside fruit, which was successfully predicted (4.2.4). The ability of Vis/NIRS coupled with chemometric analysis to cluster fruit based on original canopy position could be a possible technology to discriminate fruit, during sorting and packaging, with high chances of developing disorder. In a project aimed at the negative impact of gamma irradiation on citrus fruit quality it was determined that most cultivars are commercially unaffected in terms of rind condition below 300Gray (4.2.2). However, the exception being 'nova' mandarin and 'Turkey' Valencia fruit. Internal quality of the fruit was not negatively affected at these levels, which indicate a potential viable commercial technology if used at maximum dose of below 300 Gray. The advances in this program will continue to be important for the industry as higher volumes of export fruit will result in a higher demand for fruit without any rind defects.

Programopsomming

Die fokus in die program bly tweeledig. Eerstens om faktore te identifiseer wat 'n vrug se sensitiwiteit t.o.v. skildefekte kan beïnvloed en tweedens om tegnologie te ontwikkel om die ontwikkeling van defekte te beperk. Die projek op gepokteskil van 'Valencia' lemoene het getoon daar is betekenisvolle verskille in sensitiwiteit tussen cultivars, produksie eenhede asook seisoene (4.2.3). Die posisie in die boom asook die laat oes van vrugte was minder van 'n faktor. Daarteen oor het 'n vertraging in tyd tussen pluk en verpakking asook die weglaat van TBZ tot betekenisvol meer pitting gelei. In 'nadorcott' mandaryn vrugte was dehydrasie na-oes ook verbind met meer gepokte skil maar die plasing van laat stikstof gedurende groeifase II het nie die skilkwaliteit benadeel in kleurontwikkeling of voorkoms van defekte nie. Die was duidelik dat culitvar keuse die sensitiewe vir koueskade betekenisvol beïnvloed en die M37 en I22 seleksies was uiters gevoelig (4.2.5). Deur gebruik te maak van Vis/NIR as 'n nie destruktiewe metode om vrugkwaliteit te toets kom vrugte geskei word volgens dra-posisie, wat koueskade sensitiwiteit beïnvloed (4.2.4). Die vermoë van Vis/NIR gekoppeld met chemometriese analises om vrugte in die paklyn te klassifiseer na gelang van posisie in die boom kan help om vrugte van 'n hoër sensitiwiteit te identifiseer. Die projek waar die negatiewe impak van gama-bestraling op sitrus vrugkwaliteit getoets is, het gevind dat die meeste cultivars bestraling onder 300 Gray kan weerstaan (4.2.2). Dit sluit egter 'Turkey' Valencia en 'nova' mandaryn uit. Die interne kwaliteit was nie negatief geaffekteer nie wat 'n aanduiding is dat hierdie lae vlakke van bestraling wel 'n potensiële kommersiële behandeling kan wees. Gedurende vordering in die program is nodig, omrede die verwagte verhoogde uitvoer volumes al meer gaan lei tot 'n fokus op vrugte sonder enige skildefekte.

4.2.2 FINAL REPORT: Effect of irradiation levels on internal and external citrus fruit quality

Project 1124/PHI-63 (April 2015 – March 2017) by Paul Cronje, Jade North, Vaughan Hattingh (CRI) and Cherin Balt (Hepro)

Summary

Irradiation of citrus fruit to sterilize insect larvae such as false codling moth and various fruit fly species could develop into a technology that would enable the reduction of the cold sterilisation protocol. It has been determined that the minimum level of irradiation to sterilize FCM larvae is 60 Gy (+ 16 days at 2.5°C) or a stand-alone treatment of 100 Gy. Due to the complexity of administering a specific target dose to all fruit in a carton or pallet various problems would need to be addressed. The distribution of dosage through a package of fruit (box or pallet) is not uniform. An irradiation treatment specifies the minimum dosage required and

inevitably many fruit in the treated package will therefore be exposed to higher dosages, generally in the order of 2 to 3 times the minimum required dosage, but this could be as high as 4 times. The min-max ratio is dependent on many factors, including source of irradiation, installation, packaging and fruit type. Earlier exploratory trials indicated that citrus fruit is sensitive to irradiation dosages in the range of 200 to 500 Gy. In this experiment 9 cultivars were exposed to 200, 300, 400 or 500 Gy and placed in cold storage at either 2 or 7°C for durations of 40 or 60 days prior to evaluation of external and internal quality. There were significant differences in responses between cultivars, seasons as well as between early and late cultivars of the same group. In general, and at this stage irradiation at 300 Gy seem to be the maximum level that most cultivars could tolerate. However, a lower irradiation dose as close to 200 Gy as possible could result in the inclusion of nearly all cultivars due to the reduction in rind disorders of high value cultivars such as lemons and most mandarins.

Opsomming

Na-oes bestraling van sitrusvrugte, om inseklarwes daarin soos die vals kodling mot (FCM) asook vrugtevlieg spesie te sterliseer, kan ontwikkel word in ʼn tegnologie en daardeur ʼn verlaging in die koue-sterilisasie protokol te kry. Dit was vasgestel dat die minimum bestralingsdosis om ʼn FCM larwe te sterliseer 60 Gy plus 16 dae by 2.5°C is of ʼn bestraling van 100 Gy sonder koelopberging. As gevolg van verskeie komplekse aspekte om die verlangende dosis toe te dien in ʼn karton of op pallet basis moet seker probleem opgelos word. Eerstes is die verspreiding van die dosis deur ʼn pallet nie uniform nie. Oor die algemeen spesifiseer bestralings dosis die minimum waaraan alle vrugte blootgestel moet word en dit kan daarna lei dat ander vrugte in die pallet/karton teen heelwat hoër dosis (2 tot 3 en soms tot 4 keer) bestraal word. Hierdie min-mak ratio is afhanklik van verskeie faktore soos die bestralings aanleg, karton asook vrugtipe. Voorlopige proewe het getoon sitrus vrugte sensitief is tussen 200 - 500 Gy. In hierdie eksperimente oor twee jaar was 9 kultivars aan bestraling van 200, 300, 400 en 500 Gy blootgestel voor opberging teen of 2° C of 7° C vir 40 of 60 dae waarna vrugte geëvalueer was vir skil en interne kwaliteit. Betekenisvolle verskille was gevind tussen cultivars, seisoene asook tussen vroe en laat oes datums van dieselfde culitvar. In die algemeen blyk 300 Gy die maksimum bestralings vlak wat die vrugte kan hanteer. Dit wil egter blyk dat deur die gebruik van n lae maksimum dosis van 200 Gy byna alle cultivars in so ʼn behandeling ingesluit kan word sonder om die te lei tot skildefekte in hoe waarde vrugte soos cultivars en mandaryne.

Introduction

The occurrence of insect larvae in citrus fruit can restrict fruit movement between exporting and importing countries due to the possibility of quarantine pests establishing a population in the importing country. Efforts to minimize and control infestation include establishing fly-free zones, chemical control (fumigation), cold and heat treatments, and irradiation (Ladaniya, 2008). Ionizing irradiation is effective in the disruption of deoxyribonucleic acid (DNA) molecules in the cell of an organism leading to the inability of the cell to replicate and resulting in sterilization or death (Diehl, 1995). The United States Animal and Plant Health Inspection Service (APHIS) has approved a generic dose of 150 Gy for Tephritidae and 400 Gy for all insects except pupae and adults of Lepidoptera (Follett, 2009 and APHIS, 2012). There is, however, an ongoing international effort to develop additional generic phytosanitary irradiation treatments and reduce the generic dose of 400 Gy (Lacroix and Follett, 2015).

To overcome constraints on ionizing radiation and cold treatment as stand-alone disinfestation treatments for False Codling Moth *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) in citrus fruit, the efficacy of a combination treatment consisting of ionizing radiation and cold, both at reduced doses, was tested at the probit-9 level. Earlier research had indicated that combination treatments involving ionizing radiation and cold, suppressed larvae and their successive development stages more effectively than the individual constituents (Hofmeyr *et al.,* 2016). The probit-9 level efficacy (at least 93 613 insects) of a treatment combination comprising 60 Gy of ionizing radiation followed by cold exposure for 16 d at 2.5 °C was evaluated. A total of 104 617 mature, 5th instar larvae were treated. Larval mortality was 99.7% and 50.2% of the subsequent pupae died. A total of 143 moths with a gender ratio of 1 female to 7.9 males survived, however, only 4.8% of the moths were able to fly. No eggs were produced by the moths in mating studies. The probit-9 level efficacy of the combination treatment was validated, indicating that it meets the efficacy requirements for utilization as a phytosanitary disinfestation treatment for *T. leucotreta* in citrus fruit in international trade (Hofmeyr *et al*., 2016ab).

Irradiation would not only affect the insect but also the fruit cellular metabolism, and at too high dose levels would be detrimental. The effect of irradiation on the quality of citrus fruit depends upon various factors such as dose, fruit maturity and cultivar (Miller *et al*., 2000; Bustos and Mendieta, 1988; Monselise and Kahan, 1966; Ahmed *et al*.,1966 and Belli-Donini *et al*., 1974). Miller *et al.* (2000) evaluated the effect of phytosanitary doses up to 450 Gy on various cultivars of oranges (*Citrus sinensis* (L.) Osbeck), including 'Washington' navel.

They observed that irradiation even at 150 Gy caused rind pitting in the 'Washington' and 'Hamlin' varieties but firmness, weight loss, juice colour, juice flavour and pulp flavour were not affected. The same study also found 'Ambersweet', 'Valencia' oranges and 'Minneola' and 'Murcott' mandarins to have good tolerance up to doses of 500–600 Gy. Storage time and conditions can also impact sensitivity of fruit to irradiation damage. O'Mahony *et al.* (1985) reported that navel oranges irradiated at 600–800 Gy were more blemished as compared to untreated oranges 5–6 weeks after treatment. In general McDonald *et al.*, (2013) found that the primary effect of irradiation on quality was external damage (pitting and visual damage) of navel oranges treated at 400 Gy and higher and that the damage observed was fairly extensive. By the end of the storage period it was judged that more that 50% of the fruit at 400 and 600 Gy sustained moderate damage or greater

In the South African citrus industry context, it is important to ascertain the impact of irradiation on the various citrus cultivars to enable further development of this technology on citrus. In addition, the effect of a combination treatment of lower irradiation dose plus a reduced cold sterilisation protocol (2°C for 18 days) is also unknown from a fruit quality perspective. An irradiation treatment specifies the minimum dosage required and inevitably many fruit in the treated package will therefore be exposed to higher dosages, generally in the order of 2 to 3 times the minimum required dosage, but this could be as high as 4 times as the distribution of dosage through a package of fruit (carton or pallet) is not uniform. The min-max ratio is dependent on many factors, including source of irradiation, installation, packaging and fruit type. Earlier exploratory trials using complete pallets to irradiate, indicated that South African citrus fruit is sensitive to irradiation dosages in the range of 200 to 500 Gy.

Objectives

It is important for all major citrus fruit types exported by SA producers that a maximum dose tolerance (D_{max}) is determined. The need for this value is important as it would determine whether irradiating a specific cultivar group at a dosage that will be determined by the importing country is actually viable and will not lead to fruit quality losses. The study was planned to determine the effect of irradiation on both the internal and external (rind condition) quality of citrus fruit, with the aim of establishing thresholds for citrus fruit tolerance of irradiation dosages and cold storage treatments. During this two season project the same dosages and evaluation parameters were used.

Materials and methods

Fruit: The fruit used in the experiment were of export quality and were packed in a commercial facility applying the recommended postharvest protection chemicals *viz*. thiabendazole, 500 $mg \cdot L^{-1}$; imazalil, 500 $mg \cdot L^{-1}$; 2,4-dichlorophenoxyacetic acid, 125 $mg \cdot L^{-1}$, and polyethylene citrus wax application, before packing it into recommended citrus cartons. Over the two seasons nine cultivars were used in the experiment:

Season 1

- Irradiation 1 – 'Washington Navel' orange [*Citrus sinensis* (L) Osbeck], 'nules Clementine' mandarin (*C. reticulata* Blanco), 'Eureka' lemon (*C. limon* (L.) Burm.) and 'Star Ruby' grapefruit (*C. paradisi* Macf.).

- Irradiation 2 – 'navelate Navel' orange, 'nadorcott' mandarin [*Citrus sinensis* (L) Osbeck], 'Eureka' lemon and 'Turkey Valencia' orange [*C. sinensis* (L) Osbeck].

Season 2

- Irradiation 1 – 'Washington Navel' orange, 'nules Clementine' mandarin, 'Eureka' lemon and Nova mandarin [*C. reticulata* Blanco].

- Irradiation2 – 'navelate Navel' orange, 'nadorcott' mandarin, lemons and 'Midknight Valencia' orange [*C. sinensis* (L) Osbeck].

For each cultivar, a pallet consisting of 60-80 cartons of fruit falling into 2-3 consecutive fruit size classes was delivered to the irradiation facility and stored at a constant 20°C until irradiation. Thereafter pallets were broken down and cartons labelled, allocated and stacked according to irradiation treatments (n=6).

Irradiation treatments and Dosimetry: All irradiation treatments were administered at the commercial facility of Hepro Cape (Pty) Ltd with a cobalt irradiator in Montague Gardens, Cape Town. This facility is USDA accredited for fruit irradiation.

Before treatments ensued, the ***exposure time*** and ***dose distribution mapping*** per cultivar/carton combination was determined in order to verify the reference position to place the dosimeter in each carton during the treatments. For the dose mapping, seven (7) dosimeters per carton were used and placed inside

the carton wall next to the fruit on each of the six sides (top, bottom, long-side right, long-side left, short side-front and short side-back), as well as in the middle of the carton of fruit. For each cultivar and for each irradiation run, the dose mapping was repeated to calculate exposure time as this differs over time due to the decay of the cobalt source. From the dose mapping values, the two reference points i.e. highest and lowest values in the two carton stack, were subsequently used in all treatment runs to verify the D_{min}:D_{max} for each carton and cultivar combination by placing four dosimeters per stack in these positions (two in the top carton and two in the bottom).

The target dosages were 0, 200, 300, 400 and 500 Gy and the fruit were irradiated at carton level. For each irradiation run, four cultivars were irradiated simultaneously by fixing two cartons per cultivar on a designated turntable. Turntables rotated for the treatment period to ensure all four sides received equal exposure to the source. After treatment the dosimeters were removed and read to determine the actual irradiation dose, whereas cartons were returned to the cold room immediately. Treated fruit were transported in a closed truck to the fruit maturity laboratory at the department of Horticultural Science, University Stellenbosch.

***Cold storage treatments*:** Upon arrival at the laboratory, 10 fruit per carton were removed to determine the initial internal (juice %, total soluble solids, expressed as °Brix, as well as citric, malic and tartaric acid content) and external quality (disorders and colour). Thereafter, half of the fruit in each carton was marked to enable unbiased selection after 40 and 60-day cold storage. Three cold storage regimes were used:

1. Commercial control: 22 days at -0.6°C, plus rest of storage at 7°C.
2. Combination treatment: 18 days at +2°C plus rest of storage at 7°C.
3. Experimental control (effect of irradiation without treatment): whole period at 7°C.

Quality measurement

At the three evaluation intervals i.e. day 0, 40 and 60, the same set of quality measurements were done and included the following:

Rind disorder incidence: Visual inspection and classification of various rind disorders particular to the cultivar was done according to a severity scale of 0-3 scale (Figure 4.2.2.1). The rating values were used to calculate the rind disorder index (RDI) according to the following formula:

$$\text{RDI} = \sum \frac{[\text{Rind disorder scale } (0-3) \text{ x number of fruit within each category}]}{\text{Total number of fruit in replicate } (n=10)}$$

Colour measurement: Visual rind colour was assessed using CRI colour charts for oranges (No.34), soft citrus (No. 36) and lemons (No.37), Citrus Research International (CRI).

Internal quality: Internal quality was determined by cutting fruit in half on the equatorial line, where after the flesh was juiced using a citrus juicer (Sunkist®, Chicago, USA). The juice was strained through a muslin cloth to remove any solid particles. Juice percentage was calculated by dividing the weight of the juice by the total weight of the fruit. Total Soluble Solid (TSS) content of the juice was measured as °Brix using an electronic refractometer (PR-32 Palette, Atago Co, Tokyo, Japan) and Citric Acid (%) as a measurement of acidity was determined by titrating 20 ml of sieved juice (Titrando 888 and 815 Robotic USB Sample Processor XL, Metrohm Ltd., Herisau, Switzerland).

Taste: A consumer panel, consisting of 10 panelists selected from the citrus industry, was used to evaluate the sensory quality of fruit. Each consumer received a taste plate consisting of 2 replicates per treatment at one time. A total of four replicates per treatment were tasted (a total of 44 consistent fruit blocks per cultivar). A 1-9 hedonic scale was used to evaluate the samples in which a score of 1 represented extremely liked and 9 represented extremely disliked. This was applied to the following fruit attributes; aroma, colour, texture, taste and juiciness. The sensory evaluations were conducted at room temperature under normal laboratory light conditions. Unsalted crackers and still water was available to panelists throughout the tasting.

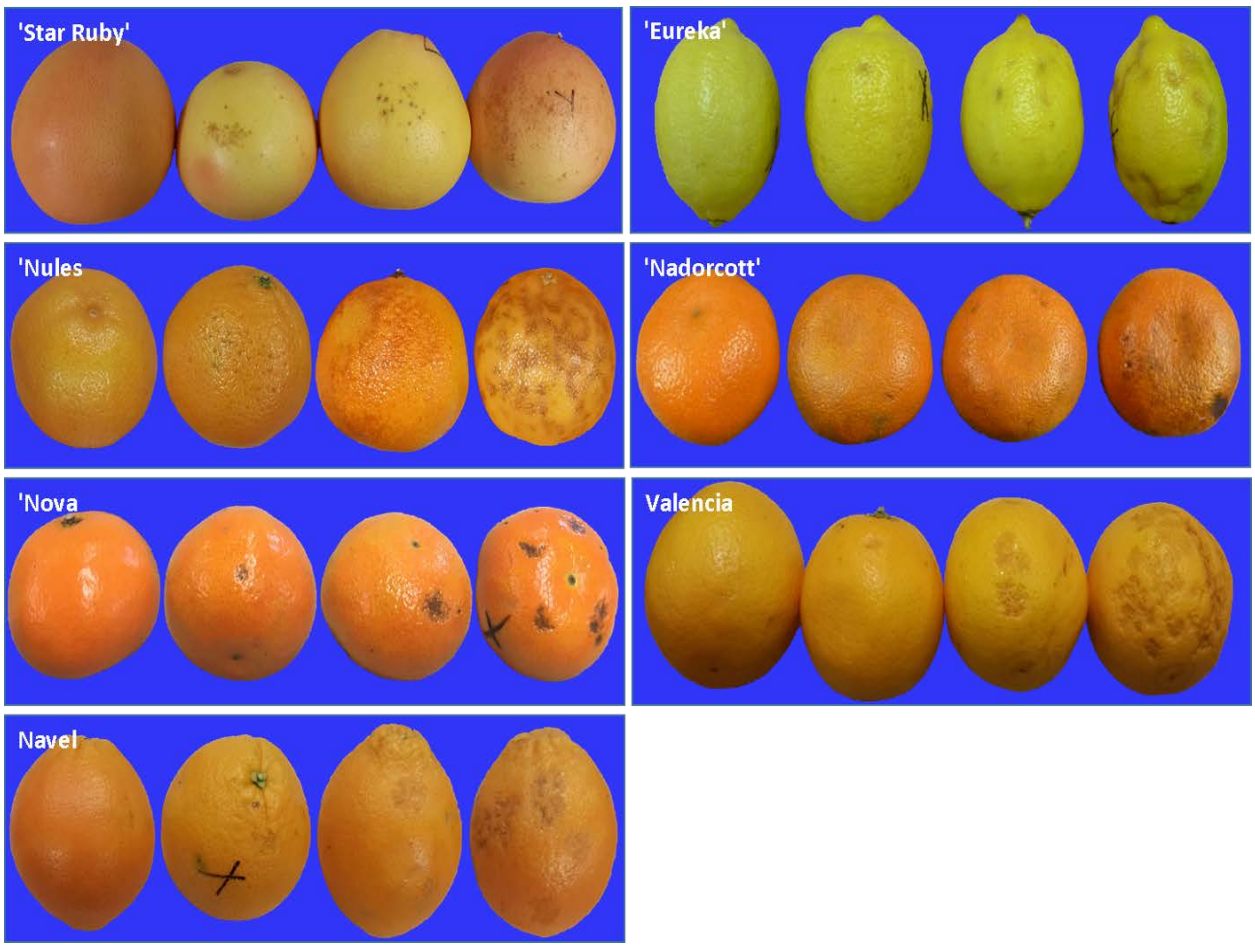

Figure 4.2.2.1. *Severity scale of 0-3 for each fruit group*

Statistical lay out and analysis: For each of these irradiation doses (5) x cold storage treatments (3), six cartons were used as replicates (n=6) per combination treatment, i.e. 300 Gy + 18 days at 2°C. The cartons were randomly allocated to these treatment combinations prior to treatment application. Fruit were stored for 40 and 60 days to simulate commercial handling, shipping and storage duration. The fruit quality data (external and internal) were analyzed with Statistic 64 as a two factorial ANOVA. Cultivars were not statistically compared. Means of treatments were separated by Fisher's least significant difference (LSD; p = 0.05). A p-value <0.05 is interpreted as a significant difference between treatments.

Results and discussion

The dosimeter data confirmed that a gradual increase from 200 to 500 Gy in irradiation levels in order to see a dose response was obtained over the two seasons. In addition, the variation within the two cartons irradiated per cultivar per run was between 1 and 1.3 on average for all treatments, indication low treatment variation in each replicate (Table 4.2.2.1). A summary of the D_{Max}:D_{Min} ratio measured in the different cultivars indicates that in all treatments except two (Early 'Eureka' @ 300 Gy and 'nules' @ 300 Gy) the average dose was above target. The ratio and average values were within the acceptable levels for irradiation of fruit and the data could be interpreted as to determine maximum dose.

The external fruit quality, evaluated as incidence of rind disorders and grouped as "pitting", indicates a progressive reaction to the increased irradiation dose. This was especially true for sensitive cultivars such as 'Turkey Valencia', 'nova' mandarin, 'Eureka' lemon and to a lesser extent 'nadorcott' mandarin and Late navel orange (Navelate) (Figures 4.2.2.2-5). In the two cultivars most affected by irradiation, the development of rind disorders was already evident at 200 Gy ('nova' and 'Turkey'), but for the second group of cultivars i.e. lemon, 'nadorcott' and 'nules', the increased negative impact only become significant after 300 Gy. Some cultivars such as the 'Midknight' Valencia and 'Star Ruby' grapefruit show a remarkable tolerance to irradiation even at 500 Gy.

Figure 4.2.2.2. Additional examples of rind disorders: **A**) 'Star Ruby' rind with a deep red pigmentation showing less damage than pale yellow rind **B**) Grey bands seen on equatorial aspect of lemons **C**) Scalding in lemons **D**) Browning of lemons **E**) Damage leading to infection during cold storage **F**) Severe rind collapse on Navel Orange **G**) Increased damage seen on paler Navel fruit indicate importance of rind colouration **H**) Colour difference seen in irradiated (left) vs no irradiated (right) 'nules' fruit **I**) Scalding in 'Nova' mandarin **J**) Severe damage in 'Nova' mandarin **K**) Different damage types pitting and staining seen in Navel orange.

The incidence of rind disorders can vary within one season for the same cultivar *viz.* lemon, or cultivar group *viz.* early vs. late Navel – which could be related to maturity of the rind influencing susceptibility. However, the fruit quality *per se*, prior to irradiation is also critical and fruit with a low quality would have a level of "normal" rind disorders developing, which increased with the additional impact of the irradiation. This observation was illustrated by the 'nadorcott' and 'nules' mandarin fruit during 2015 season (Fig. 3) which started at a relatively high pitting incidence of 0.2, which increased thereafter to 0.5 for 'nadorcott' mandarin and for 'nules' an increase of 0.6 to 0.7 in the Pitting index. Variation in rind condition between seasons will impact the reaction of fruit to irradiation. Visually comparing the results of 'nules' and 'nadorcott' mandarin from 2015 with 2016, a reduction in the pitting incidence was seen. One further aspect which could not be evaluated in this project was comparison of cultivars in the same season from different production areas i.e. Valencia oranges from Limpopo vs. Western Cape

Table 4.2.2.1. *The D_{Max}:D_{Min} ratios achieved for the different target doses (200 to 500 Gy) of 16 pallets of citrus fruit that were irradiated over two seasons.*

Season 1													
Early season Cultivars							Late season Cultivars						
Cultivar	Target Dose	Absorbed dose Min (Gy)	Absorbed dose Max (Gy)	Max:Min	Average value	Over/under target	Cultivar	Target Dose	Absorbed dose Min (Gy)	Absorbed dose Max (Gy)	Max:Min	Average value	Over/under target
Star Ruby' Grapefruit	200	205	275	1,3	240	40	Nadorcott' mandarin	200	264	355	1,3	309,5	109,5
Star Ruby' Grapefruit	300	271	499	1,8	385	85	Nadorcott' mandarin	300	310	462	1,5	386	86
Star Ruby' Grapefruit	400	429	602	1,4	515,5	115,5	Nadorcott' mandarin	400	362	516	1,4	439	39
Star Ruby' Grapefruit	500	482	718	1,5	600	100	Nadorcott' mandarin	500	469	669	1,4	569	69
Eureka' Lemon	200	217	269	1,2	243	43	Eureka' Lemon	200	286	320	1,1	303	103
Eureka' Lemon	300	252	331	1,3	291,5	-8,5	Eureka' Lemon	300	359	460	1,3	409,5	109,5
Eureka' Lemon	400	456	520	1,1	488	88	Eureka' Lemon	400	416	558	1,3	487	87
Eureka' Lemon	500	510	701	1,4	605,5	105,5	Eureka' Lemon	500	467	668	1,4	567,5	67,5
Nules' Clementine	200	205	248	1,2	226,5	26,5	Turkey' Valencia	200	245	350	1,4	297,5	97,5
Nules' Clementine	300	237	327	1,4	282	-18	Turkey' Valencia	300	362	434	1,2	398	98
Nules' Clementine	400	400	520	1,3	460	60	Turkey' Valencia	400	355	506	1,4	430,5	30,5
Nules' Clementine	500	451	588	1,3	519,5	19,5	Turkey' Valencia	500	493	608	1,2	550,5	50,5
Washington Navel'	200	240	290	1,2	265	65	Robyn Navel'	200	243	323	1,3	283	83
Washington Navel'	300	263	344	1,3	303,5	3,5	Robyn Navel'	300	360	427	1,2	393,5	93,5
Washington Navel'	400	431	525	1,2	478	78	Robyn Navel'	400	393	554	1,4	473,5	73,5
Washington Navel'	500	529	652	1,2	590,5	90,5	Robyn Navel'	500	478	597	1,3	537,5	37,5
Season 2													
Early season Cultivars							Late season Cultivars						
Cultivar	Target Dose	Absorbed dose Min (Gy)	Absorbed dose Max (Gy)	Max:Min	Average value	Over/under target	Cultivar	Target Dose	Absorbed dose Min (Gy)	Absorbed dose Max (Gy)	Max:Min	Average value	Over/under target
Nova mandarin	200	255	406	1,6	330,5	130,5	Nadorcott' mandarin	200	223	358	1,6	290,5	90,5
Nova mandarin	300	356	534	1,5	445	145	Nadorcott' mandarin	300	320	557	1,7	438,5	138,5
Nova mandarin	400	411	624	1,5	517,5	117,5	Nadorcott' mandarin	400	409	678	1,7	543,5	143,5
Nova mandarin	500	479	787	1,6	633	133	Nadorcott' mandarin	500	521	851	1,6	686	186
Eureka' Lemon	200	258	391	1,5	324,5	124,5	Eureka' Lemon	200	253	356	1,4	304,5	104,5
Eureka' Lemon	300	363	517	1,4	440	140	Eureka' Lemon	300	351	456	1,3	403,5	103,5
Eureka' Lemon	400	421	615	1,5	518	118	Eureka' Lemon	400	434	642	1,5	538	138
Eureka' Lemon	500	523	739	1,4	631	131	Eureka' Lemon	500	540	797	1,5	668,5	168,5
Nules' Clementine	200	252	398	1,6	325	125	Midknight' Valencia	200	219	324	1,5	271,5	71,5
Nules' Clementine	300	328	496	1,5	412	112	Midknight' Valencia	300	305	458	1,5	381,5	81,5
Nules' Clementine	400	410	592	1,4	501	101	Midknight' Valencia	400	396	547	1,4	471,5	71,5
Nules' Clementine	500	477	675	1,4	576	76	Midknight' Valencia	500	519	777	1,5	648	148
Washington Navel'	200	*	*				Robyn Navel'	200	214	336	1,4	275	75
Washington Navel'	300	*	*				Robyn Navel'	300	300	503	1,7	401,5	101,5
Washington Navel'	400	*	*				Robyn Navel'	400	407	588	1,4	497,5	97,5
Washington Navel'	500	*	*				Robyn Navel'	500	532	948	1,8	740	240

* Data lost due to computer faliare

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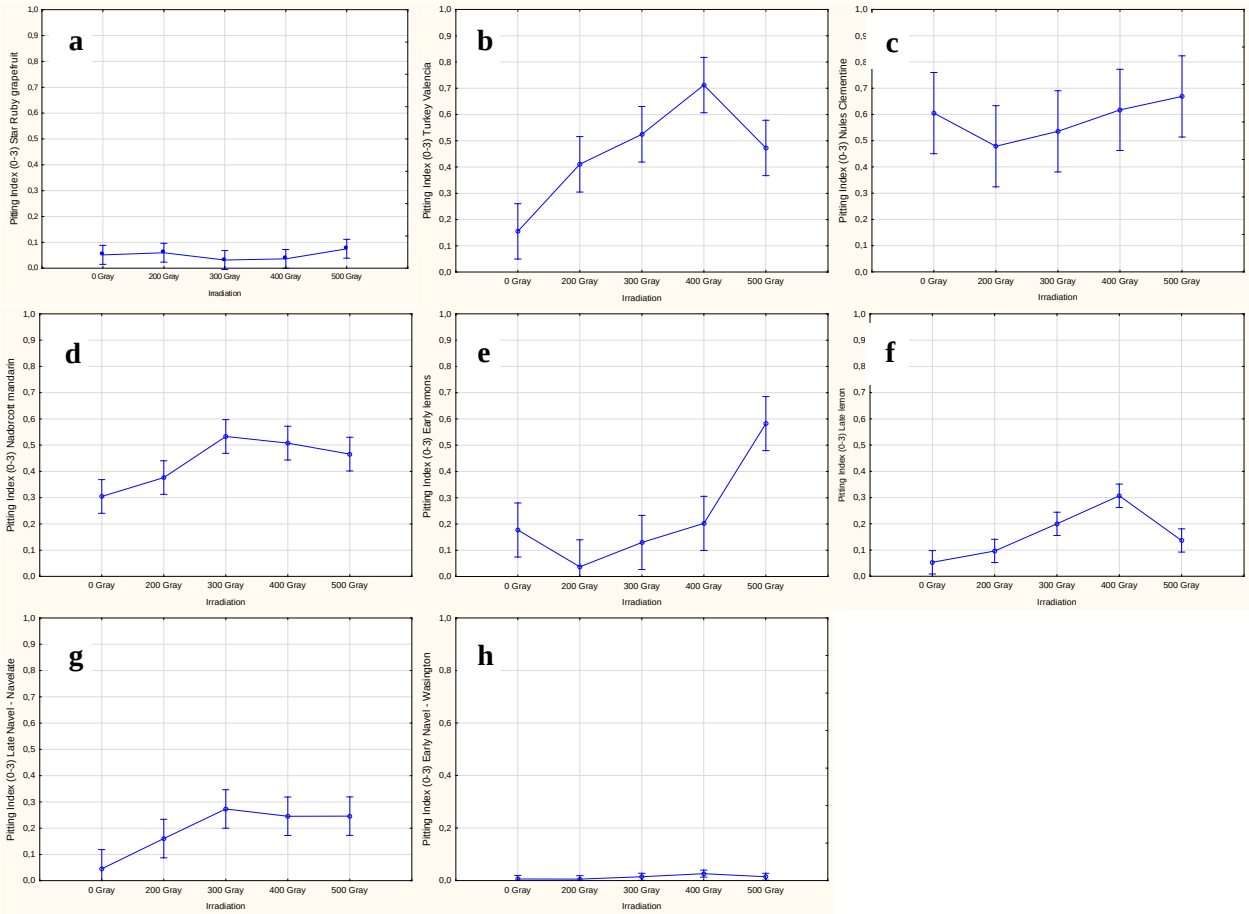
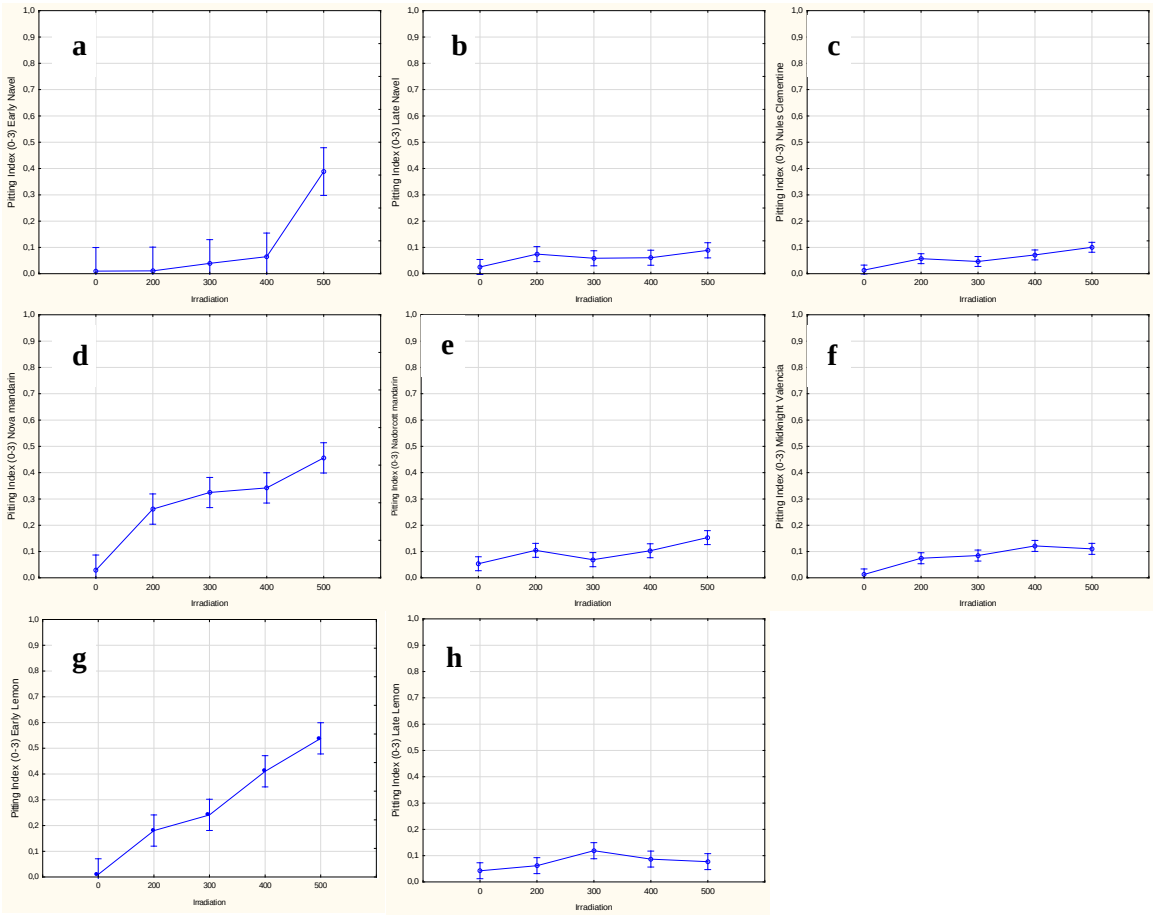


Figures 4.2.2.3 a-h. *Impact of increase in irradiation dose (0 to 500Gy) on incidence of rind disorders in (a) Star Ruby grapefruit, (b) Turkey Valencia orange, (c) Nules Clementine, (d) Nadorcott mandarin, Eureka lemon (e) early and (f) late, Navel orange (g) late Navelate and (h) early Washington during 2015. All cultivars were evaluated for pitting severity on a scale of 0 to 3 (Figure 1). Note all graphs of the different cultivars have the same y-axis value. Vertical bars denote 0.95 confidence intervals.*

Postharvest conditions after irradiation i.e. cold storage at either 2°C for 18 days + 7°C for the remainder of cold storage duration, or 7°C for the whole period, contributed to the incidence of rind disorders although not always significantly (Tables 4.2.2.2 and 3). In general, the fruit quality was better at the 2°C cold storage compared to the 7°C, indicating a possible positive impact of the lower temperature on fruit reaction to the irradiation. Storage duration of 40 and 60 days was chosen to represent the commercial market window from arrival until the maximum cold storage duration before quality is drastically compromised. The higher incidence of disorder after 60 days compared to 40 days was expected. However, the difference was not always significant at a 95% level. The general trend indicates that irradiation compromises the potential of the fruit for undue long cold storage period such as 60 days.



Figures 4.2.2.4 a-g. *Impact of increase in irradiation dose (0 to 500 Gy) on incidence of rind disorders on six cultivars; Navel orange (a) Early Washington (b) Late-Navelate, (c) Nules, (d) Nova, (e) Nadorcott mandarin, (f) Midknight Valencia and Eureka lemons (g) early and (h) late during 2016 season. All cultivars were evaluated for pitting severity on a scale of 0 to 3 (Figure 1). Note all graphs of the differ cultivars have the same y-axis value. Vertical bars denote 0.95 confidence intervals.*

Table 4.2.2.2. *Summary of the impact of cold storage duration (40 vs. 60 days) and cold storage temperatures (2°C vs. 7°C) on the incidence of rind disorders after irradiation. All statistical analysis was done within a cultivar. Different lettering denotes 0.95 confidence intervals.*

Cultivar	Cold storage period 40 vs. 60 days	Cold storage temperature 2°C vs. 7°C
Star Ruby grapefruit	Non-significant	2°C (0.07) a 7°C (0.03) b
Nules Clementine	40 d (0.1) a 60 d (1.1) b	Non-significant
1st (Early) Navel - Washington	40 d (0.01) a 60 d (0.02) b	Non-significant
1st (Early) Lemon	Non-significant	Non-significant
Nadorcott Mandarin	Non-significant	2°C (0.49) a 7°C (0.39) b
2nd (Late) Navel - Navelate	Non-significant	Non-significant
2nd (Late) Lemons	40 d (0.1) a 60 d (2.2) b	2°C (0.13) a 7°C (0.19) b
Turkey Valencia orange	Non-significant	Non-significant

Table 4.2.2.3. *Summary of the impact of cold storage duration (40 vs. 60 days) and cold storage temperatures (2°C vs. 7°C) on incidence of rind disorders after irradiation during 2016. All statistical analysis was done within a cultivar. Different lettering denotes 0.95 confidence intervals.*

Cultivar	Cold storage period	Cold storage temperature

	40 vs. 60 days	2°C vs. 7°C
Nules Clementine mandarin	40 d (0.95) a 60 d (0.2) b	2°C (0.07) a 7°C (0.03) b
Nadorcott mandarin	40 d (0.7) a 60 d (0.12) b	Non-significant
Nova mandarin	Non-significant	2°C (0.4) a 7°C (0.2) b
1st (Early) Navel -Washington	Non-significant	Non-significant
2nd (Late) Navel - Navelate	40 d (0.04) a 60 d (0.08) b	2°C (0.04) a 7°C (0.08) b
1st (Early) Lemon	40 d (0.44) a 60 d (0.31) b	2°C (0.37) a 7°C (0.15) b
2nd (Late) Lemons	Non-significant	Non-significant
Midknight Valencia orange	40 d (0.06) a 60 d (0.1) b	Non-significant

From previous studies the impact of irradiation on the internal quality was indicated to be less affected compared to the external quality (rind). The long postharvest period SA fruit has to endure in order to reach all the markets, is an important aspect in maintaining market share. The normal internal quality attributes i.e. Total Soluble Solids (°Brix), citric acid content (%), juice % as well as rind colour were evaluated for all treatments and a summary of the results for the two seasons is supplied in Tables 4.2.2.4 and 5.

The data indicates that internal quality i.e. TSS or Acid could be negatively impacted by irradiation. The impact is always more negative in those cultivars susceptible to irradiation damage *viz.* Nova, lemon and Turkey. Whereas juice % was not significantly altered by the treatments, °Brix and citric acid content can be reduced by increasing irradiation doses (Fig. 4.2.2.5 a-f; Fig. 4.2.2.6 a-f). Rind colour was not negatively affected in any of the cultivars used.

At Day 40 of cold storage, no obvious differences in taste and other sensory attributes were recorded. Results from the consumer panel showed little differences between irradiation and cold storage treatments and would be commercially acceptable. Sensorial-perceived off-flavours were recorded, however, this was not treatment specific and also occurred within control treatments (Table 4.2.2.6). According to Della-Modesta (1994), colour is a characteristic well recognized by the consumer. In order to eliminate pre-conceived perceptions, fruit blocks were free of citrus rinds. Thus, sensory attributes were not influenced by the external condition and colour of the treated citrus fruit.

Table 4.2.2.4. *Summary of the impact of irradiation and cold storage on internal quality of citrus fruit.*

Cultivar	Total soluble solids (°Brix)	Citric acid	Juice %	Rind colour
Star Ruby grapefruit	Non-significant	Non-significant	Non-significant	Non-significant
Nules Clementine	Non-significant	Non-significant	Non-significant	Non-significant
1st (Early) Navel - Washington	Non-significant	Non-significant	Non-significant	Non-significant
1st (Early) Lemon	Significant (Fig.5b)	Significant (Fig. 5a)	Non-significant	Non-significant
Nadorcott Mandarin	Significant (Fig.5f)	Non-significant	Non-significant	Non-significant
2nd (Late) Navel - Navelate	Non-significant	Non-significant	Non-significant	Non-significant
2nd (Late) Lemons	Non-significant	Significant (Fig.5c)	Non-significant	Non-significant
Turkey Valencia orange	Significant (Fig.5d)	Significant (Fig.5e)	Non-significant	Non-significant

Table 4.2.2.5. *Summary of the impact of irradiation and cold storage on internal quality of citrus fruit during 2016.*

Cultivar	Total soluble solids (°Brix)	Citric acid	Juice %	Rind colour
Nules Clementine mandarin	Significant (Fig.6a)	Significant (Fig.6b)	Non-significant	Non-significant
Nadorcott mandarin	Significant (Fig.6c)	Significant (Fig.6d)	Non-significant	Non-significant
Nova mandarin	Significant (Fig.6h)	Significant (Fig.6i)	Non-significant	Non-significant
1st (Early) Navel - Washington	Significant (Fig.6e)	Significant (Fig.6f)	Non-significant	Non-significant
2nd (Late) Navel - Navelate	Non-significant	Non-significant	Non-significant	Non-significant
1st (Early) Lemon	Significant (Fig.6g)	Significant (Fig.6h)	Non-significant	Non-significant
2nd (Late) Lemons	Non-significant	Non-significant	Non-significant	Non-significant
Midknight Valencia orange	Non-significant	Non-significant	Non-significant	Non-significant

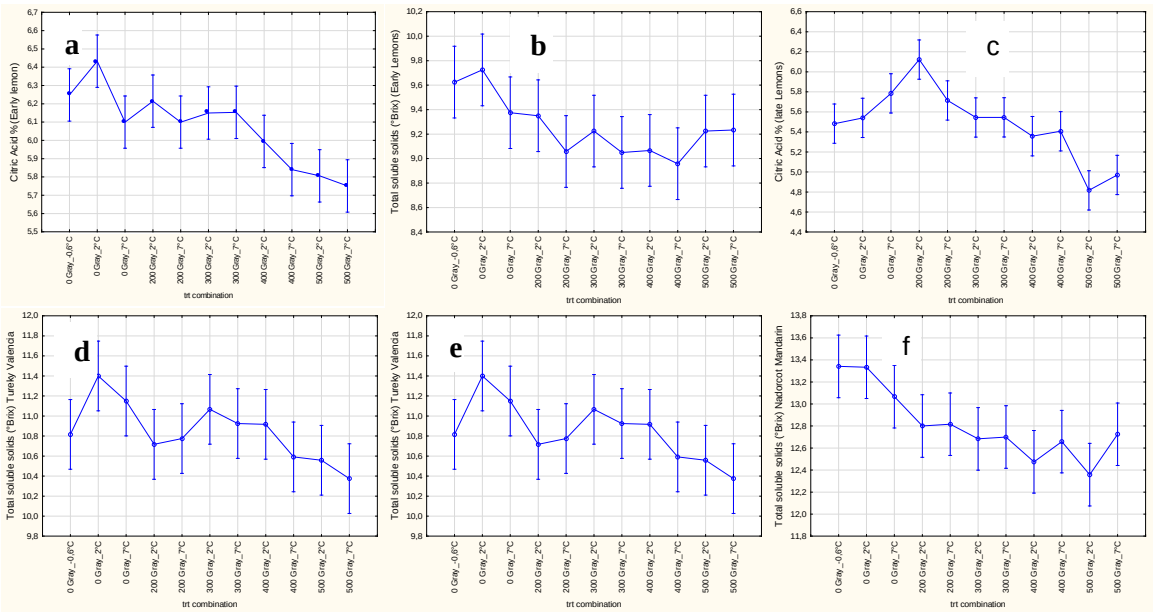
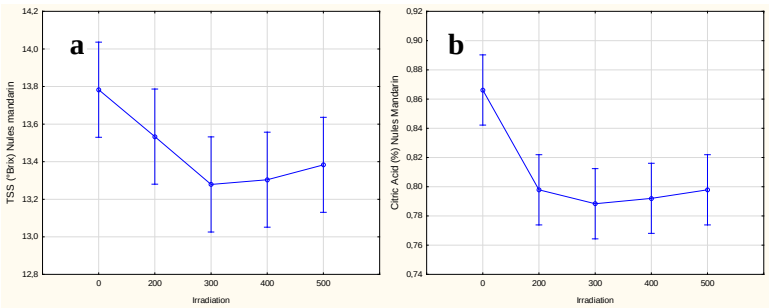


Figure 4.2.2.5 a-f. *Impact of irradiation and cold storage on citric acid (%) and total soluble solids (°Brix) of lemon (a-c), Turkey Valencia orange (d and e) and Nadorcott mandarin (f). Vertical bars denote 0.95 confidence intervals.*



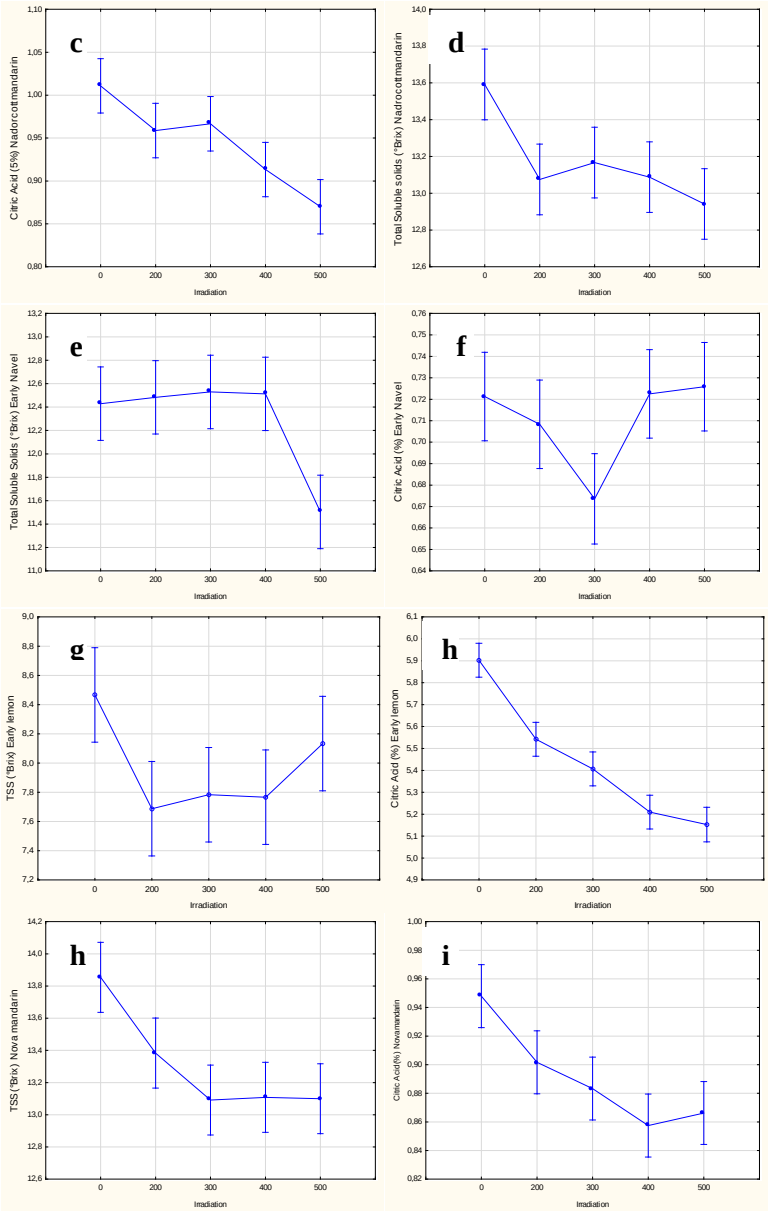


Figure 4.2.2.6 a-i. *Impact of irradiation and cold storage on citric acid (%) and total soluble solids (°Brix) of Nules Clementine mandarin (a and b), Nadorcott mandarin (c and d) Early Navel (e and f) early lemon (g and h) and Nova mandarin (h and i) during 2016. Vertical bars denote 0.95 confidence intervals.*

Decay incidence was increased due to irradiation in those cultivars susceptible to rind damage (Figure 7ab). It is thought that the damaged rind could offer a point of penetration for spores present on the surface of the fruit.

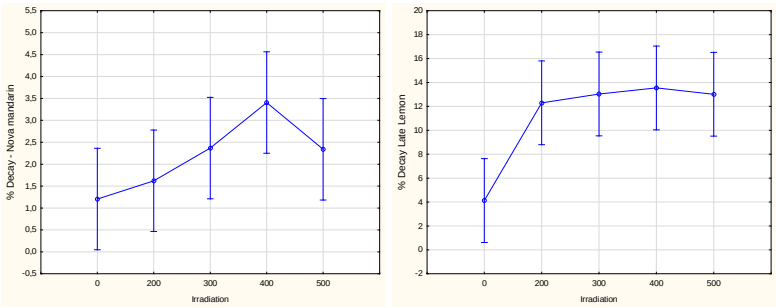


Figure 4.2.2.7 ab. *Increased incidence of decay of 'nova' mandarin and late 'Eureka' lemon fruit after irradiation with an increasing dose (200 to 500Gy). Vertical bars denote 0.95 confidence intervals*

Discussion

The aim of this project was to determine the effect of a progressive irradiation dosage range from 200 to 500 Gy on a selection of commercially important cultivars. This study was the next step in a novel attempt to develop a new phytosanitary treatment which does not rely on one factor only i.e. temperature or irradiation alone, but rather a combination of these two (Lacroix and Follett, 2015*)*. It is thought that by combining two phytosanitary control measures, the negative reaction of each as a stand-alone treatment could be negated: i.e. by using a lower irradiation dose and a higher temperature. In extensive experiments it was confirmed that FCM moth sterility could be obtained by exposure to 60 to 100 Gy and fecundity was totally suppressed above 70 Gy (Hofmeyr *et al.*, 2016cd). The results from these treatments successfully complied with the probit-9 level for FCM. By combining the lowest level of efficacy i.e. 60 Gy with an 18-day exposure to 2°C, which is the standard cold protocol for fruit flies, an effective combination effect was identified which complies with the probit-9 level of efficacy (Hofmeyr *et al.*, 2016ab).

Citrus fruit is known to be generally susceptible to irradiation from 400 to 800 Gy. In a commercial situation irradiation will be done on pallet level, which will result in the actual administered dose being 3 times the target dose to ensure penetration through the pallet to the middle point. The minimum dose of 150Gy for Tephritidae and 400Gy for all insects except pupae and adults of Lepidoptera (Follet 2009; USDA APHIS PPQ, 2012), is received in the middle of the pallet and can result in prohibitively adverse effects in citrus fruit quality. However, by using the combination treatment, a lower target dose of 60 Gy will result in a 180 Gy maximum (1:3 D_{min}:D_{max}) which could result in most cultivars being able to be irradiated without prohibitive damage and quality loss.

Various researchers have evaluated the impact of irradiation on citrus fruit quality, however, most often using only one cultivar. During this project 20 tons of citrus fruit divided into nine cultivars were evaluated over the two season to offer the best opportunity to determine the possible commercial viability of this technology.

The results of this study on ‘Star Ruby’ grapefruit confirm those of Patil *et al*. (2004) as well as Spalding and Davis (1985) and Miller and McDonald (1996), who reported minimal injury to the fruit at a dose of 300 Gy. Furthermore, these studies reported that ‘Rio Red’ grapefruit exposed to irradiation doses of up to 500Gy did not affect soluble solids (%), titratable acidity, appearance and organoleptic quality compared to untreated fruit (Hallman and Martinez, 2001). The same results were seen in the current data set.

Figure 4.2.2.8. *A visual example of the progressive nature of irradiation damage in citrus fruit during a 30-day storage period at room temperature. Note the colour changes in the fruit during the 5 day intervals.*

In a study by Miller *et al*. (2000) evaluation of doses up to 450 Gy reported rind damage in two cultivars 'Washington' and Hamlin orange at 150 Gy, however, 'Valencia' orange, 'Minneola' and 'Murcott' mandarin had a tolerance of up to 500-600 Gy. MacDonald *et al.* (2014) found the primary negative impact on 'Lane Late' navels to be rind damage at 400-600 Gy. The low incidence of pitting in the 'Washington' and 'navelate' navel, as well as the 'nules' and 'nadorcott' mandarin below 300 Gy concurs with these findings. The exceptionally high incidence of rind disorder in the 'nova' mandarin was unexpected as it was thought that fruit with a good orange rind colour are less susceptible. These results indicate the caution that needs to be taken in classifying a cultivar group, for example the mandarins, as more or less tolerant. This aspect was also shown by the high difference between 'Turkey' and 'Midknight' Valencia oranges. 'Eureka' lemons, being very susceptible to chilling injury, has also been known to be susceptible to irradiation damage (Maxie *et al*., 1964) and the lemon fruit rind was susceptible to damage from 100-400 Gy in addition to a dramatic loss in ascorbic and citrus acid in the extracted juice.

In general, a reduction in TSS (°Brix) and Citric acid was seen in most cultivars, however not always significantly. The cultivars that were most susceptible to rind damage i.e. 'nova', 'Turkey' and lemon had a more dramatic loss in citric acid and °Brix as irradiation dosage increased, which could have been a result of increase fruit respiration. However, in some instances very low rind damage was seen but a reduction in acid and TSS was recorded which is indicative of a direct effect.

Table 4.2.2.6. *Means of sensory attributes of 'Washington' and 'navelate' Navels, 'Turkey' Valencia, 'Nules' Clementine and 'Star Ruby' grapefruit after irradiation and cold storage treatments. All cultivars were evaluated on a 1-9 hedonic scale in which a score of 1 represented extremely liked and 9 represented extremely disliked.*

	Irradiation and cold storage treatments	0Gy 22d @-0.6°C + 7°C	0Gy 18d @2°C +7°C	0Gy 7°C	200Gy 18d @2°C+7°C	300Gy 18d @2°C+7°C	400Gy 18d @2°C+7°C	500Gy 18d @2°C+7°C	200Gy 7°C	300Gy 7°C	400Gy 7°C	500Gy 7°C
'Nules' Clementine	**Aroma**	4.55	4.52	4.59	4.34	4.55	4.48	4.80	4.27	4.39	4.25	4.61
	Colour	3.41	2.93	3.34	3.23	2.82	3.11	3.25	3.27	3.07	3.11	3.55
	Texture	4.30	3.80	4.20	3.80	3.57	4.16	4.02	3.82	4.02	4.14	4.16
	Taste	5.93	5.64	6.48	5.00	4.95	5.75	5.50	4.77	5.70	5.43	5.89
	Juiciness	4.30	3.77	4.45	3.95	3.48	4.18	3.95	3.93	3.80	4.05	4.07
'Washington Navel' orange	**Aroma**	4.07	3.70	3.89	3.91	3.84	3.86	4.16	3.75	3.98	3.91	4.16
	Colour	3.77	3.39	3.18	3.70	3.36	3.25	3.59	3.30	3.48	3.43	3.61
	Texture	3.61	3.18	3.84	3.52	3.07	3.36	3.32	3.45	3.32	3.64	3.50
	Taste	5.14	4.11	4.86	5.16	3.73	4.61	4.57	4.89	4.18	5.14	4.89
	Juiciness	3.39	2.93	3.48	3.48	3.14	3.09	3.09	3.16	3.20	3.41	3.25
'Star Ruby' grapefruit	**Aroma**	3.45	3.50	3.50	3.41	3.61	3.52	3.64	3.50	3.55	3.43	3.57
	Colour	3.43	3.39	3.14	3.32	3.09	3.41	3.39	3.41	2.68	3.14	3.45
	Texture	3.25	3.30	3.48	3.09	3.18	3.34	3.45	3.27	3.07	3.25	3.39
	Taste	3.77	4.05	4.23	3.86	4.09	4.18	4.64	4.20	4.11	4.18	5.39
	Juiciness	2.84	3.02	2.89	2.68	3.07	2.80	3.14	3.02	2.86	2.91	3.00
'Navelate' orange	**Aroma**	4.42	4.33	4.69	4.53	4.47	4.31	4.97	5.00	4.72	4.94	4.28
	Colour	3.50	3.58	3.31	3.33	3.58	3.44	3.44	3.42	3.47	3.33	3.22
	Texture	4.00	3.53	3.61	3.47	3.42	3.36	3.58	3.83	3.53	3.72	3.31
	Taste	5.14	4.17	5.06	4.78	4.75	4.44	5.14	5.44	5.03	5.50	4.36
	Juiciness	3.67	3.33	3.44	3.39	3.25	3.42	3.50	3.69	3.42	3.44	3.14
'Turkey Valencia' orange	**Aroma**	4.44	4.39	4.67	4.78	4.78	4.83	4.39	4.75	4.61	4.72	4.69
	Colour	3.64	3.61	3.69	3.56	3.61	3.61	3.61	3.50	3.47	3.61	3.47
	Texture	3.83	3.83	3.72	3.75	3.53	3.92	3.67	3.86	3.86	3.81	3.78
	Taste	5.31	4.50	4.97	5.58	5.00	5.42	4.86	5.44	4.94	5.53	5.33
	Juiciness	3.81	3.72	3.81	3.53	3.56	3.72	3.64	3.69	3.81	3.58	3.67
Nadorcott' mandarin	**Aroma**	3.72	3.53	3.39	3.92	4.08	4.31	4.17	3.75	4.03	4.14	4.14
	Colour	2.75	2.69	2.78	2.75	2.69	2.83	2.69	2.89	2.89	2.83	2.81
	Texture	3.31	3.08	3.08	3.14	3.58	3.44	3.22	3.19	3.39	3.31	3.31
	Taste	3.50	3.08	3.17	3.69	4.00	4.50	4.28	3.97	4.42	4.39	4.58
	Juiciness	2.92	2.81	2.86	2.97	3.08	3.25	2.94	3.00	2.89	3.08	3.11

CONCLUSIONS

The two seasons confirmed that significant variation exists between irradiation doses with an increased susceptibly from 300 Gy upwards. In addition, cultivars respond differently to the same irradiation dose from an external as well as internal view point. The consumer panels did not report the taste being commercially negative. The postharvest storage temperature of 2°C after treatments was in general more favourable to retard symptom development and resulted in better fruit quality in terms of rind disorders compared to 7°C.

These cultivars used in this project could be grouped into four according to susceptibility:

- Very low susceptibility – Star Ruby grapefruit and Midknight Valencia orange
- Low susceptibility – Early and Late Navels (Washington and Navelate)
- Medium susceptibility - Nadorcott, Nules mandarin and lemons
- Very High susceptibility - Turkey Valencia orange and Nova mandarin.

Future research

This study confirmed the possible viability of low levels of irradiation to be developed into commercial phytosanitary protocol. However only once a custom build irradiation facility is build could additional research projects initiated to further optimise the use of irradiation without negating quality.

Technology transfer

CRI research symposium. Champagne Castel sports Resort, Aug 2016.
PHI-3 Research feedback symposium Nov 2016.

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4.2.3 FINAL REPORT: Studies on aspects concerning rind pitting/staining citrus fruit.

Project 958 (2009/10 – 2014/5) by P Cronje, Jade North (CRI), Jacques Ehlers, Jeanine Joubert and Helene Marias (SU-Hort)

Summary: Pitting of Mandarin citrus fruit

'nules Clementine' and 'nadorcott' mandarin are commercially important *Citrus* cultivars in South Africa. Both cultivars are prone to develop rind breakdown and pitting which are considered non-chilling related post-harvest physiological rind disorders. The progressive and erratic nature of these rind disorders result in high financial losses. The incidence of a rind disorder is thought to be associated firstly with an increased susceptibility, as influenced by pre-harvest aspects and secondly with a trigger in the post-harvest environment. A study was conducted over two seasons to determine the effect of late nitrogen application (stage II and after summer flush), pre-harvest water stress and postharvest handling. The rind quality of 'nules Clementine' and 'nadorcott' mandarin fruit harvested from Citrusdal and Riebeeck Kasteel was evaluated. Soil applications of nitrogen at 20 $kg \cdot ha^{-1}$ and 40 $kg \cdot ha^{-1}$ were done on 21 January and 26 March 2014/2015, respectively. This was in addition to the standard 300 $kg \cdot ha^{-1}$ nitrogen provided by the producer. During 2015 a 1% urea foliar application was sprayed on 26 March. During the post-harvest period all fruit were dehydrated at 25 °C and 60 to 80% RH (0.7 to 1.1 kPa vapour pressure deficit) for two days, followed by rehydration at 100% RH for one day. Subsequently fruit were stored at either -0.6 °C or 4 °C for a 30-day period. There were no significant differences in fruit colour or size between the different nitrogen treatments. No increase in rind disorders or negative impacts on internal fruit quality were noted. To determine the impact of pre-harvest water stress, the soil below the trees was covered with plastic sheets three weeks prior to harvest to exclude rainfall or irrigation. The effect of postharvest stress was established by dehydrating and rehydrating fruit at 0.7-1.1 kPa vapour pressure deficit for different periods after harvest. Wax was applied on day 5 and thereafter fruit was stored at 4 °C for 30 days. The results indicated that pre-harvest water stress did not have a detrimental

effect on fruit susceptibility to disorders. By early wax application, however, a decrease in moisture loss was recorded, coinciding with lower incidences of rind disorders. The final part of the study was aimed at determining whether post-harvest handling lending to water loss, as induced by high vapour pressure, could increase disorders. From the results of the trials it can be concluded that exposing fruit to dehydration increased rind disorder susceptibility. Dehydration prior to wax application on day 5 also increased pitting and rind breakdown, whereas an early wax application, 2 to 3 days after harvest, reduced incidence. This study serves as a step to resolve the impact of factors predisposing the citrus fruit rind to progressive post-harvest disorders.

Opsomming: Gepokteskil van Mandaryn vrugte

'Nules Clementine' en 'nadorcott' mandaryne is kommersiële belangrike *Citrus* kultivars in Suid-Afrika. Beide kultivars is geneig tot skilafbraak en gepokte skil wat as na-oes fisiologiese skildefekte beskou word en is onafhanklik van koueskade tydens opberging. Die progressiewe- en wisselvallige aard van hierdie skildefekte het groot finansiële verliese tot gevolg. Vermoedelik hou die voorkoms van 'n skildefek eerstens verband met 'n verhoogde vatbaarheid soos beïnvloed word deur voor-oes aspekte; en tweedens met 'n sneller vanuit die na-oes omgewing. Die invloed van 'n laat stikstof toediening (fase II en na die somer groei), voor-oes waterstres en na-oes hantering op skildefekte is oor twee seisoene bepaal. 'Nules Clementine' en 'nadorcott' mandaryn vrugskilgehalte, geoes vanaf Citrusdal en Riebeeck Kasteel, is geëvalueer. Toediening van korrelkunsmis (stikstof) teen 20 kg·ha^{-1} en 40 kg·ha^{-1} is gedoen op 21 Januarie en 26 Maart 2014/2015, respektiewelik. Dié toedienings was bykomend tot die produsent se standaardbehandeling van 300 kg·ha^{-1} stikstof. 'n Blaartoediening van 1% ureum is addisioneel op 26 Maart 2015 aangewend. Alle vrugte was na-oes gedehidreer teen 25 °C en 60 tot 80% RH (0.7 tot 1.1 kPa dampdrukverskil) vir twee dae, gevolg deur 'n een dag rehidrasie periode van 100% RH. Vrugte is vervolgens opgeberg by óf -0.6 °C óf 4 °C vir 30 dae. Geen beduidende verskil in kleur en grootte van die vrugte tussen die verskillende stikstof behandelings of toename in skildefekte of negatiewe effek op interne vrugkwaliteit was waargeneem nie. Die impak van voor-oes waterstres op na-oes skilgehalte is bepaal deur grond onder die eksperimentele bome drie weke voor oes met plastiekseile te bedek om sodoende die invloed van reënval en besproeiing uit te skakel. Die effek van na-oes stres was geëvalueer in vrugte wat gedehidreer en re-hidreer was teen 0.7 tot 1.1 kPa dampdrukverskil. Die na-oes waks behandeling was op dag 5 toegedien, waarna vrugte opgeberg was by 4 °C vir 30 dae. Resultate dui daarop dat voor-oes waterstres geen negatiewe impak op die vrugte se vatbaarheid vir skildefekte gehad het nie. 'n Afname in vogverlies is wel gemeet met die wakstoediening wat ooreenstem met 'n laer voorkoms van skildefekte. Die finale gedeelte van die studie was daarop gemik om te bepaal of na-oes waterverlies, soos geïnduseer deur 'n hoë dampdruk verskil, die voorkoms van skildefekte kan induseer. Resultate dui aan dat die blootstelling van vrugte aan dehidrasie wel die vatbaarheid vir skildefekte verhoog het. Dehidrasie voor 'n wakstoediening op dag 5 het ook verhoogde skilafbraak en gepokte skil tot gevolg gehad, terwyl 'n vroeë waks, 2 tot 3 dae na oes, die voorkoms verlaag het. Hierdie studie dien as 'n skakel om die impak van faktore wat skil-vatbaarheid ten opsigte van fisiologiese defekte verhoog verder te ontrafel en dus sodoende aan te spreek.

Summary: Post-harvest rind pitting on 'Valencia' Oranges

Post-harvest rind pitting is a non-chilling related physiological rind disorder that affects various citrus cultivars and reduces fruit value. This disorder is characterised by the collapse of the flavedo sub-epidermal cells, whilst the main cause of this disorder is unknown, it is aggravated by changes in relative humidity (RH) and rind water status. Studies were conducted on 'Turkey' and more susceptible 'Benny' Valencia oranges in Limpopo and Mpumalanga South Africa. The effect of fruit position, maturity and size on fruit susceptibility to this disorder was investigated and it was found that fruit from the outside of the canopy are more susceptible to this disorder probably due to greater exposure to variation in environmental conditions than fruit from the inside of the canopy. More mature fruit were also found to be slightly more susceptible, however, size did not influence incidence of this disorder. Various plant growth regulators were also evaluated to prevent pitting. The application of the synthetic auxins 2,4-dichlorophenoxy acetic acid (2,4-D) and 3,5,6 trichloro-2-pyridiloxyacetic acid (3,5,6 TPA) at 50 % petal drop (2,4-D) or after physiological fruit drop (2,4-D or 3,5,6-TPA) reduced the incidence of post-harvest pitting. Application of s-abscisic acid 1 week before harvest was also found to reduce incidence of this disorder, however, gibberellic acid applied in January did not reduce the incidence of post-harvest rind pitting. A systemic fungicide thiabendazole (TBZ) which reduces the incidence of chilling injury also reduced post-harvest pitting incidence when applied before fruit were subjected to stress inducing environmental conditions. Pre-harvest foliar application of TBZ 1 week before harvest and post-harvest dip treatments directly after harvest reduced post-harvest weight loss and incidence of this disorder. A citrus industry survey was conducted to estimate the financial impact of this disorder at foreign and local markets for producers. Markets generating higher prices had a lower tolerance for incidence of post-harvest rind pitting than lower priced markets and are therefore seen as high-risk. Due to this large reduction in market price for fruit with the disorder, treatments found during this study might be cost effective.

Opsomming: Na-oes gepokteskil van 'Valencia' lemoene.

Gepokteskil is 'n na-oes fisiologiese skildefek wat nie met koueopberging by lae temperature gedurende geassosieer word nie en kan verskeie sitruskultivars affekteer en die waarde van vrugte verminder. Die defek word gekenmerk deur die ineenstorting van die sub-epidermale flavedo selle en alhoewel die hoof oorsaak van die defek nie bekend is nie word dit vererger deur variasie in relatiewe humiditeit (RH) en skil water status in die na-oes omgewing. Hierdie studie was op 'Turkey' en die meer vatbaar 'Bennie' Valencia lemoene in Limpopo en Mpumalanga Suid-Afrika gedoen. Die effek van voor-oes faktore soos vrug posisie, -volwassenheid en -grootte op die vatbaarheid van vrugte vir hierdie defek is ondersoek. Dit is bevind dat die vrugte aan die buitekant van die blaardak meer vatbaar is vir hierdie defek, waarskynlik as gevolg van groter blootstelling aan variasie in omgewings toestande as vrugte vanaf die binnekant van die blaardak. Daar is ook gevind dat meer volwasse vrugte 'n hoër vatbaarheid vir die defek het, maar dat vruggrootte dit nie beïnvloed nie. Die effektiwiteit van verskeie plant groei reguleerders om die voorkoms van gepokteskil te verminder is geëvalueer. Die toediening van sintetiese ouksiene 2,4-dichlorofenoksie asynsuur (2,4-D) en 3,5,6 trichloro-2-piridiloksi asynsuur (3,5,6 TPA) by 50 % blomblaarval (2,4-D) of na fisiologiese vrug val (2,4-D en 3,5,6-TPA) verminder die voorkoms van na-oes gepokteskil. So ook het die toediening van absisiensuur een week voor-oes die voorkoms van hierdie defek verlaag, maar daarteenoor het gibberelliensuur in Januarie geen effek op die voorkoms van na-oes gepokteskil gehad nie. Dit is al voorheen bewys dat 'n sistemiese swamdoder thiabendazole (TBZ) koueskade kan verminder, en TBZ het ook die voorkoms van na-oes gepokteskil verminder mits dit voor stres geïnduseerde omgewings toestande aangewend word. TBZ toediening een week voor-oes as 'n blaar bespuiting of as 'n doop behandeling direk na oes verminder gewig verlies en die voorkoms van die afwyking. 'n Sitrusbedryf opname was gedoen om die geskatte finansiële impak van gepokteskil in buitelandse en plaaslike markte op produsente te bepaal. Markte wat hoër pryse aanbied het 'n laer toleransie vir die voorkoms van na-oes gepokteskil as markte wat laer pryse aanbied en word gesien as hoë risiko. As gevolg van 'n drastiese afname in markprys vir vrugte met gepokteskil kan van die behandelings in die studie moontlik koste effektief wees.

Introduction

Post-harvest rind pitting or staining of Valencia orange and mandarin fruit in South Africa results in lost market opportunities and increased costs for producers. Unfortunately, very little is known about the impact of post-harvest practices on the occurrence of physiological disorders under Southern African conditions. Data published by Spanish and Floridian researchers on non-chilling postharvest pitting and staining of grapefruit and 'navel' orange, gives a clear indication of the importance of rind water content (Alférez, *et al*., 2003; 2005). In these studies, the dehydration of the rind during low relative humidity (RH) conditions followed by a re-hydration at high RH conditions, can result in turgor-stress in the transition cellular zone between the flavedo and albedo. This stress is thought to cause cellular collapse followed by the visible staining or pitting lesions.

The extent to which the citrus rind loses water in the period after picking and before packing is unknown for citrus cultivars in South Africa. In the case of fruit, the movement of water is more often than not out of the fruit due to its high water content, towards the drier atmosphere. The temperature of the fruit and ambient environmental conditions (wet and dry bulb temperature and RH) are the primary factors to influence the VPD value and the eventual water loss (determined as weight loss). The citrus fruit, due to being a hesperidium berry with a leathery rind, primarily loses water from the rind and very seldom from the pulp. The water content of the rind is therefore directly related to any water loss from the fruit.

In a preliminary study during 2012 it was found that post-harvest physiological rind disorders (pitting and staining) in 'nadorcott' mandarin (*Citrus reticulata* Blanco) were influenced by type of rootstock (known to influence water supply to fruit), as well as post-harvest handling practices (low vs. high RH). The results indicated a significantly higher susceptibility of fruit from rough lemon rootstocks compared to fruit from Carrizo citrange. The post-harvest dehydration prior to wax application induced significantly higher levels of rind disorders compared to fruit that was waxed within 24 h after harvest. The data concurs with findings on different citrus rind disorders, where a dramatic water loss due to high vapour pressure deficit (VPD) resulted in an inadequate adjustment of the water status of the rind, leading to cellular collapse and tissue damage (Agusti *et al*., 2001). It was hypothesized that rough lemon rootstocks result in a rind with a reduced ability to prevent water loss and therefore a higher rind disorder development. In addition, post-harvest handling practices could aggravate the incidence of rind disorders. Therefore, known and implementable postharvest practices such as removal of field heat and reduction of fruit VPD could decrease post-harvest citrus rind disorders.

Most research on water relations in citriculture has focused on increased yield during the pre-harvest stage. However, very seldom has the link been made with post-harvest quality (Cronje *et al*., 2001; 2013). Furthermore, the influence of pre-harvest water stress on the citrus fruit rind as it undergoes the various

postharvest stresses such as dehydration and packaging was unknown, and was the primary focus of this project for the next two years.

It was also hypothesised that incorrect N fertilisation could result in an increased sensitivity of mandarin fruit to post-harvest physiological rind disorders. The objective of this study was to ascertain whether additional N, applied later than the current practice, during the phase II and III fruit growth periods, could negatively impact on rind colour and susceptibility to rind disorders of 'nules Clementine' and 'nadorcott' mandarin.

Objectives

1. To gather information on the impact of post-harvest water stress on pitting of citrus fruit.
2. To determine whether variation in sensitivity exists between producers of the same cultivars in an area.
3. To determine the impact of pre-harvest water stress on rind sensitivity of mandarin.
4. Determine the impact of Nitrogen application on rind quality.
5. Determine the efficacy of thiabendazole application on pitting of Valencia orange fruit.

Materials and methods

Orchard sites

Over two seasons 'Valencia' orange (Turkey and Benny Valencia) (*Citrus sinensis*) were sampled from commercial orchards from 2-3 different production units in Letsitele (Limpopo) and Nelspruit (Mpumalanga). 'nadorcott' mandarin (*C. reticulata* Blanco) was sampled from trees in Riebeeck Kasteel, Citrusdal, and de Doorns (Western Cape). All experiments were laid out as randomised block designs on a per tree level.

Postharvest stress treatment to be used in all experiments

The fruit was subjected to high VPD for 3 days (25°C) prior to rehydration (100% humidity for 6 h), followed by postharvest wax and cold storage. The control fruit was immediately waxed and cold stored for 20 days at 4°C (non-chilling temperature). Fruit was weighed at each handling stage to determine % water loss.

Pre-harvest water stress and nitrogen application

In order to determine the combined impact of higher and lower irrigation supply (water stress), the delivery was changed to 1/2 X and 2X, with X being what the producers normally supply. In addition, N was applied (40 kg/ha) as an additional factor to determine the combined impact (Table 4.2.3.1). Nitrogen (LAN) was soil-applied at 20 and 40 $kg^{-1}\cdot ha$ in February and March on 10 trees per treatment in two orchards each, in addition to 250-300 $kg^{-1}\cdot ha$ applied commercially, to 'nules Clementine' and 'nadorcott' mandarin over two season (Table 4.2.3.3 and 4.2.3.4). These cultivars are harvested in May and August, respectively, in South Africa, and are susceptible to postharvest rind disorders. Fruit from the N-treated trees were stored at -0.6°C or 4°C for 30 days to determine the impact of postharvest stress on the external and internal quality.

Table 4.2.3.1. Summary of Irrigation and Nitrogen treatments in a 'nadorcott' mandarin orchard in Citrusdal.

Treatment	**Irrigation & Nitrogen (N)**
Standard irrigation	X
Increased irrigation water supplied	2X
Decreased irrigation water supplied	0.5X
Standard irrigation with added nitrogen (40kg/ha)	X & N
Increased irrigation with added nitrogen (40kg/ha)	2X & N
Decreased irrigation with added nitrogen (40kg/ha)	0.5X & N

Foliar treatments: Plant growth regulators (PGR's)

At fruit thinning stage, Corasil.P (15 ppm) was applied in mid-November (after fruit set). To increase rind strength 2,4-D application (5 ppm) was done in September after petal drop, mid-November after fruit set, and in June two weeks prior to harvest. In addition, Gibberellic acid (20ppm) and prohexadione-Ca were applied as contrasting treatments in order to prevent senescence of rind in Jan. and 2 weeks prior to harvest. Abscisic acid (15,30 and 40 ppm) was applied 1, 2 weeks prior to harvest to reduce water loss.

Foliar treatments: mineral nutrients

In both seasons, calcium arsenate, monoammonium phosphate (MAP) and potassium nitrate (KNO_3) were applied. The treatments were applied to trees as foliar sprays with a motorised knapsack sprayer until runoff. Each tree received approximately 2.25 L of the mixture or 2250 L/ha, which is comparable to commercial foliar applied mineral nutrients at a low volume coverage (1500 L – 3000 L/ha). Summarised treatment details are supplied, with the average fruitlet diameter at the time of spraying 130-135 mm (Table 4.2.3.2).

Table* 4.2.3.*2. *Mineral nutrient products applied, application time in reference to flowering and harvest and concentrations sprayed in 2015/16.* *WAFB = Weeks after full bloom; **WBH = Weeks before harvest.

Commercial name	Product	Application time	Concentration	
			2015 season	**2016 season**
Calcium arsenate, **Plaaskem (Pty) Ltd**	Calcium arsenate ($Ca_3(AsO_4)_2$)	7-8 WAFB*	0.05%	0.1%
Vitassol™ MAP, **SQM VITAS**	Monoammonium phosphate (MAP) ($NH_4H_2PO_4$)	7-8 WAFB	0.8%	1.5%
		7-8 WAFB	1.5%	2%
		7-8 WAFB	0.8% x 2	1.5% x 2
		7-8 WAFB		2% x 2
		6 WBH**	1%	1%
Multi-K GG, **Haifa Chemicals**	Potassium nitrate (KNO_3)	7-8 WAFB	2%	4%

Postharvest Thiabendazole (TBZ) application of Valencia orange

In order to determine the most opportune timing, TBZ was applied at four different stages during the post-harvest stress treatment, and for controls no TBZ was applied. Furthermore, an ideal practice was compared and reported separately to the control. For the *ideal practice* fruit were dipped, waxed and placed into cold storage directly after harvest; this treatment did not receive the pitting inducing stress treatment. The treatments were as follows:

- Directly *after harvest*, fruit were dipped before undergoing the pitting inducing stress treatment and waxed afterwards without TBZ and placed into cold storage.
- During the pitting inducing stress treatment *after dehydration* for 3 days at 25°C and 50% RH, fruit were dipped before being rehydrated for 1 day at 20°C at 99% RH. After rehydration, fruit were waxed without TBZ and placed into cold storage.
- *After rehydration*, fruit were dipped and waxed without TBZ and placed into cold storage.
- After the pitting inducing stress treatment, fruit were waxed with *TBZ in the wax* and placed into cold storage.
- The *control fruit* underwent the pitting inducing stress treatment and were waxed afterwards without TBZ and placed into cold storage.

TBZ was applied as a post-harvest dip bath and/or wax treatment according to the recommended rates on the product label.

For the dip bath: A bath was filled with 50 L of water and 200 ml TBZ to obtain a concentration of 2000 $\mu g \cdot mL^{-1}$. The bath was heated to 35°C and thoroughly mixed before and during each dip treatment to prevent any precipitation of TBZ. Fruit were dipped in the bath for 1 minute and then placed at ambient temperature (18°C) for 2 hours to dry.

Wax application: For the two treatments with TBZ in the wax, 8 ml TBZ (4000 $\mu g \cdot mL^{-1}$) was added to 1 L of 18 QDP wax [John Bean Technologies (Pty) Ltd.]. The wax application with and without TBZ were done with separate cartons of fruit and brushed to avoid TBZ contamination.

All fruit received similar cold storage conditions and were placed into cold storage at 4.5°C for 21 days before evaluation for post-harvest pitting incidence after cold storage.

Quality measurements

Internal quality

The internal quality was quantified as total acidity (TA), total soluble solids (TSS as °Brix), TSS:TA and juice content. Fruit were juiced with a mechanical citrus juicer (Sunkist®, Chicago, USA). The total acidity as well

as the citric acid was determined using an automatic titrator (Metrohm Titrando with an 815 Robotic USB Sample Processor and tiamo software, Metrohm, Switzerland). °Brix was determined using an electronic refractometer (PR-32 Palette, Atago Co, Tokyo, Japan) to quantify TSS.

HPLC measurements. In addition to the automatic titrator and electronic refractometer, the citric was determined using High Performance Liquid Chromatography (HPLC) (Agilent 1100 Series, Agilent Technologies, Santa Clara, CA, United States). Extracted citrus juice, from samples during the season, at harvest and after storage, was frozen in 2 ml Eppendorf tubes at -40°C. To enable extraction, the samples were defrosted. Juice samples were mixed thoroughly with a Vortex before centrifugation at 4000 rpm for 4 minutes (5417 R centrifuge, Eppendorf, Hamburg, Germany). After centrifugation, 100 µl of the clear supernatant was pipetted into 50 ml falcon tubes followed by 9900 µl of distilled water. A 100 times dilution was made of the extracted juice. The supernatant with water was mixed with a vortex before being filtered through a 0.45 µm syringe filter into HPLC vials to remove solid particles. In the HPLC (Agilent 1100 Series, Agilent Technologies, Santa Clara, CA, United States), for separation, a Rezex ROA-Organic Acid H+ (8%) column (150×7.8mm, 8µm) [Phenomenex, Torrance, Calif., U.S.A.) was thermostated at 30°C as solid phase and 2 mM H_2SO_4 solution as mobile phase with an isocratic flow rate of 0.3 $ml.min^{-1}$. A diode array detector (DAD) set at 210 nm. Standard solutions of citric acid were used for peak identification. The quantification was done using the standard curves of the standards used.

External quality

Pitting incidence: The deliverable in all experiments was to determine if pitting incidence was significantly reduced by a treatment and was calculated as follows. For mandarins the fruit were rated from 0-3 and for Valencia 0-4 on severity (Figure 1).

$$Pitting\ index\ (0-3/4) = \sum \frac{[Pitting\ (scale\ 0-3/4) \times number\ of\ fruit\ in\ each\ class]}{Total\ number\ of\ fruit\ in\ rep}$$

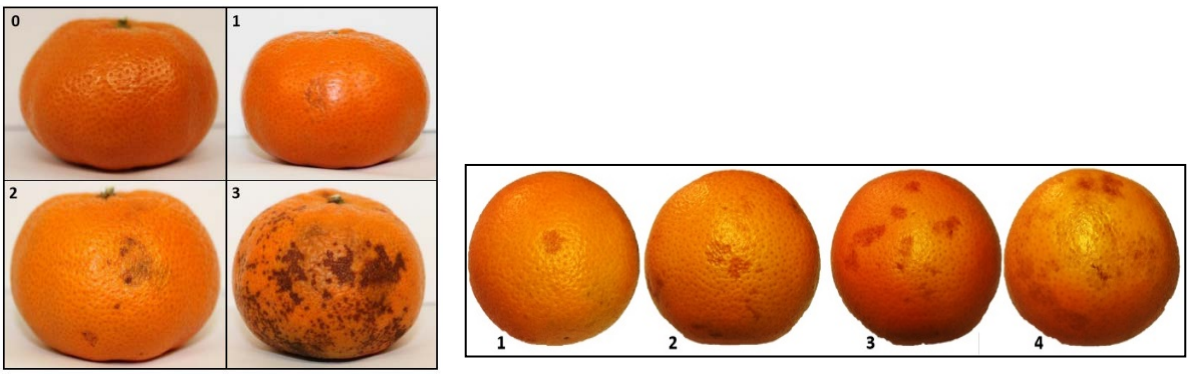


Figure 4.2.3.1. Pitting/staining incidence of 'nadorcott' mandarin (left) was scored according to a severity of 0 to 3 as the affected area increased. Post-harvest pitting rating for Valencia orange (on right) was rated from 0-4.

The external quality was quantified by measuring rind colour with a colorimeter (Konica Minolta CR-400, Tokyo, Japan) on two sides of the fruit and expressed as lightness, chroma and hue angle (Hue°). Fruit diameter and rind thickness was determined using an Electronic Fruit Size Measure (GÜSS Manufacturing (Pty) Ltd., Strand, South Africa) and an electronic calliper (CD-6"C, Mitutoyo Corp, Tokyo, Japan), respectively.

Microscopy imaging and analysis

In order to identify the concentration as well as locality of nutrients in the rind, samples at harvest were prepared for scanning electron microscopy (SEM) studies. Thin, uniform rind pieces (approximately 0.125 cm^3) were cut with a razor blade and fixed in 1:1 ($^v/_v$) solution of 2.5% glutaraldehyde and 2.5% formaldehyde for 1 to 2 hours at room temperature. The samples were then rinsed three times with distilled water. The material was then dehydrated in a graded (50%, 60%, 70%, 80%, 90%, 100%) ethanol series for 2 h. The material was thereafter washed with acetone, before being placed in a critical point drier in liquid carbon dioxide.

Imaging of the samples was taken using a Leo® 1430VP Scanning Electron Microscope at the Central Analytical Facilities, Stellenbosch University, South Africa. Prior to imaging, the samples were mounted on SEM stubs with double sided carbon tape. The mounted material was coated with a thin layer of gold in a Polaron E-6100 sputter coater. This was done in order to increase the electrical conductivity of the sample

surface. SEM was performed on a LEO 1450VP scanning electron microscope (SEM), at an acceleration voltage of <30 kV. The Scanning Electron (SE) image detects the surface structure of inspected material. Beam conditions during surface analysis were 7 kV and approximately 1.5 nA, with a spot size of 150.

Experimental design and statistical analysis

The data were analysed using the one-way Anova, two-way Anova (test interaction) and non-parametric one-way Anova (Kruskal-wallis test, when data was not normally distributed) with SAS Enterprise guide v.5.1 (SAS institute, Cary, NC, USA). Each treatment was compared with the other using Fischer least significance differences (LSD) and *P*-values smaller than 0.05 were deemed significant unless otherwise indicated.

Development of sensitivity analysis of pitting in various export markets

A citrus industry survey (CIS) was done in collaboration with BPAF at UP/US (Dr Marnus Gouse and Mr. Johan Boonzaaier) through a questionnaire which was designed to identify the cost of production, packing and transport of fruit to the various markets. All the questions related to costs were posed on a Likert scale to shorten answering time, however prices at markets were open ended questions. Seven markets were chosen to include most of South Africa's major export markets, i.e. Canada, China, EU, Hong Kong, ME, Singapore and the UK. Citrus fruit are exported to these markets at 4°C and therefore at conditions not related to chilling injury. The United States of America (USA) was excluded from this study as not all South African citrus production regions are allowed to export to the USA and all fruit are exported at -0.6°C for 32 days and therefore exposed to conditions that could lead to chilling injury.

In order to facilitate calculations a few assumptions were made:

- Exchange rate of R 14.00 to the US Dollar
- Bin of fruit has a mass of 380 kg
- Pallet consists of 80 cartons and with a mass is 15.5 kg
- Average pack out percentage of 71 %
- 4.6 bins of fruit are needed to produce 1 pallet of fruit.

The data from the CIS 2015 survey were pooled to give an overall industry view of cost and prices. Only enough information was obtained for EU, ME and UK markets and these results are reported further in this study. The findings are however relevant, as in 2013 over 50 % of South Africa's oranges were exported to these three markets (CGA, 2013) and there is little reason to believe that volume per country exports for 2015 was much different. The information obtained was used to calculate the production, packing and transport costs to deliver a pallet of fruit to the inland port. Prices were obtained at delivered in port (DIP) level and net income was estimated for each market per carton of fruit for the following pitting severities: zero, low (15 %) or high (30 %). The prices for these fruit were also estimated for local markets at the pack-house level.

With this information a hypothetical 'Valencia' farm of 100 ha was created that produced 251 935 cartons of exportable fruit annually and four hypothetical scenarios were created as follows:

- The 1st scenario 100 % of fruit had no pitting incidence.
- The 2nd scenario 80 % of fruit had no pitting incidence, while 10 % of fruit had low and 10 % high pitting incidence.
- The 3rd scenario 60 % of fruit had no pitting incidence while 20 % of fruit had low and 20 % high pitting incidence.
- The 4th scenario 50 % had no pitting incidence while 30 % of fruit had low and 20 % of fruit had high pitting incidence.

Results and discussion

Pitting of mandarin fruit

One of the main objectives of this part of the study was to determine the influence of N application during the later stages II and III of mandarin fruit development. For both cultivars, in all the orchards, no significant differences due to N-application, with respect to fruit coloration or rind disorders were recorded. The late application of nitrogen had no negative effect on the fruit rind colour, contrary to expectations (Figure 4.2.3.2). The late N application, following the summer flush, can thus be considered as an option to increase tree N content, as rind condition was not negatively affected. However, results from this study should be regarded as preliminary and further research is required to address relevant questions on the possible impact that a late N application may have on flower and fruit set in the subsequent season. In addition, more studies on the

influence of N-status on the citrus rind of various cultivar and production areas and its impact on disorders are needed.

Table 4.2.3.3. External fruit quality (n=10) of 'Nules Clementine' mandarin cultivated at Riebeeck Kasteel, quantified as diameter and colour (scoring by chart and hue°), at harvest and after cold storage at -0.6 °C or 4 °C for 30 days, following a range of additional soil LAN (limestone ammonium nitrate) nitrogen treatments as well as foliar urea (%) treatments, applied either early (21 January) or late (26 March) over two seasons

Nitrogen treatment [N (kg.ha^{-1}) or Urea (%)]	**External fruit quality**						
	Diameter (mm)	**Colour Chart (1-6)**			**Hue°**		
	Harvest	**Harvest**	**-0.6 °C**	**4 °C**	**Harvest**	**-0.6 °C**	**4 °C**
2014/5							
Control	73.12^{ns}	3.2^{ns}	1.90^{ns}	1.50ab^{y}	77.81^{ns}	68.88^{ns}	70.76^{ns}
20 kg.ha^{-1} Early	58.95	3.75	2.0	1.10b	78.59	71.51	69.46
40 kg.ha^{-1} Early	59.33	3.50	2.2	1.30ab	79.02	70.22	71.05
20 kg.ha^{-1} Late	54.99	3.40	1.7	1.30ab	76.51	68.17	71.15
40 kg.ha^{-1} Late	54.49	3.50	2.3	1.90a	78.81	71.09	71.48
p-value	*0.2015*	*0.8419*	*0.2588*	*0.0342*	*0.7302*	*0.9329*	*0.1071*
2015/6							
Control	60.92b	2.70ab	1.00^{ns}	1.00^{ns}	77.25b	73.44^{ns}	72.55^{ns}
20 kg.ha^{-1} Early	62.08b	2.80ab	1.00	1.00	78.06b	74.69	72.13
40 kg.ha^{-1} Early	66.70a	2.10b	1.00	1.00	77.16b	73.20	72.60
20 kg.ha^{-1} Late	63.60ab	3.10a	1.00	1.00	78.30b	74.20	73.61
40 kg.ha^{-1} Late	66.70a	2.60ab	1.00	1.00	83.30a	78.56	76.88
1% Urea Late	63.02ab	2.00b	1.00	1.00	80.42ab	76.35	74.37
p-value	*0.0146*	*0.0057*	---^{x}	---	*0.0313*	*0.0976*	*0.0636*

^{ns} No significant differences.
^{y} Means with a different letter within a column differ significantly at the 5% level (LSD).
^{x} P-value is non-significant.

Table 4.2.3.4. External fruit quality (n=10) of 'Nadorcott' mandarin cultivated at Riebeeck Kasteel, quantified as diameter and colour (scoring by chart and hue°), at harvest and after cold storage at -0.6°C or 4°C for 30 days, following a range of additional soil LAN (limestone ammonium nitrate) nitrogen treatments as well as foliar urea (%) treatments, applied either early (21 January) or late (26 March)

Nitrogen treatment [N (kg.ha^{-1}) or Urea (%)]	**External quality**						
	Diameter (mm)	**Colour Chart (1-6)**			**Hue°**		
	Harvest	**Harvest**	**-0.6 °C**	**4 °C**	**Harvest**	**-0.6 °C**	**4 °C**
2014/5							
Control	73.02a^{y}	1.20^{ns}	1.00^{ns}	1.00b	---^{z}	---	---
20 kg.ha^{-1} Early	73.45a	1.50	1.20	1.10ab	---	---	---
40 kg.ha^{-1} Early	67.35b	1.90	1.10	1.00b	---	---	---
20 kg.ha^{-1} Late	65.59b	1.80	1.70	1.50a	---	---	---
40 kg.ha^{-1} Late	68.26ab	1.00	1.40	1.30ab	---	---	---
p-value	*0.0002*	*0.0567*	*0.1050*	*0.0090*	---	---	---
2015/6							
Control	66.64ab	1.40^{ns}	1.00^{ns}	1.00^{ns}	55.20^{ns}	55.94^{ns}	55.53^{ns}
20 kg.ha^{-1} Early	67.80ab	1.50	1.00	1.00	55.35	55.82	55.81
40 kg.ha^{-1} Early	65.34b	1.60	1.00	1.00	55.98	56.13	56.09

20 kg.ha^{-1} Late	69.16a	1.40	1.00	1.00	54.99	55.63	55.43
40 kg.ha^{-1} Late	65.39b	1.80	1.00	1.00	57.29	57.39	56.92
1% Urea Late	68.68ab	2.10	1.00	1.00	56.04	56.43	56.16
p-value	*0.0029*	*0.2980*	---[x]	---[x]	*0.1670*	*0.7338*	*0.6214*

[ns] No significant differences.
[z] Measurements not performed.
[y] Means with a different letter within a column differ significantly at the 5% level (LSD).
[x] P-value is non-significant.

Figure 4.2.3.2. Example of 'nadorcott' mandarin fruit at harvest from the N-treatments: (A) Control, (B) 20 kg·ha^{-1} Early, (C) 40 kg·ha^{-1} Early, (D) 20 kg·ha^{-1} Late, (E) 40 kg·ha^{-1} Late and (F)1% Urea.

The combined application of higher or lower irrigation volume compared with the normal practise resulted in no increase in pitting on its own or if N was applied. In addition, no negative impact on internal quality (°Brix) or Citric acid was found (Table 4.2.3.5). The only negative impact was the combined treatments of 2X water plus late N, which resulted in a higher °Hue, indicating a less orange and more green fruit rind (Figure 4.2.3.3).

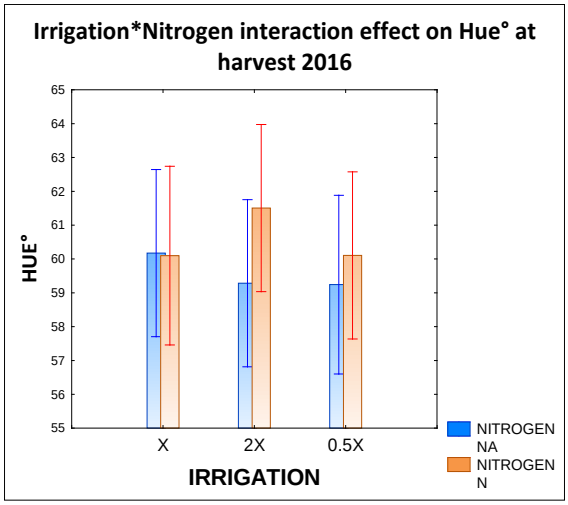


Figure 4.2.3.3. The impact of irrigation (1/2X, X and 2X) (orange bars) with or without N (blue bars at 40 kg/ha on rind colour, measured as Hue° on 'Nadorcott' mandarin ($p \leq 0.05$)

Table 4.2.3.5. The impact of irrigation (1/2X, X and 2X) with or without N (40kg/ha) on internal quality as well as pitting incidence of 'Nadorcott' mandarin ($p \leq 0.05$)

Treatments	**Citric Acid (%)**		**Total Soluble sugar (°Brix)**		**Ration: Ta/TSS**		**Pitting (0-3)**
	2015	**2016**	**2015**	**2016**	**2015**	**2016**	
X	1,70a	1,38 NS	15,6 NS	13,7 NS	9,4 NS	10,0 NS	0
X + N	1,75a	1,35	16,0	13,4	9,3	10,0	0
2X	1,66ab	1,50	15,4	14,4	9,4	9,6	0
2X + N	1,48b	1,28	15,1	13,3	10,3	10,4	0
0.5X	1,67ab	1,49	15,6	14,4	9,4	9,7	0
0.5X + N	1,86a	1,48	16,5	14,8	8,9	10,1	0

Foliar application of MAP and Ca-arsenate resulted in a decrease in citric acid and an increase in the sugar to acid ratio (Figure 4.2.3.4). Ca-arsenate was the most effective in reducing citric acid with MAP (0.8% and 1.5%) less effective, but still numerically better than the control. The °Brix was not affected (data not shown) indicating the direct influences on the increase in the ratio can be attributed to the lower acid content. The fruit were not negatively affected from a rind condition perspective, and they were fully coloured and showed no increase in susceptibility to disorders (data not shown). The change in citric acid and ratio was not significant at 95%, however the increase in ratio from 8 to 10 is commercially significant.

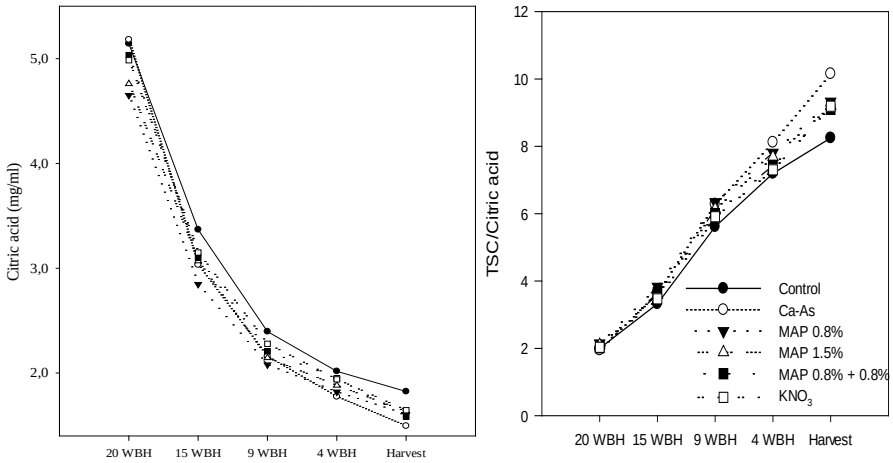


Figure 4.2.3.4. The citric acid content, ratio of °Brix:Citric acid as measured with the automatic titrator during fruit development in 2015 after foliar applications (see legend). WBH = weeks before harvest.

Microscopic analysis of the Mandarin fruit rind

Low magnification images of the cuticle, epidermis, flavedo and albedo layers of 'nadorcott' mandarin fruit rind, following storage at -0.6 °C for 30 days, projected the rind structure as intact with well-compacted cells, showing no signs of cell disruption or collapse in either epidermal or flavedo cells (Fig. 4.2.3.5A). Fruit that was harvested from trees which received the additional 40 kg·ha^{-1} N soil application prior to harvest showed no cellular damage occurring within the rind sections, but provided images of a healthy and gradual transition from densely packed flavedo cells to loosely congregated albedo cells. Rind breakdown lesions manifest in 'nadorcott' mandarin fruit as clearly collapsed regions with the flavedo and albedo (Fig. 4.2.3.5B). A similar condition was reported by Alquézar et al. (2010) on 'navelate' oranges which were stored postharvest at various relative humidity, which ranged from 45% to as high as 95% RH. Our studies also reported collapsed oil glands of 'nadorcott' mandarin fruit which developed pitting lesions after 30 days of storage at -0.6 °C (Fig. 4.2.3.5C). Alférez et al. (2003) stated that occasionally with these rind disorders the underlying oil glands remain intact, or at least during the initial stages of the disorder, before further collapsing in the flavedo-albedo intercellular zone, in-conjunction with the collapsed oil gland (Fig. 4.2.3.5D). The affected cells appear twisted and compressed, forming a layer of collapsed cells between healthy intact cells of the flavedo and albedo. Leakage of essential oils from oil glands in the flavedo proceeds to oxidize the albedo and then finally the epidermis, where oxidized tissue characteristically is detected as dark-brown coloured areas.

After the scanning electron microscope (SEM) gave inconsistent readings on the direct impact of Ca and Mg on rind due to large variation within the rind, Fluorescence SEM microscopy was investigated as a method to

determine whether consistent results could be obtained to visually show the location and distribution of Ca and Mg in the rind. By using two florescent dyes it was attempted to quantify Ca Mg concentration and distribution in citrus rind. A protocol for the use of fluorescent dyes was evaluated but no effective method was developed even though initial results with a calcium dye (fura-2, pentapotassium salt) gave promising results (Figs. 4.2.3.6-8). The magnesium fluorescent dye that was used were mag-fura-2, tetrapotassium salt but did not result in any results.

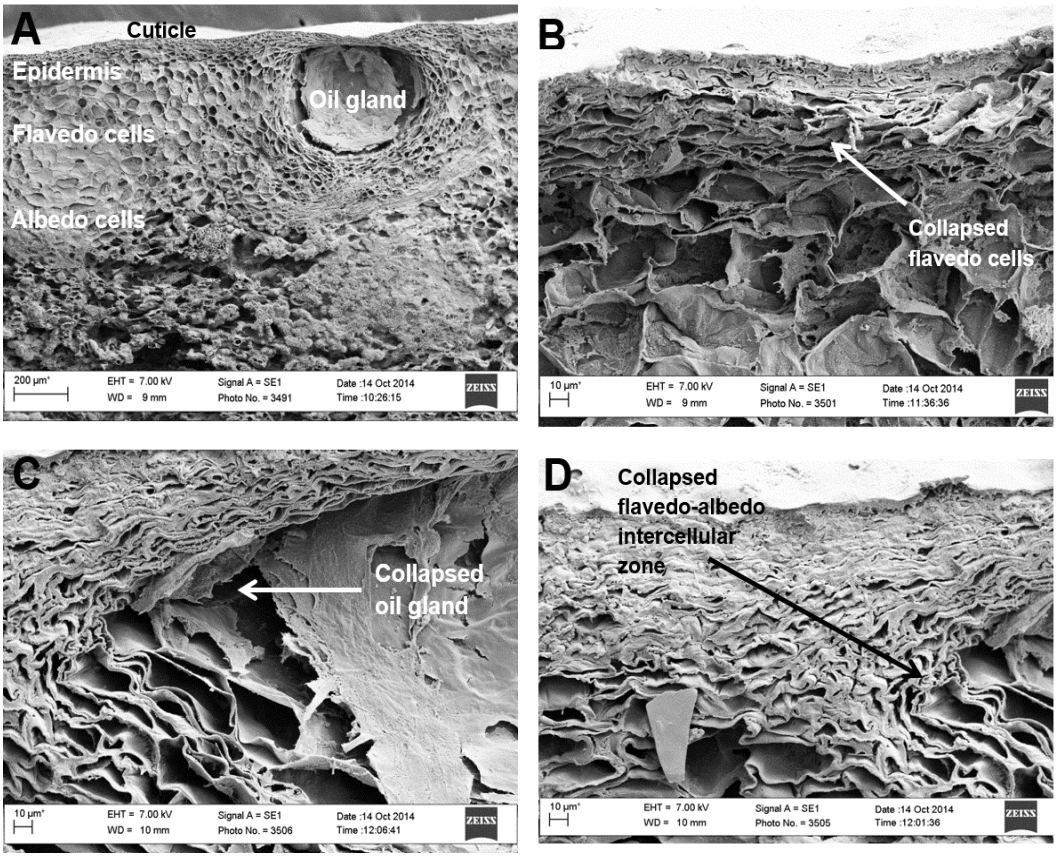
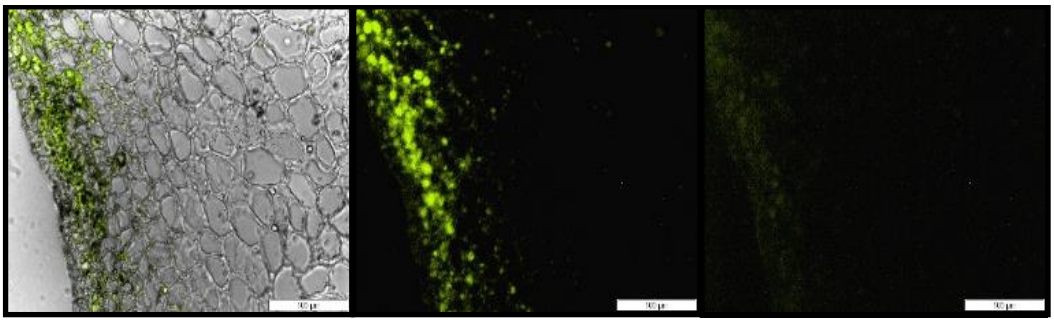


Fig. 4.2.3.5A-D. Scanning electron microscopy (SEM) comparative cross sections of different cellular sections of 'nadorcott' mandarin fruit rind after storage at -0.6 °C for 30 days, showing either no rind disorder lesions (A) or where it manifested with distinct rind disorder lesions (B-D). Fruit, produced in Riebeeck Kasteel in 2014, were subjected to the additional application (Early- 21 Jan. 2014; Late- 26 March 2014) of various soil N LAN treatments prior to harvest. **A.** No cellular damaged occurred within the rind sections of fruit which received a Late LAN soil application at a rate of 40kg.ha^{-1}. The gradual transition from the densely packed flavedo cells to the loosely congregated albedo cells is visible; **B.** Rind breakdown lesions, with collapsed flavedo and albedo sections evident; **C.** A collapsed oil gland which developed into a pitting lesion after cold storage; **D.** A collapsed flavedo-albedo intercellular zone, in conjunction with a disintegrated oil gland.



Figures 4.2.3.6-8. The Ca fluorescent dye stained (fura-2, pentapotassium salt) rind sections (Figs. 6-7. Fura340 with CFP filter; Fig. 8. Fura340 with UBG filter) using Carl Zeiss LSM 780 confocal microscope.

Postharvest pitting of Valencia orange

Postharvest pitting of 'Benny' Valencia orange increased by 90%, compared to fruit that were kept at a constant high RH and did not receive the rehydration postharvest stress (Figure 9). This significant increase in pitting is the first recorded data on 'Valencia' orange and a vital step forward in developing a strategy to reduce the incidence of pitting on a large scale. Over the two seasons, differences between the same cultivars at 2 or more orchards in their susceptibility to pitting injury were recorded, indicative of the problematic nature of identifying and quantifying the various contributing sources of variation to pitting susceptibility.

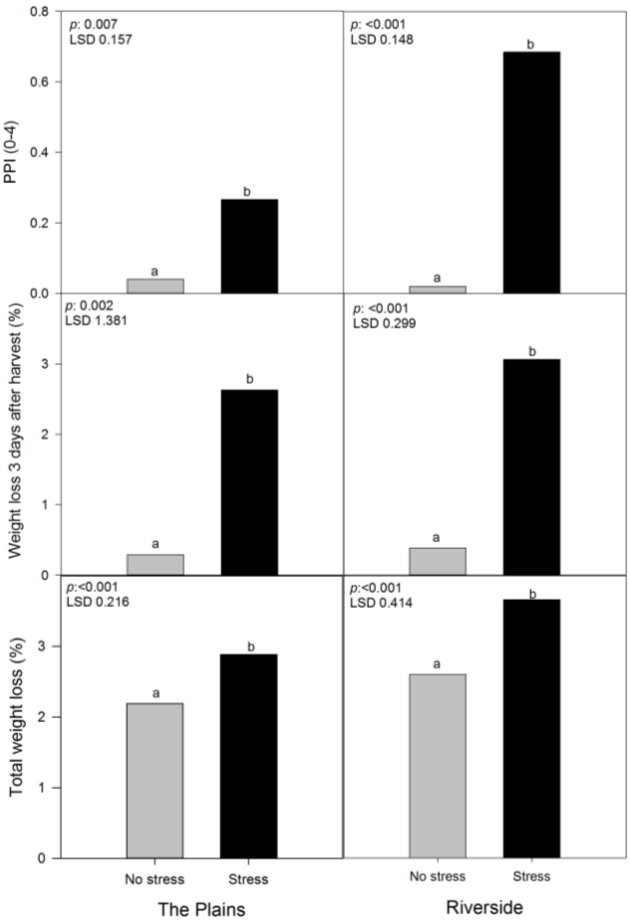


Figure 4.2.3.9. Post-harvest pitting index (PPI) (0-4) of 'Benny' Valencia fruit evaluated after 20 days' storage at 4.5°C. Weight loss 3 days after harvest (%) and total weight loss (%) after 21 days of storage at 4.5°C of fruit that received the stress treatment (stressed) and fruit which did not receive the stress treatment (no stress). Different letters indicate significant difference at 95 % level ($p \leq 0.05$).

The results over the two seasons and application in multiple orchards show a novel use for various plant growth regulators to reduce the incidence of pitting in Valencia orange and possibly Navel orange as well. The application of 2,4-D (10 mg·L^{-1}) at 50% petal drop or after physiological fruit drop, reduced incidence of post-harvest rind pitting by 38% and 56% respectively (Figure 4.2.3.10). Application of ABA (400 mg·L^{-1}) (Protone®) and TBZ (4000 mg·L^{-1}) (ICA-TBZ®) 14 days before harvest reduced the incidence of this disorder by 43% and 51% respectively (Figure 4.2.3.11). No negative impacts due to the foliar sprays in the form of chemical burn or loss of fruit quality were recorded. These results offer a new direction in the possible control of the incidence of this postharvest physiological disorder by reducing susceptibility prior to harvest.

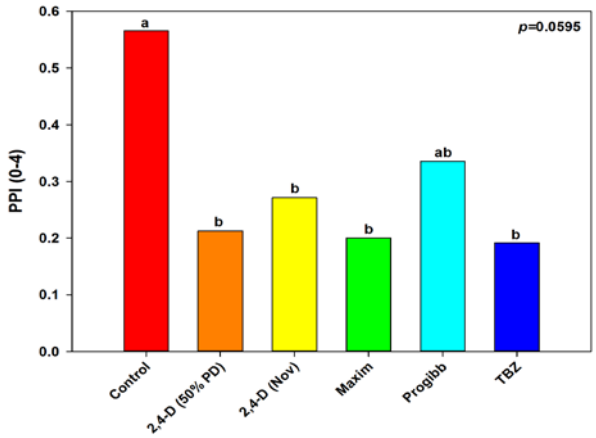


Figure 4.2.3.10. Post-harvest pitting index (PPI) (0-4) of 'Benny' Valencia fruit treated with four different PGR's (2,4-D, Maxim, Progibb and TBZ) as foliar sprays. Different letters indicate significant difference at 95 % level (p≤0.05)

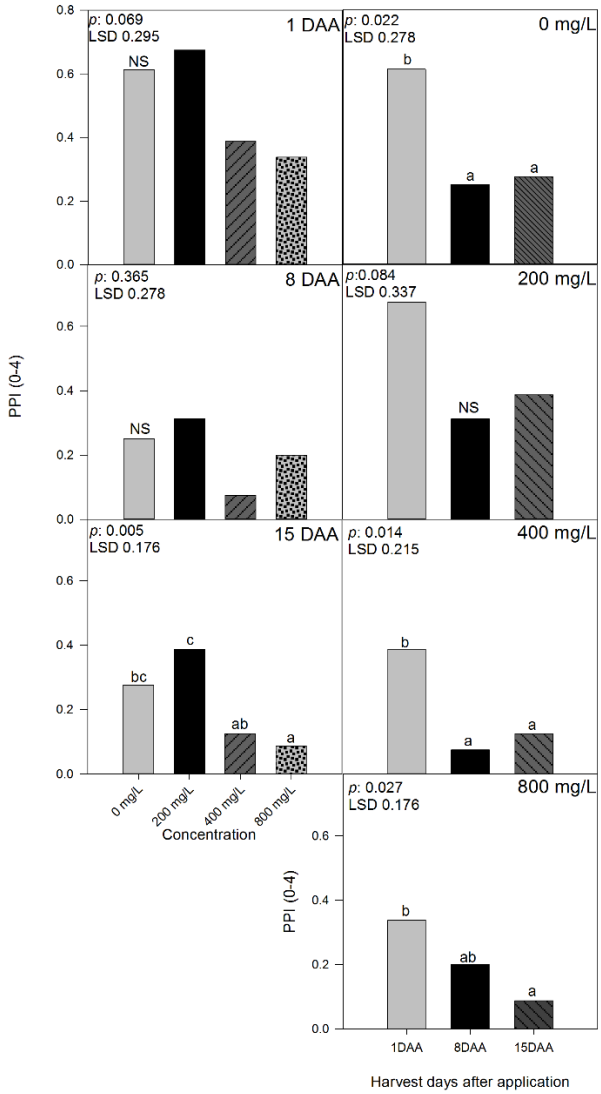


Figure 4.2.3.11. Post-harvest pitting index (PPI) (0-4) of 'Benny' Valencia fruit harvested 1 Day after application (DAA), 8 DAA and 15 DAA, treated with four different concentrations of s-ABA 0.0g·L^{-1}, 0.2g·L^{-1}, 0.4g·L^{-1} and 0.8g·L^{-1}. Different letters indicate significant difference at 95 % level (p≤0.05). NS=No significant difference at 95 % level (p≤0.05)

The application of TBZ reduced post-harvest rind pitting when applied before the dehydration/rehydration stress treatment. This trend was evident in all experiments, however, only when the severity of this disorder was sufficiently high enough did TBZ significantly reduce this disorder (Figure 4.2.3.12).

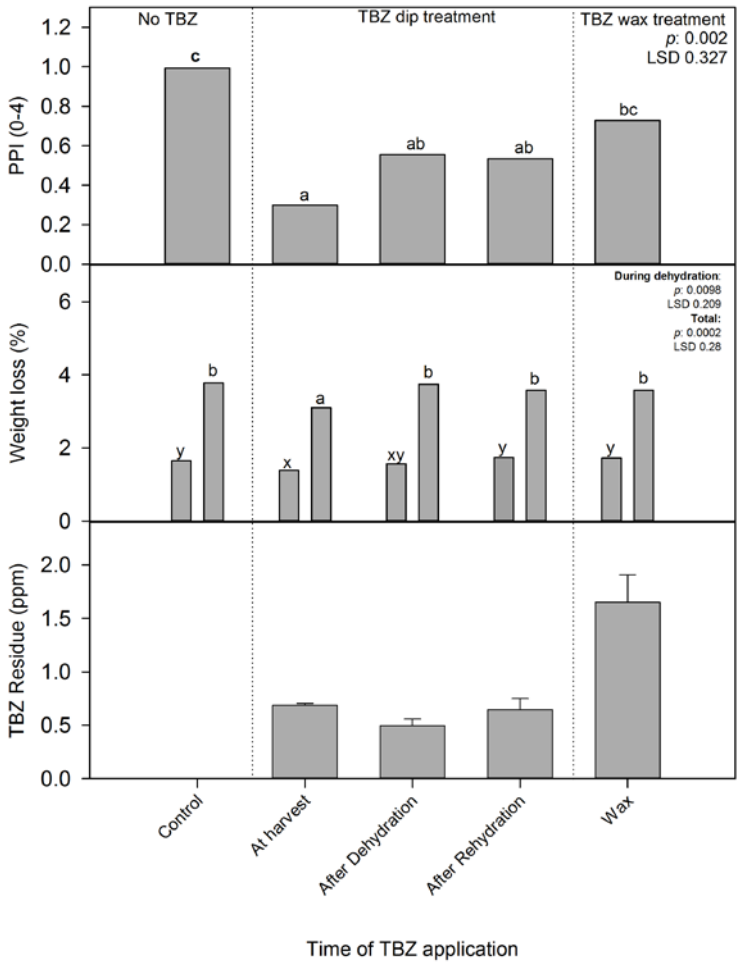


Figure 4.2.3.12. Post-harvest pitting index (PPI) (0-4), weight loss (%) during three day's dehydration, total weight loss (%) during post-harvest handling and thiabendazole (TBZ) residue analysis with standard error of fruit treated with TBZ post-harvest at different times throughout a stress treatment. Different letters indicate significant difference at 95 % level ($p \leq 0.05$).

Financial impact of pitting of Valencia orange- sensitivity analysis of pitting in various export markets

Based on assumptions and information obtained from the CIS 2015, the average cost to produce a carton of 'Valencia' oranges for export was R 63.31 during the 2014 season. This included costs associated with production, packing and transport to an inland port. The average cost to produce a bin of fruit for the local market was R 522.50. The estimated DIP price and income for a carton of 'Valencia' orange fruit in September without pitting, with low or high pitting incidence is summarised in Table 4.2.3.6. The highest price for a carton of fruit with no pitting was obtained in the UK market followed by the EU and lastly the ME market. The price realised per carton dropped below the cost to produce and deliver a carton if fruit had low pitting incidence, with the highest reduction in price in the UK market. As pitting incidence increased, the realised price was reduced even more and higher losses were incurred (Figure 4.2.3.13). On the local market an estimated loss of R 97.50 and R 137.50 occurred when a bin of fruit was sold for fresh or processing use, respectively. This is a loss of R 5.62 or R 10.78 per carton fruit, respectively.

As the percentage pitting increased, so did the potential losses that could occurred in the market. In the most severe scenarios *viz.* 3 and 4, more than half of the potential income of the hypothetical farm was lost due to pitting incidence. However, even though the price reduction was greatest in the UK market, the highest income for each scenario was when 100 % of fruit were sent to the UK (Table 4.2.3.6).

Table 4.2.3.6. Estimated income and potential losses incurred in four hypothetical scenarios due to pitting at EU, ME and UK markets.

	Scenario			
Pitting severity	**1**	**2**	**3**	**4**
Zero	100	80	60	50
Low (15 %)	0	10	20	30
High (30 %)	0	10	20	20
	Income (Rand)			
EU	3 946 987	2 857 534	1 768 081	1 312 287
ME	3 245 031	2 303 360	1 361 688	1 016 819
UK	7 485 001	5 166 732	2 848 464	1 726 721
	Losses (Rand)			
EU	0	-1 089 453	-2 178 906	-2 634 699
ME	0	- 941 672	-1 883 344	-2 228 212
UK	0	-2 318 268	-4 636 537	-5 758 279

Source: Own calculations from CIS, 2015.

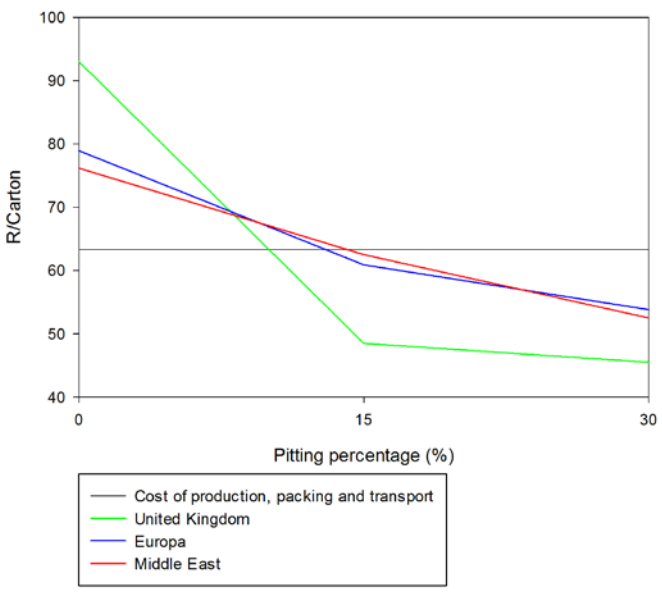


Figure 4.2.3.13. Cost of production, packing and transport of one carton 'Valencia' orange until inland harbour. Delivered in port (DIP) price of one carton of 'Valencia' oranges at United Kingdom, Europa, and Middle East markets with different levels of pitting incidence.

Discussion and conclusion

Pitting of Mandarin citrus fruit

The nitrogen trial was conducted in an attempt to clarify whether late N fertilization viz. 20 and 40 kg·ha^{-1} early (Jan.) and late (March) has a negative impact on rind quality. If no negative impact exists, it would enable producers to increase N-levels in trees which could potentially improve flower quality. This is difficult to do via the standard N foliar application on 'nadorcott' which is harvested late in the season. In general, no significant differences in fruit colour or size were recorded across the areas and season studied. Furthermore, no consistent differences were seen among the N treatments after the fruit had been stored at either -0.6°C or 4°C. Mineral nutrient analysis recorded that N applications did not increase the N levels in the leaves, indicating that these N amounts used (which were chosen on advice from nutrition consultants) could be increased in future projects to determine a threshold level. From this study, the expected decrease in rind colour due to increased nitrogen application was therefore not confirmed. Although the incidence of pitting was recorded, it was generally at levels too low for all the treatments to warrant being included in the data analysis and therefore

could not be correlated with N treatments. However, the lack of disorders did indicate that these two treatments (N and storage temperature) did not lead to higher pitting susceptibly. It was also not possible to link pre-harvest rind mineral content with the eventual incidence of rind pitting during subsequent storage, with additional N applications. The interaction between N application and irrigation indicates that available soil moisture plays a role in N uptake. Studies on post-harvest disorders are made extremely difficult due to their erratic (and low) incidence between seasons. Within this study certain correlations could be made between factors influencing rind quality (climatic influence, moisture loss and colour) and rind disorder incidence of mandarin fruit. However, no single factor could be identified as the main underlying cause of rind disorders. Even though some results lack explanation, the "induction" of rind disorders in some instances by stressing the fruit pre-harvest or the lack of expected decreased rind colour due to increased nitrogen application, can be regarded as a positive result. These data can, therefore, help serve in unravelling the factors predisposing the citrus fruit rind to progressive post-harvest disorders.

Post-harvest pitting of Valencia oranges

Post-harvest pitting of Valencia oranges is a physiological rind disorder aggravated by changes in post-harvest RH and temperature. Waxing of fruit has been shown not to be the cause of pitting, but can enhance the incidence of the disorder. Large variation exists between cultivars, seasons, farms, within orchards and within trees. During this study it became clear that this cultivar is highly influential in determining pitting susceptibility with 'Benny' Valencia being more susceptible than 'Turkey' Valencia. More mature fruit on the outside of the canopy seems to be more affected, possibly due to more exposure to changes in environmental conditions resulting in increased water stress pre-harvest. This aspect should receive more focused research attention. The synthetic auxins 2,4-D applied at 50 % PD or after physiological fruit drop and 3,5,6-TPA applied after physiological fruit drop significantly reduced the disorder when applied at 10 $mg \cdot L^{-1}$, possibly due to increased sink strength, increased transport of water and nutrients and increased cell growth of the rind. In a novel approach, the application of s-ABA and the fungicide TBZ reduced the disorder. An application of 10 $mg \cdot L^{-1}$ 2,4-D or 3,5,6-TPA after physiological fruit drop is recommended to reduce susceptibility of fruit. In addition, fruit should be packed and placed within the cold chain as soon possible to minimise post-harvest environmental variation and water stress, resulting in dehydration followed by rehydration of the rind which results in pitting development in susceptible fruit. Pre- or post-harvest application of the systemic fungicide TBZ before stress related to environmental conditions, reduced post-harvest pitting of 'Benny' Valencia oranges. Post-harvest dip treatment at 2000 $\mu g \cdot mL^{-1}$ for 1 minute directly after harvest reduced incidence of this disorder. In contrast TBZ applied in wax after stress treatment did not reduce incidence of this disorder. The mode of action of TBZ to reduce this disorder is unknown, however, a reduction in weight loss was found when fruit were treated before dehydration. This could be due to changes in epicuticle wax structure and morphology brought on by adjuvants added to TBZ solution. In future more attention should be given to the effect of TBZ on cuticle wax, as well as how to implement TBZ application in commercial practices to have the largest reduction in post-harvest pitting.

GENERAL CONCLUSION

Even though the symptom development or lesions of rind pitting look similar, the mechanism responsible for the cellular collapse is probably unrelated in the different cultivars, even within cultivar groups such as mandarin and Valencia orange groups. In general, it is seen that high moisture loss during the time from pick to pack is problematic and influences pitting incidence. From these results the indications are that TBZ does have a clear mitigating impact on pitting and should be used as advised. To reduce potential financial losses due to post-harvest pitting, producers should first determine the likelihood or susceptibility of fruit to develop post-harvest pitting. This should be done for each orchard by factoring in knowledge on cultivar, orchard history and environmental conditions and then attempt to minimise this risk through management practices such as reducing time between harvest, application of TBZ and optimising the cold chain. Secondly, after the risk of fruit to develop pitting has been determined, the producer and exporter should formulate a strategy in order to manage supply of fruit to markets with a certain risk attached to them. This is necessary as the highest rate of return was found in a high risk market i.e. the UK, with a known zero to little tolerance for this disorder. Fruit from orchards with a history of developing pitting should then rather be exported to the ME or EU markets as even though lower prices are realised in these markets, the penalty is less if the fruit develop pitting prior to arrival. If a specific orchard has been identified as a very high risk it would make more financial sense to sell the fruit at the pack-house to the local market for a substantially lower loss than what would be incurred if fruit were exported.

TECHNOLOGY TRANSFER

- Cronje, P.J.R. 2015.Prevention of pitting in citrus. 27-30 Jan. (Polokwane, Loskopdam), 11-13 Feb (Port Elizabeth, Durban), 17-18 (Stellenbosch)

- Cronje, P.J.R. 2016. Prevention of pitting in citrus. 29-311 Jan. (Polokwane, Loskopdam), 13-14 Feb (Port Elizabeth, Durban), 20-21 (Stellenbosch)

HUMAN RESOURCES DEVELOPMENT/TRAINING

From this project two MSc Agric (Horticulture) theses were written and are available on the University of Stellenbosch library website for download.

- **Jacques Louis Ehlers** Post-harvest rind pitting on 'Valencia' Oranges: http://hdl.handle.net/10019.1/98751
- **Jeanine Joubert** Influence of rind water content on mandarin citrus fruit quality. http://hdl.handle.net/10019.1/98607
- **Helen Marias,** will complete her MSc in Horticulture by March 2017 on this project.

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4.2.4 **FINAL REPORT: Non-destructive prediction and monitoring of postharvest rind quality of citrus fruit using Vis/NIR spectroscopy**
Project PHI 02/2014 (Jan 2015 – Dec 2016) by Lembe Magwaza (University of KwaZulu-Natal)

Summary

The overall aim of the study was to develop non-destructive methods to predict the quality of citrus fruit. This project was set out to validate the ability of visible to near infrared spectroscopy (Vis/NIRS) models to predict rind and internal quality of citrus fruit. The study consisted of three experiments. The first experiment examined the relationships between fruit canopy position, fruit physico-chemical composition and susceptibility to rind physiological disorders during postharvest storage. Fruit from inside and outside the canopy were harvested from South Africa's major citrus fruit producing provinces, KwaZulu-Natal, Limpopo, Mpumalanga, Eastern and Western Cape. Secondly, models for predicting internal and external quality parameters of 'Marsh' grapefruit were developed. Thirdly, the relationship of parameters to rind disorders were also examined as a way to predict the possibility that fruit could develop rind disorders. The results obtained in this study confirmed the role that pre-harvest conditions, notably canopy position, play in maintaining the rind and internal quality of mandarins, oranges and grapefruit. Grapefruit that grew inside the canopy in the two orchards in Limpopo and KwaZulu-Natal, had higher chilling injury incidence (27% and 24%) compared to their counterparts outside the canopy (12% and 0%). In both sites, rind pitting was lower on inside (13% and 2% respectively) than outside canopy fruit (19% and 9%).

We successfully developed Vis/NIRS models to predict internal quality parameters and susceptibility of fruit to rind disorders based on total antioxidant capacity, carotene, total carotenoids, chlorophyll a, chlorophyll b, dry matter, sucrose, glucose and fructose. Rind pitting, for example, was predicted with an accuracy of 78%.

This study provided baseline information to improve understanding of how canopy position affects rind quality and antioxidant composition, which is hypothesised to play a role in grapefruit ability to withstand postharvest rind physiological disorders. Robust, stable and accurate models for integrated prediction of citrus fruit internal

quality and susceptibility to rind physiological disorders were developed. The ability of Vis/NIRS coupled with chemometric analysis to cluster fruit based on original canopy position was recognised and recommended as a secondary approach to discriminate fruit, during sorting and packaging, with high chances of developing RP, since its occurrence was high on fruit from outside canopy.

Introduction

Citrus is the biggest component of South African exported subtropical fruit. Globally, the South African citrus industry is ranked the third largest exporter of fresh citrus fruit after Spain and Turkey, respectively (Citrus Growers' Association of Southern Africa, 2012), worth over 6.5 billion Rand (ZAR) ($733 million; April 2013) annually (Bonorchis, 2013). The position of the industry in the international market is strengthened by the production of a wide range of cultivars over an extended period of time - from March through to November. However, citrus fruit are prone to develop various types of progressive physiological rind disorders, manifested by a multitude of symptoms during handling and storage (Magwaza et al., 2013a). The amount of fruit affected by these disorders can reach up to 60% of total production in the worst seasons (Agustí et al., 2001). Limited knowledge of the physiological mechanisms underlying these disorders hinders the development of cost-effective solutions to minimize losses, and assure a consistent supply of quality fruit.

Among physiological rind disorders affecting citrus, include chilling injury, rind pitting of oranges and grapefruit, rind breakdown disorder (RBD) of 'nules Clementine' mandarins and peteca spots of lemons (Magwaza et al., 2013a,b). Although these physiological disorders are superficial and do not compromise the edible internal portion of the fruit, they, however, dramatically decrease fruit market value, since external appearance is the primary specification used to evaluate the quality of fresh citrus fruit (Alquezar et al., 2010). One of the main difficulties related to rind physiological disorders such RBD is its progressive nature. The disorder does not manifest during harvest or fruit grading in the packing house, but only starts developing symptoms during storage, about three to five weeks postharvest (van Rensburg and Bruwer, 2000; van Rensburg et al., 2004; Cronje et al., 2011a, b; Magwaza et al., 2013b). The manifestation of RBD symptoms during the later stages of postharvest handling is, in essence, the main difficulty that faces the industry and importers alike, because if fruit are shipped over long distances, symptoms of the disorder usually coincide with the commercial shipping period and/or point of sale. This is extremely problematic as rind disorders can lead to tremendous financial losses and customer complaints.

In order for the South African citrus industry to maintain its competitive edge and increase its market share in the lucrative export markets, there is a need to develop non-destructive methods that can be used to monitor and predict citrus fruit susceptibility to rind physiological disorders. Among non-destructive quality assessment techniques, Vis/NIRS is arguably the most advanced with regard to instrumentation, applications, accessories, and chemometric software packages. As such, Vis/NIRS and hyperspectral spectroscopy has become one of the most used candidates for non-destructive evaluation of a wide range of postharvest quality assessments of fruit and vegetables (Magwaza et al., 2012a). Therefore, the overall aim of the study is to develop non-destructive methods to predict the quality of citrus fruit.

Objectives

- Validate and test the robustness of Vis/NIRS PCA and PLS models developed for 'Nules Clementine' mandarin fruit to predict rind quality 'Benny Valencia' oranges and 'Marsh' grapefruit.
- Investigate robustness of Vis/NIRS PCA and PLS models developed for Western Cape Province to predict rind quality of citrus fruit harvested from other citrus growing regions of South Africa.
- Investigate the feasible of hyperspectral spectroscopy to characterise rind quality and structural changes associated with chilling injury, rind breakdown and rind pitting disorders.

Materials and methods

Experiment 1

This study was conducted to examine the relationships between fruit position within the tree canopy, fruit physico-chemical composition and susceptibility of 'Marsh' grapefruit to rind physiological disorders during postharvest storage. Fruit from inside (IC) and outside canopy (OC) were harvested from two of South Africa's major grapefruit producing provinces, KwaZulu-Natal and Mpumalanga.

During cold storage, 'Marsh' grapefruit were scored biweekly for the incidence of rind pitting, over eight weeks. RBD incidence was scored on a subjective scale from 0 = no breakdown to 3 = severe breakdown. Figures 4.2.4.1, 2 and 3, respectively show examples of scoring of rind pitting, stem-end rind breakdown and decay observed during the study.

Figure 4.2.4.1. Different levels of rind pitting on 'Marsh' grapefruit after 9 weeks of postharvest storage. Left to right shows increasing intensity; from no (0), little (1), moderate (2) and severe rind pitting (3).

Figure 4.2.4.2. Different levels of stem-end rind breakdown on 'Marsh' grapefruit after 9 weeks of postharvest storage. Left to right shows increasing intensity of stem-end rind breakdown; from no (0), little (1), moderate (2) and severe (3).

Figure 4.2.4.3. Different levels of decay on 'Marsh' grapefruit after 9 weeks of postharvest storage at ±0.5 °C. Left to right shows increasing intensity; from no (0), little (1), moderate (2) and severe (3) decay.

Experiment 2

Initial studies on grapefruit and orange fruit were conducted to determine the optimum condition for Vis/NIRS measurements and to evaluate the accuracy of this technique and associated chemometric analysis. In this

study, the capability and conditions of visible to near infrared spectroscopy to non-destructively quantify BrimA, TSS, TA and TSS: TA ratio of intact 'Valencia' orange and 'Star Ruby' grapefruit was evaluated.

The research was carried out during 2014/15 season using 'Valencia' orange (*Citrus sinensis*) and 'Star Ruby' grapefruit (*Citrus x paradisi* Macfad). A total of 120 grapefruit and 120 oranges were randomly picked, mixing all possible sizes of mature fruit, from two orchards at Olifant's River Estates, a commercial citrus farm, located at Hoedspruit in Limpopo Province, South Africa (24°23'39.02"S; 30°49'20.65"E). Harvested fruit were transported in ventilated vehicle to the Postharvest Technology Laboratory of the University of KwaZulu-Natal where experiments for Vis/NIRS and destructive analyses were performed. Upon arrival at the laboratory, fruit were equilibrated at room temperature (21±1 °C; 65±1% RH) for 24 h before Vis/NIR spectra was acquired. Vis/NIR spectral data was acquired in reflectance mode using a laboratory bench-top monochromator NIR Systems Model XDS spectrometer (FOSS NIR Systems, Inc.; Maryland, USA) equipped with a quartz halogen lamp and lead sulfide (PbS) detector. The chemometric analysis was performed using Vision software (Vision® Single and Multi-User ("S/M") Version 3.5.0.0, FOSS NIRSystems, Inc.; Maryland, USA). Reference measurements and spectral datasets were subjected to partial least square (PLS) regression analysis.

Experiment 3

In this study of the capability of Vis/NIR and hyperspectral spectroscopy to predict RP, spectral data was acquired from fruit just after harvest. 'Marsh' grapefruit (*Citrus x paradisi* Macfad) for this study was harvested during 2015/2016 season. A total of 240 mature fruit, mixing all possible sizes, were harvested from inside or outside canopy position of randomly selected trees from the following two orchards: Hoedspruit in Limpopo Province (24°23'39.02"S; 30°49'20.65"E) and at Enkwalini in KwaZulu-Natal province (32°75'28.S; 35°89'31.E), South Africa. The fruit were transported with a ventilated vehicle to postharvest physiology laboratory of the University of KwaZulu-Natal where analysis was done. Upon arrival at the laboratory, fruit treatments simulating commercial chain were applied. The data was acquired in reflectance mode using a laboratory bench-top monochromator Vis/NIR Systems Model XDS spectrometer equipped with a quartz halogen lamp and lead sulfide (PbS) detector. The data was correlated with the visual scores of RP disorder development after six weeks in cold storage and a week in shelf life. PLS regression models were developed using Unscrumbler® chemometric software.

Results and discussion

Experiment 1

The results show that the physico-chemical properties of fruit are affected by position in the canopy (Figure 4.2.4.4). The fruit inside the canopy were greener and less mature and weighed less than fruit on the outside. Fruit position within the canopy had a significant effect ($p < 0.01$) on fruit susceptibility to chilling injury (Figure 4.2.4.5) and rind pitting (Figure 6) physiological disorders.

The difference was aligned with parameters determining physiological status of fruit, which differed with fruit canopy position on the tree. Fruit that grew inside the canopy in the two orchards in Limpopo and KwaZulu-Natal, had higher chilling injury incidence (27% and 24%) compared to their counterparts outside the canopy (12% and 0%) (Figure 4.2.4.5). In both sites, rind pitting was lower on inside (13% and 2% respectively) than outside canopy fruit (19% and 9%) (Figure 4.2.4.6). Fruit from inside the canopy had higher concentrations of sucrose, fructose (Figure 4.2.4.7) and antioxidant activity (Figure 4.2.4.8), and lower dry matter, compared with outside canopy fruit.

The relationships (correlations) of parameters to rind disorders were also examined for the possibility to develop techniques for pre-symptomatic prediction of the disorders. Parameters with positive correlations to rind pitting did not show a positive correlation to chilling injury, which prompted the idea of using fruit parameters to predict the possibility of the disorders (Figure 9). Although the correlations of parameters to disorders were weak, it was important that the relationship, whether negative or positive, of each parameter and the disorders, was explored. Fruit from different canopy position had different susceptibility to disorders and the content of physico-chemical parameters differed significantly. PCA-based discrimination of fruit from different canopy positions was also successful. This can enable the prediction of parameters related to disorders for estimation of disorder developing chances.

This study further examined the effect of canopy position on rind biochemical composition of 'nules Clementine' mandarin fruit during postharvest cold storage. The composition of these compounds in flavedo and albedo of the rind were separately examined. Fruit from inside canopy (IC) and outside canopy (OC) were harvested during 2015 season from commercial farms in Eastern Cape (EC) and Western Cape (WC) Provinces in South Africa. Fruit were cold stored at 7.5 ± 0.5 ºC for nine weeks and data were taken every three weeks on both

flavedo and albedo of the rind. Results showed that CP had significant ($p < 0.05$) effects on total carotenoid content (TCC), DM and chlorophyll an of the fruit rind, with OC fruit having more of the compounds than IC fruit. The radical scavenging activities (RSA, 59.7, 67.2, 58.6, 51.3) % and TCC (13.0, 15.7, 11.6, 14.4) µg/g DW of the flavedo are higher compared to albedo (49.3, 51.3, 51.1, 50.1) % and (9.0 13.1, 10.0, 10.1) µg/g DW at weeks 0, 3, 6, 9, respectively. However, total carbohydrates and vitamin C are more concentrated in the albedo than flavedo throughout the period of storage. Overall, the rind biochemical composition of the fruit declined postharvest but its antioxidant status still prevented the incidence of rind physiological disorder throughout the period of storage as none was recorded throughout the experiment.

This study provided baseline information to improve understanding of how canopy position affects rind quality and antioxidant composition, which is hypothesised to play a role in grapefruit ability to withstand postharvest rind physiological disorders.

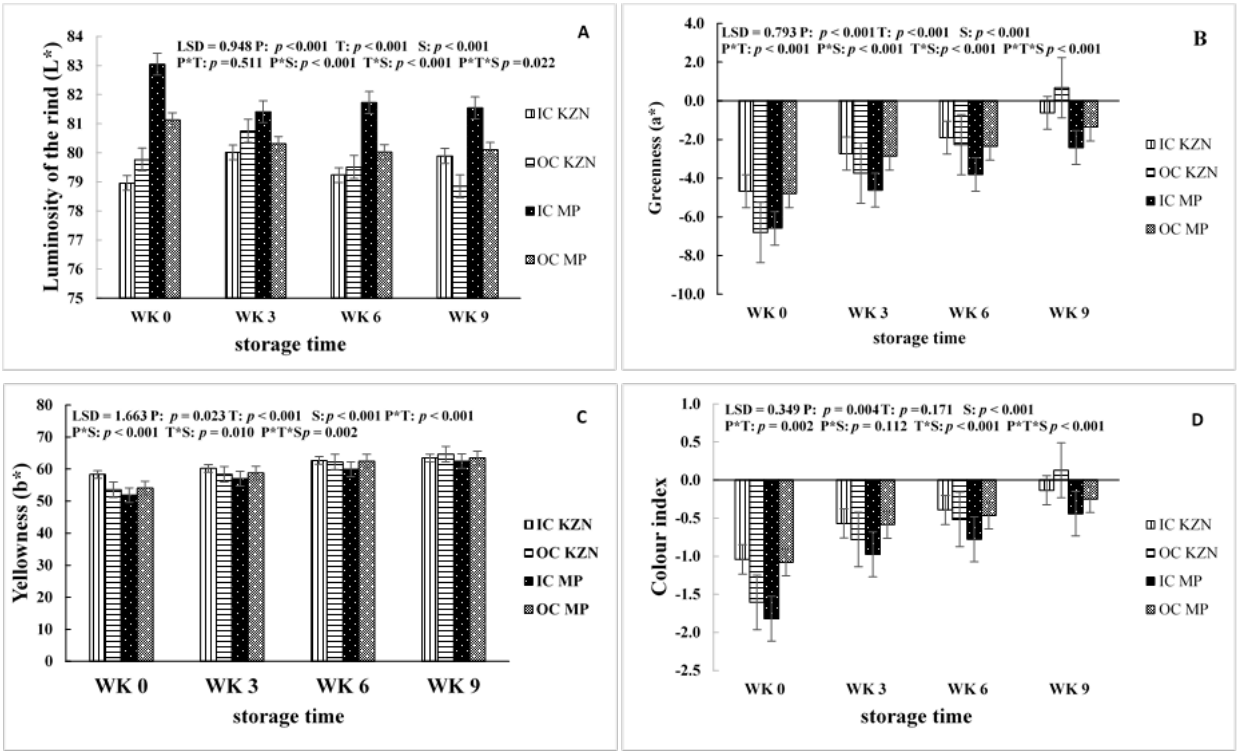

Figure 4.2.4.4. Effect of canopy position (inside canopy (IC) and outside canopy (OC)), on luminosity (L*) (A), greenness (a*) (B), yellowness (b*) (C) and colour index (D) of 'Marsh' grapefruit from KwaZulu-Natal (KZN) and Mpumalanga (MP) provinces during postharvest non-chilling storage (Weeks 0, 3, 6 and 9). LSD: least significant difference; P: canopy position; T: postharvest storage time; S: production location; * represent an interaction between factors.

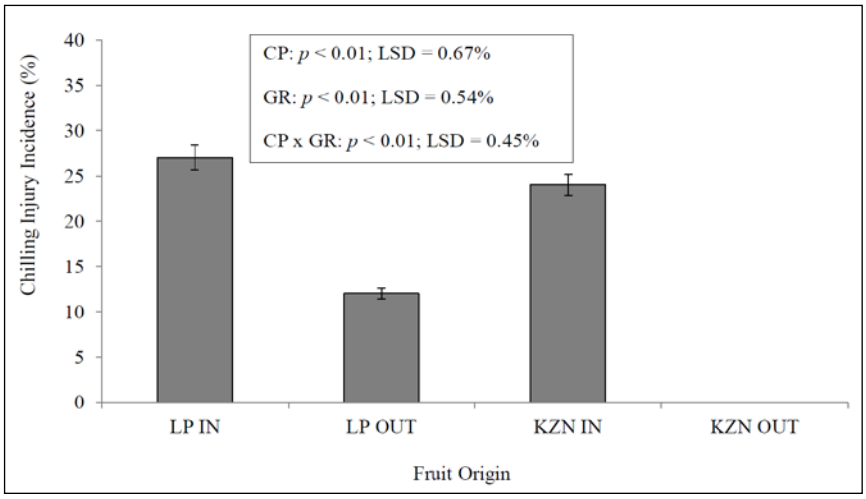

Figure 4.2.4.5. The chilling injury incidence difference based on fruit origin. Data presented as mean ±standard deviation (SD); LP, Limpopo; LP IN, fruit from inside canopy of the orchard in Limpopo; LP OUT, fruit from

outside canopy position of the orchard in Limpopo; KZN, KwaZulu-Natal; CP, canopy position; GR, growing region.

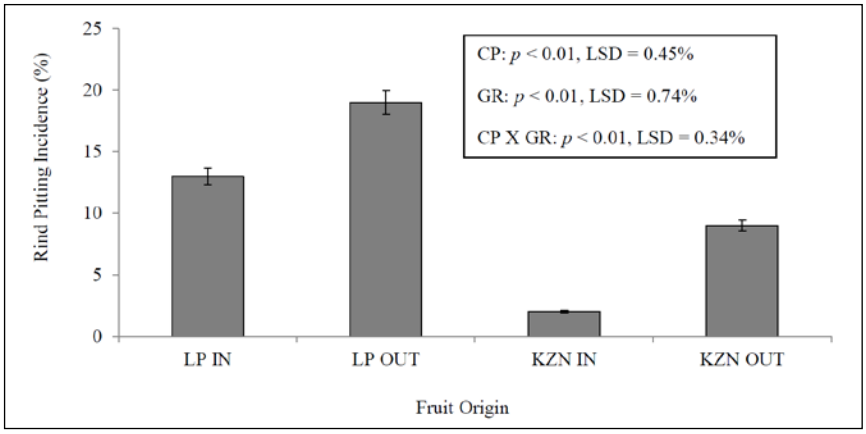

Figure 4.2.4.6. The Rind pitting incidence difference based on fruit origin and canopy position. Data presented as mean ± 5% standard error; LP, Limpopo; LP IN, fruit from inside canopy of the orchard in Limpopo; LP OUT, fruit from outside canopy position of the orchard in Limpopo; KZN, KwaZulu-Natal; CP, canopy position; GR, growing region.

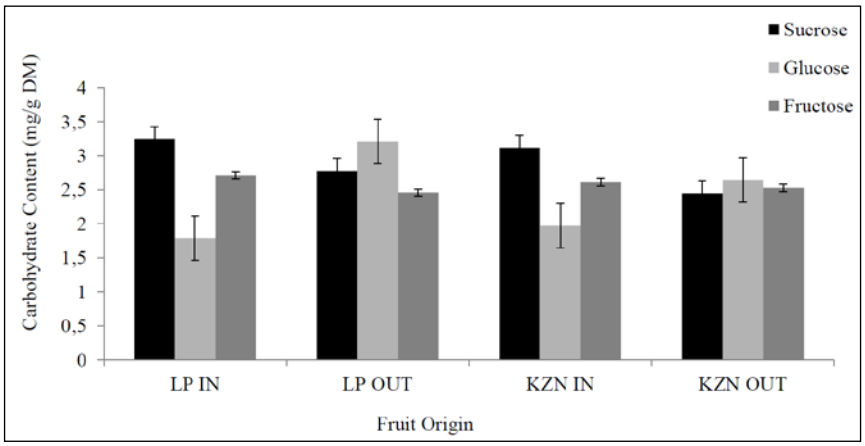

Figure 4.2.4.7. The amount of rind non-structural carbohydrates of fruit from different origins. Data presented as mean ± SD; LP, Limpopo; LP IN, fruit from inside canopy of the orchard in Limpopo; LP OUT, fruit from outside canopy position of the orchard in Limpopo; KZN, KwaZulu-Natal; DM, dry matter basis; CP, canopy position; GR, growing region.

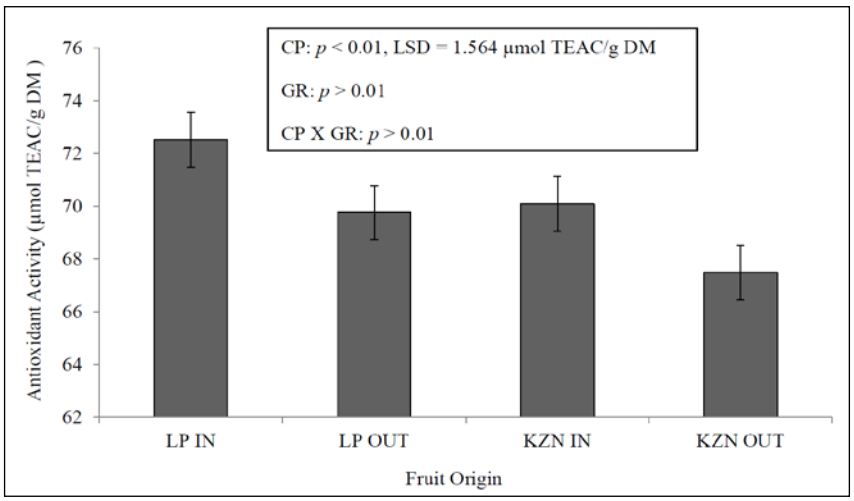

Figure 4.2.4.8. Trolox equivalent antioxidant capacity (TEAC) based on DPPH scavenging activity of 'Marsh' grapefruit from different origins based on canopy position. Data presented as mean ± standard error; LP, Limpopo; LP IN, Limpopo inside canopy; LP OUT, Limpopo outside canopy; KZN, KwaZulu-Natal; DM, dry mass basis; CP, canopy position; GR, growing region.

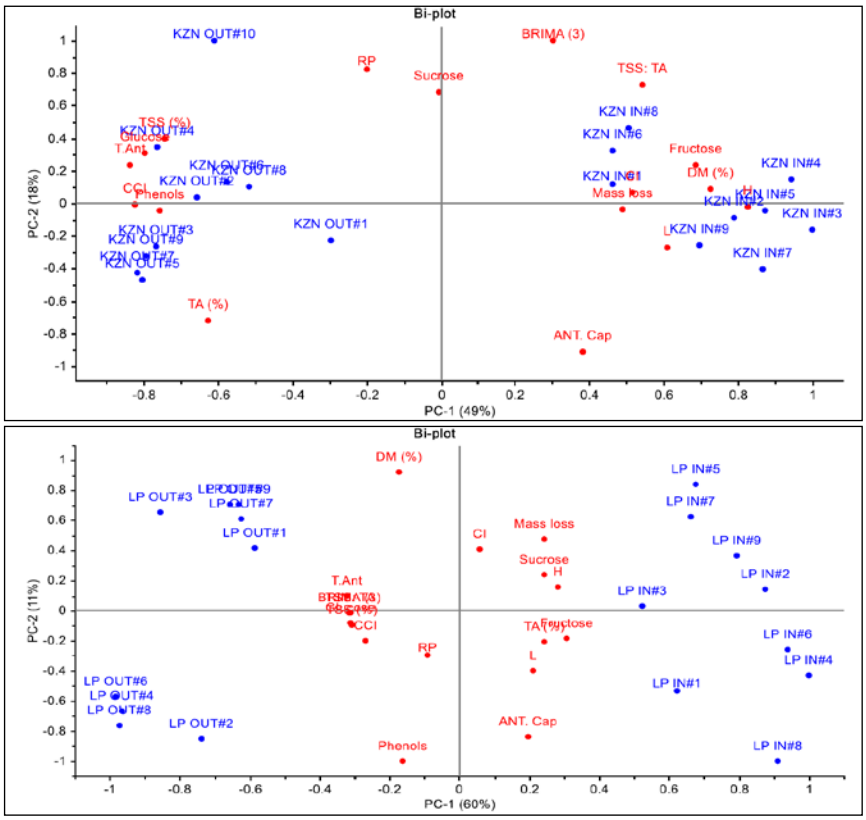

Figure 4.2.4.9. The principal component analysis-based correlation of parameters to fruit origin. KZN, KwaZulu-Natal; KZN IN, KwaZulu-Natal inside canopy fruit, KZN OUT, KwaZulu-Natal outside canopy fruit; LP, Limpopo; LP IN, Limpopo inside canopy fruit, LP OUT, Limpopo outside canopy fruit; DM (%), percentage rind dry matter; CI, chilling injury; H, hue angle; L, Lightness; TA (%), titratable acidity percentage; ANT. Cap, antioxidant capacity; RP, rind pitting; CCI, citrus colour index; T. Anti, total antioxidant capacity; TSS (%), total soluble solutes

Experiment 2

The prediction statistics obtained from best models developed for all sweetness and flavour quality parameters of both 'Valencia' oranges and 'Star Ruby' grapefruit are presented in Table 4.2.4.1. The best prediction models were observed for BrimA of 'Valencia' oranges with the coefficient of determination (R^2) = 0.958; root mean square error of prediction (RMSEP) = 0.006 and residual predictive deviation (RPD) = 3.96, followed by TSS: TA ratio (R^2 = 0.958; RMSEP = 0.605; RPD = 4.92). Good models for predicting flavour of grapefruit were also attained, with TSS having the best model (R^2 = 0.896, RMSEP = 0.308 and RPD = 2.94), followed by BrimA (R^2 = 0.858; RMSEP = 0.429; RPD = 2.45) (Table 4.2.4.2). Scatter plots of the relationships between conventionally measured quality parameters and those predicted using NIR models developed for 'Valencia' oranges are presented in Figure 10. These results demonstrated the ability of Vis/NIRS to non-destructively predict sweetness and flavour attributes of oranges and grapefruit. Vis/NIRS was recommended as a possible fast and accurate technique to be used for fruit discrimination based on flavour parameters during packing and for pricing of fruit in the market.

Table 4.2.4.1. The statistics of the values predicted by models chosen based on a higher prediction of 'Valencia' orange parameters.

Parameter	Mean Pred	$Mean_{ref}$	R^2	SD_{pred}	SD_{ref}	$CV\%_{pred}$	$CV\%_{ref}$	RPD	RMSEC	RMSEP	Bias
TSS	10.24	10.24	0.927	1.053	1.01	10.29	9.91	3.57	0.283	0.283	0.027
TA	0.66	0.66	0.929	0.063	0.066	9.58	9.95	3.88	0.017	0.017	0.002
TSS:TA	15.48	15.48	0.958	2.480	2.534	16.02	16.37	4.92	0.515	0.605	0.049
BrimA3	8.18	8.18	0.957	1.326	1.357	16.22	16.58	4.83	0.281	0.011	0.027
BrimA4	7.52	7.52	0.957	1.089	1.081	14.34	14.23	3.92	0.276	0.006	0.026
BrimA5	6.93	6.93	0.958	1.104	1.096	15.93	15.81	3.96	0.277	0.006	0.027

TSS, total soluble solutes; TA, titratable acidity; Mean $_{pred}$, mean of predicted data; Mean $_{ref}$, mean of reference data values; R^2, correlation coefficient between Vis/NIRS predicted and measured values; SD $_{pred}$, Standard deviation of predicted data values; SD $_{ref}$, standard deviation of the reference data; RPD, residual predictive deviation; CV% $_{ref}$, the coefficient of variation for the reference data; CV% $_{pred,}$ the coefficient of variation for the predicted data; BrimA3,4,5, BrimA with *k* = 3,4 and 5 respectively; RPD, residual predictive deviation; RMSEC, root mean square error of calibration; RMSEP, root mean square error of prediction.

Table 4.2.4.2. Statistics of the values predicted by models chosen based on the higher prediction accuracy of 'Star Ruby' grapefruit parameters.

Parameter	$Mean_{Pred}$	$Mean_{Ref}$	R^2	SD_{pred}	SD_{ref}	$CV\%_{pred}$	$CV\%_{ref}$	RPD	RMSEC	RMSEP	Bias
TSS	10.32	10.32	0.896	0.910	0.958	9.28	8.79	2.94	0.308	0.308	0.021
TA	1.430	1.431	0.835	0.149	0.163	10.40	13.66	2.46	0.066	0.066	0.003
TSS:TA	7.310	7.311	0.812	0.939	1.042	12.84	14.25	2.32	0.449	0.451	0.027
BrimA3	6.041	6.041	0.858	0.902	0.974	14.93	16.13	2.68	0.364	0.429	0.022
BrimA4	4.608	4.608	0.846	0.965	1.049	20.94	22.77	2.45	0.410	0.411	0.025
BrimA5	3.217	3.224	0.810	0.973	1.077	30.24	33.40	2.19	0.498	0.498	0.029

TSS, total soluble solutes; TA, titratable acidity; BrimA3, 4, 5, BrimA with *k* = 3, 4 and 5 respectively; Mean $_{pred}$, mean of predicted data; Mean $_{ref}$, mean of reference data values; R^2, correlation coefficient between Vis/NIRS predicted and measured values; SD $_{pred}$, Standard deviation of predicted data values; SD $_{ref}$, standard deviation of the reference data; RPD, residual predictive deviation; CV% $_{ref}$, the coefficient of variation for the reference data; CV% $_{pred,}$ the coefficient of variation for the predicted data; RPD, residual predictive deviation; RMSEC, root mean square error of calibration; RMSEP, root mean square error of prediction.

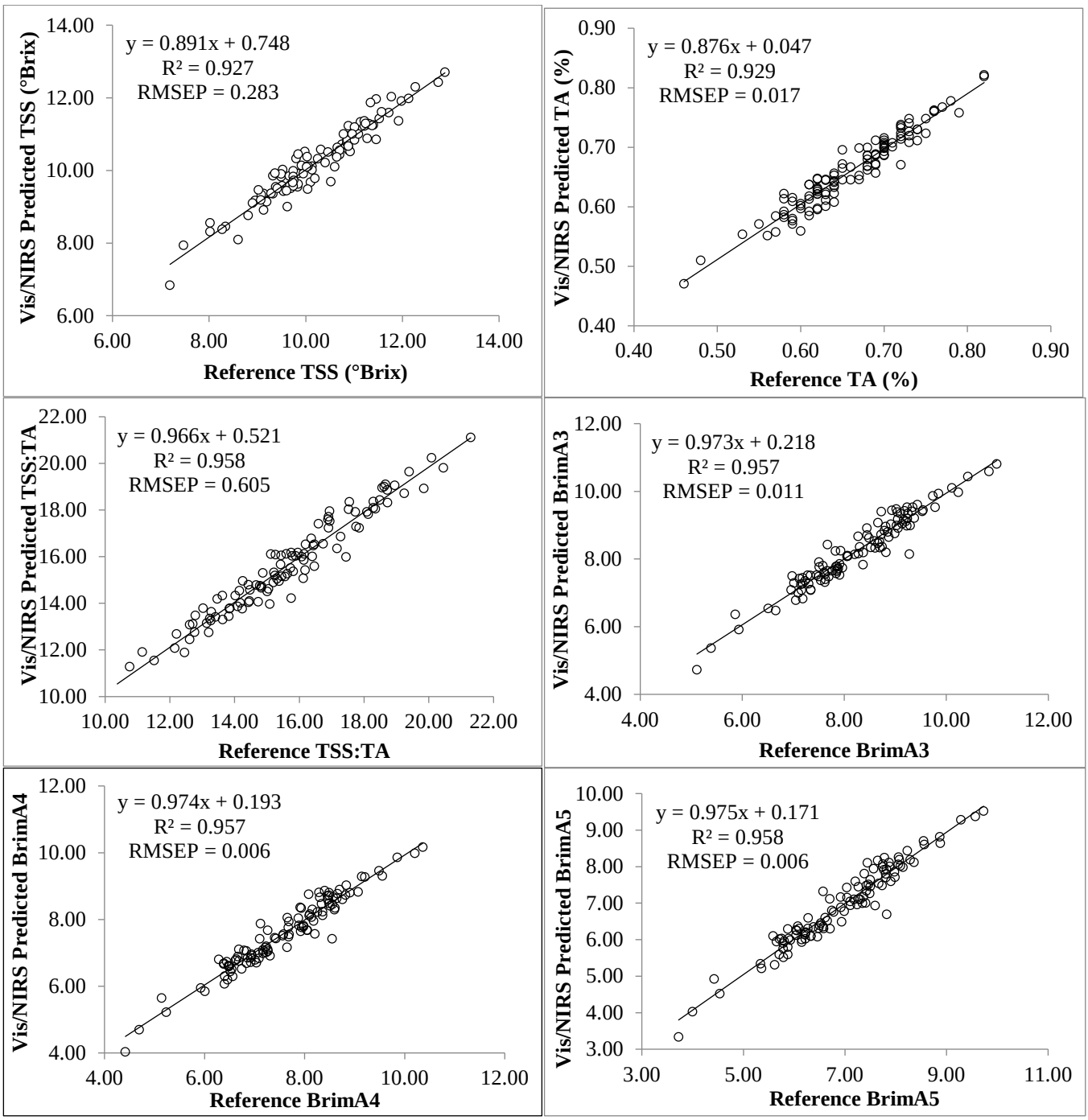

Figure 4.2.4.10. Scatter plot of Vis/NIRS predicted versus measured or calculated values of 'Valencia' oranges Total Soluble Solutes (TSS) (A), Titratable Acidity (TA) (B), TSS: TA ratio (C), BrimA with k = 3(BrimA3) (D), BrimA4 (E) and BrimA5 (F). RMSEP, root mean square error of prediction; R^2, the regression coefficient of Vis/NIRS predicted data to reference data; Vis/NIRS, visible to near infrared spectroscopy.

Experiment 3

Good prediction of RP was obtained (R^2_p = 0.78; RPD = 2.03; RMSEP = 1.41) (Figure 4.2.4.11). Prediction models of rind quality parameters successfully developed included total antioxidant capacity (RMSEP = 0.04; R^2_p = 0.95), β carotene (RMSEP = 0.002; R^2_p = 0.99), total carotenoids (RMSEP = 2.51; R^2_p = 0.92), chlorophyll a (RMSEP = 0.008; R^2_p = 0.89), chlorophyll b (RMSEP = 0.008; R^2_p = 0.93), dry matter (RMSEP = 0.297; R^2_p = 0.88), sucrose (RMSEP = 0.021; R^2_p = 0.91), glucose (RMSEP = 0.013; R^2_p = 0.93) and fructose (RMSEP = 0.023; R^2_p = 0.94). PLS regression models for internal quality parameters were also developed successfully. Principal component analysis (PCA) scores and loadings for the first two principal components (PCs) displayed sample projection using spectra acquired from 'Benny Valencia' orange from different canopy positions (Figure 4.2.4.12). Clear separation was observed demonstrating the effects of canopy position on spectral and physico-chemical properties of orange fruit. The first two PCs explained 98% (PC1 = 95%, PC2 = 3%) of the

total variance. These clusters allowed distinction between fruit from different preharvest canopy positions with an accuracy of 98%. The distribution of the clusters clearly showed that the canopy position effect was directed along the first PC and a very small amount (3%) contributed by the second PC. Similar clusters with the accuracy of 100% (99% for PC1 and 1% from PC2) were also observed in the PCA similarity map developed using Vis/NIRS signal of 'Marsh' grapefruit (Figure 4.2.4.13). The ability of Vis/NIR and hyperspectral spectroscopy coupled with chemometric analysis to cluster fruit based on original canopy position (Figures 4.2.4.12 and 13) was recognised and recommended as a secondary approach to discriminate fruit, during sorting and packaging, with high chances of developing RP, since its occurrence was high on fruit from outside canopy.

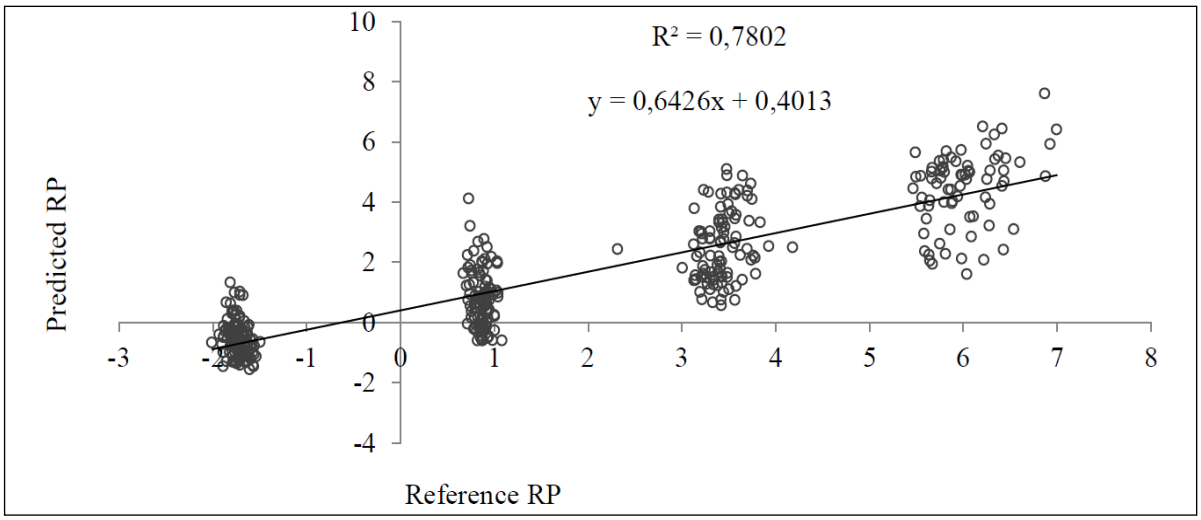

Figure 4.2.4.11. The correlation of rind pitting values predicted using Vis/NIRS compared to subjective visual scoring.

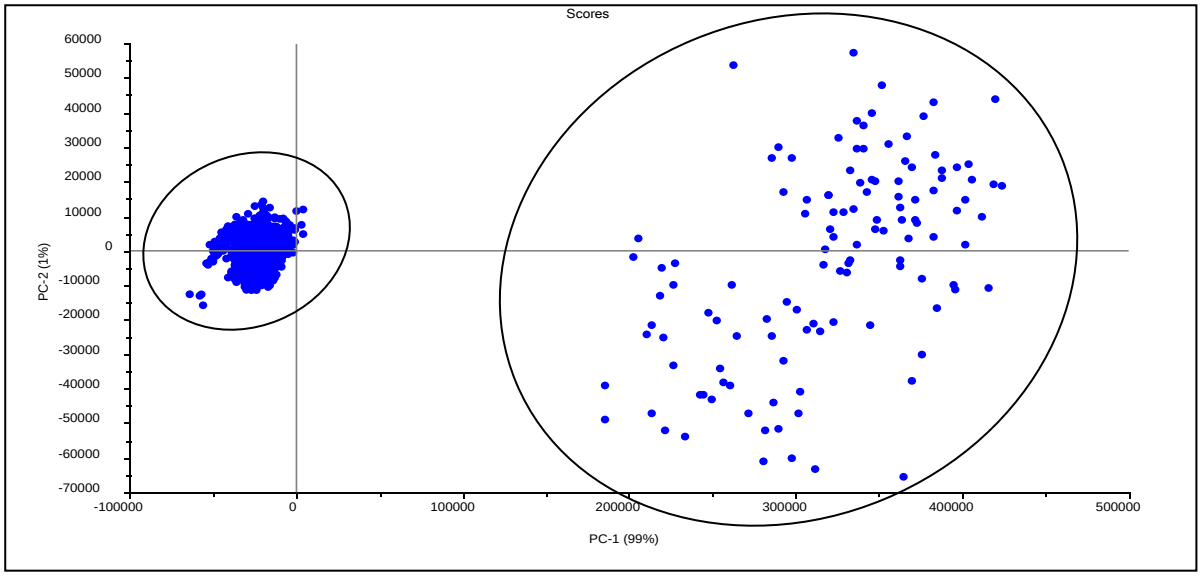

Figure 4.2.4.12. The principal component analysis (PCA) similarity map determined by principle components (PCs) 1 and 2 showing clear clusters of 'Valencia' oranges harvested from different canopy positions.

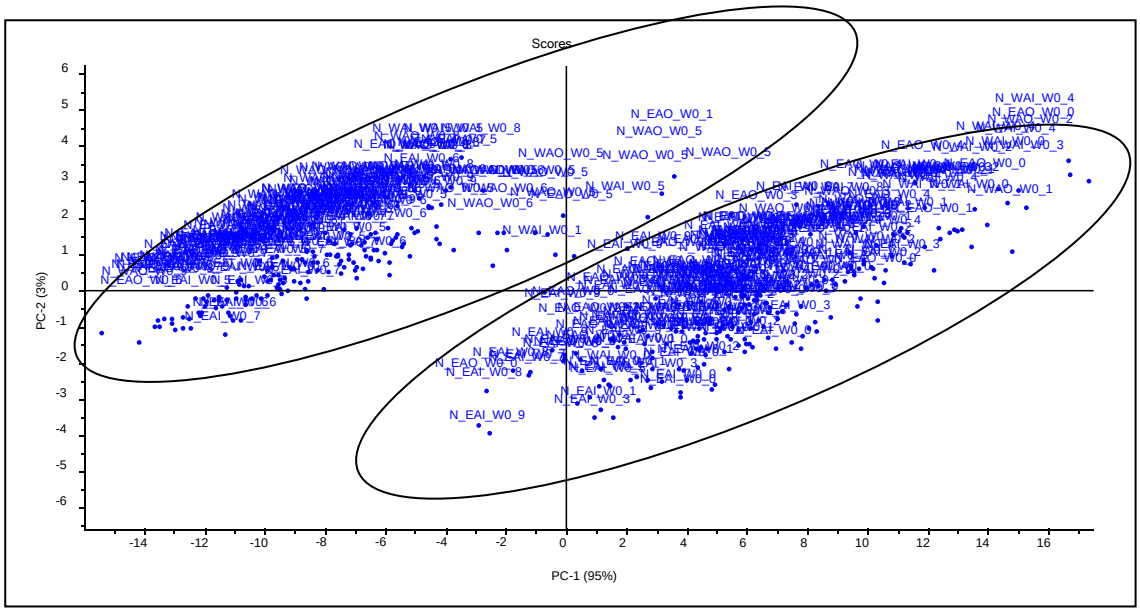

Figure 4.2.4.13. Principal component analysis (PCA) similarity map determined by principle components (PCs) 1 and 2 showing clear clusters of 'Marsh' grapefruit harvested from different canopy positions.

Conclusion

This study showed a significant difference on fruit susceptibility based on canopy position. Fruit from inside canopy suffered higher CI and fruit from outside canopy suffered higher RP. The disorder occurrence was aligned with the difference in a microclimate that the fruit developed in. Fruit from inside canopy had higher rind moisture content probably because of lower exposure to sunlight and reduced aeration inside the tree. Therefore, when those fruit got damaged it resulted to CI which was characterised by affected parts of rind becoming water soaked patches. When fruit from outside canopy got damage, they suffered from RP which was characterised by dry patches of dead cells on the flavedo.

Rind physiological disorders of 'Marsh' grapefruit, 'nules Clementine' mandarins and 'Benny Valencia' oranges were predicted successfully. The models for predicting internal and rind parameters were also developed. PCA-based fruit spectra grouping technique was recommended as a secondary tool for canopy based fruit discrimination during sorting and packaging. The technique may be applied in/online with the fact that 'Marsh' grapefruit from outside canopy showed higher susceptibility to rind pitting.

Vis/NIR spectroscopy and hyperspectral spectroscopy were explored as tools to predict rind physiological disorders. The tools did not only predict the pitting but also successfully predicted rind quality parameters. The combination of Vis/NIRS, to non-destructively collect accurate and fruit-specific spectra, and chemometric software to specify and cluster spectra into groups based on fruit origin was also noticed.

The grouping technique was highly recommended as it allows canopy based discrimination during sorting and packaging. Canopy-based sorting is good news to farmers and postharvest managers as it assures that most of their harvest reaches a consumer. Fruit with higher chances of developing disorders may be sent to local markets for eliminating cold storage exposure that normally takes place during shipping or they can be processed to other products such as juices, dried fruits and sweets which have a lifespan longer than the optimum shelf life of fresh fruit.

Future research

Now that these models have been developed and validated for accuracy, stability and robustness, funds for the manufacturing of a prototype will be acquired for a practical and semi-commercial in-line application of Vis/NIR spectroscopy. We will be approaching manufacturers of fruit sorting, sizing and grading equipment such as MafRoda Robotics RSA and Compac® to help manufacture a semi-commercial packing line.

Technology transfer

a) PRESENTATIONS/PAPERS DELIVERED

Magwaza LS. (2016) Near Infrared (NIR) Spectroscopy applications for external and internal quality analysis of citrus fruit: What we know and future prospects. Presentation at the 9th Citrus Research Symposium at the Champagne Sports Resort in the Drakensberg, 21st to 25th August 2016.

Magwaza LS. (2016) Visible to near Infrared (Vis/NIR) spectroscopy applications for external and internal quality analysis of citrus fruit: What we know and future prospects. Presentation at the 2nd Postharvest Innovation Symposium at Spier Wine Farm, Stellenbosch, 21st to 22nd November 2016.

Ncama K, Magwaza LS, Fawole OA, Tesfay SZ, Opara UL. (2016) Non-destructive prediction of rind pitting and quality of 'Marsh' grapefruit (*Citrus x paradisi* MacFad) using Vis/NIR spectroscopy. Presentation at the 2nd Postharvest Innovation Symposium at Spier Wine Farm, Stellenbosch, 21st to 22nd November 2016.

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4.2.5 FINAL REPORT: Investigating cold storage potential of new mandarin citrus selections/cultivars and the effect of ethylene degreening on rind disorders

Project PHI 64/2015 (Jan 2015 – March 2017) by Nhlanhla Mathaba (ARC)

Summary

The main objective of the study was to evaluate the effect of ethylene degree and cold sterilization on internal and external quality of new mandarin citrus selections/cultivars. To achieved this objective, degreened and non-degreened 'Sonnet, Nova and Nova ARC, B17, I22 and M37' mandarin citrus fruit were sourced for Addo research farm in the Easter Cape during 2015 and 2016 harvest season. Mandarin fruits (Sonnet, Nova and Nova ARC, B17, I22 and M37) were ethylene degreened (3 ppm), 90% RH, and 20-22°C for 72 hours, thereafter, waxed with polyethelyene citrus wax (Citrushine ®), packed and transported to ARC-TSC in Nelspruit. At ARC-TSC laboratory, fruit were re-packed into smaller boxes, which contained fifty fruit per boxes, with three boxes per storage temperature (-0.5, 2.0 and 4.5°C) for 28 days. After withdrawal for cold storage, fruit were kept at ambient temperature for 7 days to allow for the development of rind disorders, mainly chilling injury. In the previous studies (2014 and 2015), only selection 'M37' showed severe external chilling symptoms, irrespective of degreening and storage temperature. However, selections/cultivars 'I22 and Nova' showed external chilling damage at -0.5 and 2.0°C during the 2016 season, irrespective of degreening treatment. Thus, selection M37 was chilling susceptible irrespective of season, while 'I22 and Nova' seem to be chilling susceptible, depending on seasonality. Furthermore, chilling susceptible selections/cultivars showed significant firmness and weight loss, and increased electrolyte leakage after withdrawal from cold storage.

Opsomming

Die hoofdoel van hierdie studie was om die effek van etileenontgroening en koue sterilisasie op eksterne en interne vrugkwaliteit van nuwe sitrus mandaryn seleksies/kultivars te ondersoek. Om hierdie doelwit te bereik was ontgroende en nie-ontgroende vrugte van die 'Sonnet', 'nova', 'nova ARC', 'B17', 'I22' en 'M37' seleksies/kultivars verkry vanaf die Addo Proefplaas, geleë in die Oos-Kaap, gedurende die 2015 en 2016 oesseisoene. Al die vrugte van die genoemde seleksies/kultivars was ontgroen vir 72 ure lank met 3 dpm etileen by 90% RH en 20 tot 22°C. Daarna was die vrugte gewaks met sitrus polietileen waks (Citrushine®), verpak en vervoer na die LNR-TSG in Nelspruit. By die LNR-TSG na-oes laboratorium was die vrugte herverpak in kleiner kartonne om vyftig (50) vrugte per karton te bevat. Vrugte is daarna vir 28 dae opgeberg by -0.5, 2.0 en 4.5°C met drie kartonne per opbergingstemperatuur. Na die koue-opbergings periode was vrugte verwyder en vir 7 dae by kamertemperatuur gehou om die ontwikkeling van skilafwykings, veral koueskade, toe te laat. Slegs seleksie 'M37' het in vorige studies (2014 en 2015) hewige koueskade ontwikkel, ongeag van ontgroening of opbergingstemperatuur. Maar gedurende 2016 het kultivar/seleksie 'I22' en 'nova' ook eksterne koueskade gekry by -0.5 en 2.0°C, ongeag van die ontgroeningsbehandeling. Dit wil dus voorkom of seleksie 'M37' altyd vatbaar is vir koueskade terwyl die vatbaarheid van 'I22' en 'nova' seisoenaal is. Verder was gevind dat seleksies/kultivars wat vatbaar was vir koueskade ook betekenisvolle fermheids- en

gewigsverlies getoon het, asook ʼn toename in elektronverlies getoon het nadat dit van koue-opberging verwyder was.

Introduction

The South African Citrus fruit must be cold sterilized against fruit fly, specifically against the new species *Bactrocera dorsails* fruit fly. However, citrus fruit are chilling susceptible, including 'Sonet' the new ARC-ITSC selection, 'B17' and 'B24', 'I22' and 'M37', ʼnova', ʼnova Seedless', 'Clemcott', 'Clemcott Seedless', ʼnadorcott', 'Tango', 'Mandalate'. These selections will increase the competitiveness of the South Africa industry, especially early selection. Chilling injury is a physiological rind damage, which manifests as dark spots or lesions, thereby reducing marketability of fruit after storage (McLauchlan *et al*., 1997). Furthermore, such findings were confirmed on ʼnules Clementine' mandarins with or without ethylene during degreening, whereby -0.5°C had significantly low chilling injury compared with fruit stored at 7.5°C (Cronjé *et al*., 2011). Therefore, to increase competitiveness of the South African citrus industry, new cultivars such as Sonet, the new ARC-ITSC selection; B17 and B24, I22 and M37 must be introduced. In addition, the storage behaviour and response to subzero temperatures (-0.5°C) must be investigated, as well as potential methods to mitigate any potential rind disorders.

Objectives

- To establish if ethylene degreening has an effect on the development of rind disorders on newly bred mandarin selections 'Sonet', B17 and B24, I22, M37, Nova, Nova Seedless and Nadorcott after cold sterilization.
- To establish the effect of cold sterilization on internal quality parameters of newly bred mandarin selections Sonet, B17 and B24, I22, M37, Nova, Nova Seedless and Nadorcott after cold storage.

Materials and methods

Degreened and non-degreened 'Sonet', ʼnova' and ʼnova ARC', 'B17', 'I22' and 'M37' citrus fruit were sourced from Addo farm in the Eastern Cape. Fruits were ethylene degreened (3 ppm), >90% RH, and 20-22°C for 72 hours, and afterwards waxed with polyethylene citrus wax (Citrushine ®, Johannesburg, South African) (Cronje *et al*., 2011), packed and transported to laboratory of the ARC-ITSC in Nelspruit. At ARC-ITSC laboratory, fruit were re-packed into smaller boxes which contains fifty (50) fruit per box, with three boxes per storage temperature (-0.5, 2.0 or 0.4°C) for up to 28 days. After withdrawal from cold storage, fruit were kept at ambient temperature for 7 days to allow for the development of rind disorders, mainly chilling injury. Fruits were evaluated for the following physicochemical parameters: chilling injury, fruit weight loss, electrolyte leakage, juice total soluble solids, titritable acidity and rind colour.

Results and discussion

In the 2015 season, cold sterilization had no effect on physical and juice quality of evaluated selections/cultivars, with an exception of selection 'M37', irrespective of degreening and cold storage temperature (Table 4.2.5.1). However, selections/cultivars 'I22' and 'Nova' also showed external chilling symptoms during the 2016 season as did 'M37' mandarin fruit (Table 4.2.5.1). Therefore, 'M37' mandarin fruit were chilling susceptible irrespective of season, while 'I22' and 'Nova' mandarin fruit susceptibility is season dependent (Figure 4.2.5.1 and Table 4.2.5.1). In both seasons (2015 and 2016), manifestation of chilling symptoms seems to correlate with high fruit weight loss and electrolyte leakage, irrespective of degreening treatment. Furthermore, high fruit weight loss for chilling susceptible mandarin selections/cultivars 'M37, I22 and 'Nova' was associated with increased Brix after withdrawal from cold storage. Moreover, increased Brix during storage can also be due to organic acids being used for energy production and alcoholic fermentation (Grierson and Ben, 1989).

Conclusion

In 2015 season, only 'M37' mandarin showed external chilling symposium after cold sterilization. However, selection 'I22' and 'Nova' also showed external chilling damage in 2016, irrespective of degreening treatment and storage temperature.

Future research

There seems to a genetically differences in chilling susceptibility of new mandarin selections/cultivars studied. Therefore, genomic, proteomic and metabolics studies are recommended in order to bench-marker chilling susceptible selections during breeding.

Technology transfer

Poster presentation:

K Mmako, T.P. Mafeo, M Masevhe, N Mathaba and J Mlimi. Response of newly developed mandarin citrus selections to ethylene degreening and cold sterilization, Combined Congress, George, South Africa, 19-22 January, 2015

Conference presentations

K Mmako, T.P. Mafeo, N Mathaba and J Mlimi, Cold sterilization of new mandarin selection developed by the ARC-ITSC, Combined Congress, Bloemfontein, Monte Bela, 19-22 January 2016

K Mmako, T.P. Mafeo, N Mathaba and J Mlimi, Impact of degreening and cold sterilization on new mandarin selections/cultivars developed by the ARC-ITSC, CRI symposium, Drankensburg, Natal Midlands, 21-23 August 2016

K Mmako, T.P. Mafeo, N Mathaba and J Mlimi, Investigating cold storage potential of new mandarin citrus selections/cultivar and the effect of ethylene degreening on rind disorders, PHI Symposium, Stellenbosch, 21-22 November 2016, South Africa

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Figure 4.2.5.1. External chilling damage on A. 'M37' B. 'I22' and C. 'nova'

Table 4.2.5.1. Effect of harvest season, ethylene degreening and cold sterilization of new mandarin selections on fruit weight loss, electrolyte leakage and chilling injury after 28 days cold storage

			Degreened			**Non-degreened**		
Cultivars/selections	Season	Storage temperature (C)	Weight loss (%)	EC (%)	Chilling injury (%)	Weight loss (%)	EC (%)	Chilling injury (%)
Sonet*	2015	-0.5	8.5ab	0.4ab	0	6.5ab	0.3ab	0
		2.0	9.1a	0.4ab	0	7.8a	0.4a	0
		4.5	7.4ab	0.4a	0	7.5a	0.4a	0
	2016	–	–	–	–	–	–	–
		–	–	–	–	–	–	—
		–	–	–	–	–	–	–
I22	2015	-0.5	4.5b	0.6b	0	7.9bc	0.6a	0
		2.0	5.3b	0.8a	0	8.0bc	0.6a	0
		4.5	5.0b	0.8a	0	7.5c	0.5a	0
	2016	-0.5	9.93b	0.6abc	97.7	13.55ab	0.3a	50.3
		2.0	17.08a	0.6abc	93.3	11.46b	0.8bc	46.7
		4.5	11.28b	0.5ab	91.0	5.40c	1.0c	0.0
M37	2015	-0.5	7.0bc	0.6b	28	7.2b	0.6a	30
		2.0	6.3c	0.6b	12	5.8b	0.6a	15
		4.5	7.2bc	0.6b	10	9.3a	0.6a	8
		-0.5	3.79d	0.6ab	28.7	11.33abc	0.6ab	66.3
	20116	2.0	4.54cd	0.6ab	68.7	15.68a	0.6ab	67.0
		4.5	5.32bcd	0.7b	23.3	12.21ab	0.5a	46.0
B24	2015	-0.5	4.3b	0.6a	0	4.8c	0.6a	0
		2.0	3.9b	0.6a	0	3.5c	0.6a	0
		4.5	5.3b	0.5ab	0	4.3c	0.5a	0
	2016	-0.5	16.99ab	0.5a	0	11.77b	0.4b	0
		2.0	19.12a	0.4b	0	4.94b	0.4b	0
		4.5	2.63b	0.4b	0	14.82ab	0.4b	0
Nova ARC	2015	-0.5	4.3cd	0.5a	0	4.9b	0.5bc	0
		2.0	2.9d	0.6a	0	5.4b	0.5ab	0
		4.5	4.3bcd	0.6a	0	5.4b	0.6a	0
	2016	-0.5	8.03b	0.4bc	0	16.48a	0.5b	0
		2.0	7.55b	0.7a	0	20.41a	0.4bc	0
		4.5	5.79b	0.3c	0	6.62b	0.4bc	0
Nova	2015	-0.5	9.0a	0.9a	0	6.5bc	0.6a	0
		2.0	12.2ab	0.6b	0	17.4a	0.6a	0
		4.5	5.9b	0.5c	0	6.1bc	0.5a	0
		-0.5	8.392ab	0.5a	26.3	5.55b	0.5a	13.3
		2.0	8.60ab	0.5a	66.7	19.72a	0.4a	6.7
		4.5	7.60b	0.5a	2.3	13.79ab	0.4a	0.0

*Sonet was not cold sterlizaed during the 2016 season due to high creasing
**Mean with different letters show significant difference at $p<0.05$

Table 4.2.5.2. Effect of harvest season, degreening and cold sterilization of quality parameters of “B24” mandarin selection before and after cold storage during 2015 and 2016 season

			Degreened			Non-degreened		
Season	Evaluation time	Storage temp (°C)	Firmness (N)	Hue °	°Brix	Firmness (N)	Hue °	°Brix
2015	Pre-storage	-0.5	**55.7b**	**35.7e**	**9.0b**	**62.0b**	**29.9d**	**10.0d**
		2.0	**55.8c**	**35.3e**	**8.9b**	**60.3b**	**29.4d**	**9.9d**
		4.5	**57.4c**	**41.8abc**	**8.8b**	**60.6b**	**29.0d**	**10.0d**
	Post-storage	-0.5	**51.6d**	**37.9cde**	**9.1b**	**57.3c**	**37.1cd**	**12.5a**
		2.0	**52.7d**	**37.2de**	**9.0b**	**55.3d**	**32.8c**	**11.0bc**
		4.5	**54.4c**	**40.4abcd**	**10.2b**	**54.9de**	**38.3bcd**	**10.6cd**
2016	Pre-storage	-0.5	**57.10ab**	**47.19a**	**5.08e**	**52.53bcde**	**48.28a**	**9.25b**
		2.0	**55.53bc**	**29.50bc**	**8.38bcd**	**49.63bcde**	**48.87a**	**6.40cde**
		4.5	**54.83bcd**	**25.76e**	**9.94b**	**53.23bcde**	**28.79bcd**	**9.79b**
	Post-storage	-0.5	**50.40bcde**	**26.37de**	**13.37a**	**47.90de**	**30.50b**	**8.52bcd**
		2.0	**55.00bcd**	**27.10cde**	**6.17de**	**48.38cde**	**29.34bc**	**6.40cde**
		4.5	**63.00a**	**26.01e**	**9.79b**	**46.10e**	**28.69bcd**	**8.86bc**

*Means with different letters were significant different at $p<0.05$

Table 4.2.5.3a. Effect of harvest season, degreening and cold sterilization of quality parameters of "I22" mandarin selection before and after cold storage during 2015 and 2016 season

			Degreened			Non-degreened		
Season	Evaluation time	Storage temp (°C)	Firmness (N)	Hue °	°Brix	Firmness (N)	Hue h°	°Brix
2015	Pre-storage	-0.5	60.9a	17.9e	9.78c	62.2a	13.9d	8.0d
		2.0	60.2a	17.0e	9.8c	62.2a	14.6d	8.1d
		4.5	61.2a	17.7e	10.0c	64.2a	13.1d	8.1d
	Post-storage	-0.5	56.2b	21.7d	9.2c	54.5b	20.4cd	9.5c
		2.0	56.3b	27.7c	11.8b	49.7b	31.2bc	9.9c
		4.5	55.1b	27.9c	9.4c	51.1b	30.4bc	9.8c
2016	Pre-storage	-0.5	59.23ab	16.45bcd	7.68d	61.77ab	36.59a	7.68d
		2.0	59.83ab	28.87ab	7.68d	64.90a	26.45abc	7.68d
		4.5	57.94ab	25.63abc	7.68d	63.77a	19.31bcd	7.68d
	Post-storage	-0.5	44.78d	27.65ab	11.47a	48.27cd	23.28abcd	10.14abc
		2.0	57.50ab	10.56d	8.36cd	41.53d	16.40bcd	9.28bcd
		4.5	45.58d	28.84ab	11.00ab	54.47bc	23.28abcd	9.36bcd

*Means with different letters were significant different at $p<0.05$

Table 4.2.5.3b. Effect of harvest season, degreening and cold sterilization of quality parameters of “M37” mandarin selection before and after cold storage during 2015 and 2016 season

			Degreened			Non-degreened		
Season	Evaluation time	Storage temp. (°C)	Firmness (N)	Hue angle (h°)	Brix	Firmness (N)	Hue °	°Brix
2015	Pre-storage	-0.5	58.6b	23.7bc	13.0ef	67.4a	28.8c	11.9c
		2.0	60.6b	23.6bc	12.7f	67.4a	36.0ab	12.1c
		4.5	59.8b	23.5bc	14.0de	66.8a	36.7ab	11.5c
	Post-storage	-0.5	56.4c	20.6bc	14.7cd	63.3b	16.7d	11.7c
		2.0	55.4c	24.3bc	10.7g	60.7c	17.3d	9.6c
		4.5	52.0d	17.9c	13.0ef	53.4d	17.6d	27.9a
2016	Pre-storage	-0.5	52.30abcde	29.86a	8.41bc	57.80a	18.52d	8.79bc
		2.0	48.17cdef	27.39ab	7.33bc	56.13ab	22.94c	8.79bc
		4.5	45.23f	29.51a	8.41bc	48.03cdef	22.59c	7.00c
	Post-storage	-0.5	47.13def	26.42b	8.73bc	50.43bcdef	25.86b	9.57b
		2.0	46.77ef	26.08	8.41bc	53.97abc	22.68c	8.39bc
		4.5	50.07bcdef	29.34a	8.68bc	53.73abcd	25.86b	12.56a

*Mean with different letters were significant different at p<0.05

Table 4.2.5.4. Effect of harvest season, degreening and cold sterilization of quality parameters of “Nova” mandarin selection before and after cold storage during 2015 and 2016 season

			Degreened			Non-degreened		
Season	Evaluation time	Storage temp. (°C)	Firmness (N)	Hue °	Brix	Firmness (N)	Hue °	Brix
2015	Pre-storage	-0.5	65.5c	38.4e	17.5a	67.0a	42.6c	17.6a
		2.0	71.1a	38.4e	17.5a	64.7b	40.3c	17.6a
		4.5	65.3c	38.3±0.3e	17.5a	64.1bc	38.8cd	17.6a
	Post-storage	-0.5	58.2f	39.6cd	16.5ab	62.3d	37.8cd	14.1c
		2.0	59.9e	38.7de	16.3b	64.9b	40.6c	15.1b
		4.5	58.4f	40.3c	12.7d	63.7bcd	40.7c	13.1cd
2016	Pre-storage	-0.5	66.72abc	16.60cd	7.86a	71.63ab	18.34bcd	7.96a
		2.0	70.83ab	21.03bc	7.86a	72.37ab	22.14ab	7.96a
		4.5	67.41ab	15.08cd	7.86a	74.73a	15.95cd	7.88a
	Post-storage	-0.5	50.33de	18.30bcd	9.75a	64.97bc	26.63a	9.25a
		2.0	44.00e	19.21bcd	7.97a	58.65cd	16.73cd	8.99a
		4.5	55.78d	17.84bcd	9.88a	50.83de	10.66e	7.96a

*Mean with different letters were significant different at $p<0.05$

Table 4.2.5.5. Effect of harvest season, degreening and cold sterilization of quality parameters of “Nova ARC” mandarin selection before and after cold storage during 2015 and 2016 season

			Degreened			Non-degreened		
Season	Evaluation time	Storage temp. (°C)	Firmness (N)	Hue °	Brix	Firmness (N)	Hue h°	Brix
2015	Pre-storage	-0.5	66.1a	46.4c	15.4b	68.92a	41.2cd	15.6b
		2.0	64.4a	46.3c	15.4b	68.6a	41.1d	15.6b
		4.5	64.7a	46.4c	15.3b	66.2b	41.3cd	15.6b
	Post-storage	-0.5	60.5b	42.9d	16.3ab	65.7b	40.3d	12.3e
		2.0	59.5b	43.6d	13.0cd	63.5c	40.5d	14.6c
		4.5	58.8bc	46.5c	15.4b	62.0cd	44.2b	15.3b
2016	Pre-storage	-0.5	67.19cd	19.98cd	8.25ab	75.04ab	7.98g	8.15ab
		2.0	69.83bc	23.33ab	8.25ab	75.17ab	21.70bc	7.59b
		4.5	67.47cd	18.95cde	8.25ab	77.07a	7.75g	7.79b
	Post-storage	-0.5	54.88fg	20.97bc	10.49a	59.29ef	17.47def	8.15ab
		2.0	49.03gh	25.16a	8.57ab	49.03gh	14.62ef	8.15ab
		4.5	54.88fg	21.55bc	9.75ab	62.79de	16.70ef	8.487ab

*Mean with different letters were significant different at p<0.05

4.3 PROGRAMME: FLOWERING AND FRUIT SET

Programme coordinator: Jakkie Stander (CRI)

4.3.1 Programme summary

Research within this program aims to optimise flowering potential and optimise fruit set. In alternate bearing trees, flowering and fruit load, root growth during spring and summer, and summer vegetative shoot development were identified as the major predictors of intensity of return bloom flowering and yield potential (4.3.3). Flowers and fruit are the major carbohydrate sinks, and disturb the balance between root growth and vegetative shoot development. A pyramid pruning treatment in young citrus trees stimulated new vegetative growth and resulted in a 30% increase in yield response compared to an open vase pruning treatment (4.3.5). In mature trees, light selective pruning treatments resulted in highest yields for 2 consecutive seasons, and severe mechanical pruning, the lowest. In flower induction experiments, foliar GA_3 ttreatments of 40 ppm during May to June resulted in an inhibition of flowering on vegetative shoots. Treatments aimed at increasing return bloom flowering on summer vegetative shoots should therefore be timed during May to June. For example, a 2-fold elevating of leaf carbohydrate content by girdling and de-fruiting during this period significantly increased return bloom flowering. Two research projects on the effects of permanent shade netting found that 20% white shade nets reduced air temperatures, leaf vapour pressure deficit (VPD), solar radiation and wind speed, and improved tree water usage efficiency (4.3.6 and 4.3.7). The shade net increased fruit yield (no. of fruit per tree), size and significantly reduced rind blemishes. Shade netting reduced fruit rind strength at time of commercial harvest, as well as rind carbohydrate contents. A novel chemical thinning agent in pome fruit, Nevis®, with the active ingredient metamitron are being evaluated in citrus. A treatment of 300 ppm metamitron at fruitlet diameter of 8 or 15 mm significantly reduced tree total fruit yield and enhanced the fruit size distribution and did not negatively influence fruit quality. A combination foliar spray treatment of 10 ppm 2,4-D amine (half the concentration of the current, unregistered treatment) and 80 ml/100L Tebuconazole at FB (2 weeks earlier than the commercial treatment) significantly increased the number of fruit with closed navel-ends, reduced the size of the navel-end openings, as well as significantly reduced Alternaria black core rot (ABCR) infection (4.3.2). The commercial treatment for ABCR, *viz.*, 80 ml/100L Tebuconazole applied at 50% and 100% petal drop was less effective. Outputs of research within this program provide novel insights into complex production problems, but at the same time practical, readily-applicable and cost-effective solutions.

Programopsomming

Navorsing in die program het ten doel om blompotensiaal en vrugset te verhoog. In alternerende drag is blom- en vruglading, wortelgroei gedurende lente en somer, en die somer-vegetatiewe groei geïdentifiseer as die belangrikste voorspellers van die intensiteit van opvolgblom- en opvolg opbrengspotensiaal (4.3.3) Dis bevind dat blomme en vrugte die hoof koolhidraatverbruikers is en die balans tussen wortelgroei en vegetatiewe loot-ontwikkeling versteur. 'n Piramied snoei-behandeling in jong sitrusbome stimuleer nuwe vegetatiewe groei en het gelei tot 'n 30% toename in opbrengs vergeleke met 'n oop-kelk snoei behandeling (4.3.5). In volwasse bome het ligte selektiewe snoei behandelings die hoogste opbrengs vir 2 agtereenvolgende seisoene tot gevolg gehad, en strawwe en meganiese snoei, die laagste. In blominduksie eksperimente het blaar GA_3 behandelings van 40 dpm gedurende Mei tot Junie gelei tot 'n inhibisie van blomontwikkeling op vegetatiewe lote (4.3.4). Behandelings wat daarop gemik is om goeie blom op die somer vegetatiewe lote te stimuleer moet dus gedurende Mei tot Junie toegedien word. 'n 2-voudige vermeerdering van blaarkoolhidraatinhoud deur ringelering en ontvrugting gedurende hierdie tydperk het 'n aansienlike toename in opvolgblom tot gevolg gehad. Twee navorsingsprojekte op die effekte van permanente skadunette het bevind dat 20% wit skadunette die lugtemperatuur, blaardampdruk tekort (VPD), sonstraling en windsnelheid verminder het, en die doeltreffendheid van boom waterverbruik verbeter het (4.3.6 en 4.3.7). Die skadunet behandeling het vrugopbrengs (aantal vrugte per boom) en tellings verhoog en voorkoms van vrugletsels verlaag.Skadunette het vrugskil sterkte verlaag, asook die inhoud van skilkoolhidrate. 'n Nuwe chemiese uitdunningsmiddel in kernvrugte, Nevis®, met die aktiewe bestandeel metamitron word tans vir effektiwiteit in sitrus geëvalueer. 'n Behandeling van 300 ppm metamitron by vrugdeursnee 8 of 15 mm het die totale oes opbrengs aansienlik verminder en die vruggrootte verspreiding verbeter, sonder om vrugkwaliteit negatief te beïnvloed. 'n Kombinasie blaarbespuiting van 10 ppm 2,4-D amien (die helfte van die konsentrasie van die huidige ongeregistreerde behandeling) en 80 ml / 100L Tebukonasool by volblom (2 weke vroeër as die kommersiële behandeling) het die aantal vrugte met toe nawel-ente verhoog, die grootte van die nawel-ent verklein, sowel as aansienlik minder Alternaria kernvrot infeksie tot gevolg gehad (4.3.2). Die kommersiële behandeling vir alternaria kernvrot, naamlik 80 ml / 100L Tebukonasool toegedien by 50% en 100% blomblaarval was minder effektief. Uitsette van navorsing in hierdie program bied nuwe insigte in komplekse produksieprobleme, maar terselfdertyd praktiese, toepaslike en koste-effektiewe oplossings.

4.3.2 **FINAL REPORT: Potential of 2,4-D as commercial solution to Alternaria black core rot (ABCR) in Navel oranges**

Project 1130 (Apr 2015-Mar 2017) by Jakkie Stander and G.C. Schutte (CRI)

Summary

Alternaria black core rot (ABCR) is a pre-harvest disease of Navel oranges. It is caused by the fungus *Alternaria citri* and causes infected fruit to colour and drop prematurely. The rupturing of the fruit style provides lesions through which the fungus can penetrate to remain quiescent in the space between the secondary and primary fruit until fungal growth is stimulated under favourable conditions and eventually causes fruit drop. The effect of 2,4-D on the structural integrity of the fruit style during blossom period, has the potential of creating a physical barrier to the natural entry point of the fungus and could be another option as control agent of ABCR in Navel oranges. The aim of this study is to evaluate the effect of full bloom application of 2,4-D on ABCR of Navel oranges, as well as the effect when combined with tebuconazole, a systemic control agent of ABCR. The combination treatment of 10 ppm 2,4-D amine and 80 ml/100L tebuconazole applied at FB provided the best control method for Alternaria black core rot infection and the subsequent development of navel-end rot. The effect of 2,4-D on the navel-end characteristics provides a physical barrier against fungi penetrating the fruit, while the addition of tebuconazole also provides a systemic control action.

Opsomming

Alternaria kernvrot is ʼn groot voor-oes probleem in Nawel lemoene. Dit word veroorsaak deur die fungus *Alternaria citri* en veroorsaak die voortydige verkleuring en val van vrugte wat lei tot oesverliese. Die afsnoering van die blom-/vrugstyl voorsien ʼn ingangspunt vir die swam om die vrug te penetreer en te ontwikkel onder gunstige omgewingstoestande. Die effek van 2,4-D om blom-/vrugstyl afsnoering te inhibeer, het die potensiaal om die penetrasie van die fungus te verhoed en kan moontlik as alternatiewe oplossing vir Alternaria kernvrot dien. Die doel van hierdie studie is om die effek van volblom-toediening van 2,4-D te evalueer vir effektiwiteit om Alternaria kernvrot te verminder en te vergelyk met huidige beheermiddel, tebuconazole. Die kombinasie behandeling van 10 dpm 2,4-D amien en 80 ml/100L tebukonasool wat by FB toegedien is, het die beste beheermaatreël vir Alternaria kernvrot infeksie en die daaropvolgende ontwikkeling van nawel-end vrot verskaf. Die effek van 2,4-D op die nawel-end eienskappe bied 'n fisiese versperring teen swamme wat die vrug binnedring, terwyl die toevoeging van tebukonasool ook 'n sistemiese beheer bied.

Introduction

Alternaria black core rot (ABCR) is a pre-harvest disease of Navel oranges. It is caused by the fungus *Alternaria citri* and causes infected fruit to colour and drop prematurely. Manifestation of the disease is linked to the formation of large secondary navels within the primary fruit, which develops at periods of temperatures >25°C and low relative humidity <20% during early fruitlet development (Schutte et al., 1994). The rupturing of the fruit style provides lesions through which the fungus can penetrate to remain quiescent in the space between the secondary and primary fruit until fungal growth is stimulated under favourable conditions and eventually causes fruit drop.

Commercial systemic control agents are currently available in the form of difenoconazole (Score®) and tebuconazole, however, recent observations on the effect of 2,4-D on navel-end openings provides a possible supplementary solution to ABCR. Natural auxins in plant tissue such as 3-indoleacetic acid (IAA) primarily regulate abscission by blocking the capacity of ethylene to stimulate the abscission of plant material (Borroto et al., 1981; Goren, 1993). The synthetic auxin 2,4-D is rapidly absorbed and translocated in the phloem to young meristematic tissue and accumulates in sink organs such as young leaves, flowers or fruitlets where it stimulates cell expansion (Ashton et al., 1991; Mitchell, 1961*)*. Mupambi (2010) found that applications of 25 $mg.L^{-1}$ at full bloom, as well as 15 $mg.L^{-1}$ and 25 $mg.L^{-1}$ at petal drop reduced the number of Navel orange fruit with open navel-ends. Similar treatments reduced fruit splitting of 'Marisol Clementine', however, styles failed to abscise from treated fruit and remained attached for a prolonged period compared to control fruit, possibly the result of the auxins blocking the capacity of ethylene to stimulate the natural abscission of the styles (Goren, 1993). The formation of a uniform abscission layer without tears between the style and stylar-end of the rind seems to be important to prevent stylar-end fruit splitting, as the small lesion is thought to be the starting point for fruit splitting (Stander, 2013).

This effect of 2,4-D on the structural integrity of the fruit style during blossom period, has the potential of creating a physical barrier to the natural entry point of the fungus and could be another option as control agent of ABCR in Navel oranges. The aim of this study is to evaluate the effect of full bloom application of 2,4-D on

ABCR of Navel oranges in the Eastern and Western Cape of South Africa, as well as its effect when combined with tebuconazole, a systemic control agent of ABCR.

Objectives

Objective A: Evaluate the effect of 2,4-D foliar applications, alone and in combination with a systemic control agent, tebuconazole on ABCR of Navel oranges in Eastern and Western Cape.

Objective B: Determine fruit residue levels of 2,4-D foliar spray treatments for possible registration trials.

Materials and methods

Plant material and experimental design: Experiments were conducted on Navel oranges at Dunbrody Estates, in the Eastern Cape, as well as in Citrusdal, in the Western Cape. Each experiment consisted of a randomized complete block design with 5 treatments and 8 blocks (n=8). Experimental trees were selected on the basis of uniform size and appearance.

The following treatments were applied in a 2014/5 preliminary experiment and were repeated in 2015/16:

Summary of treatments

Treatment	Timing
Control (Water + Breakthru)	Full bloom
2,4-D @ 20 ppm + Breakthru	Full bloom
Tebuconazole @ 80 ml/100L + Breakthru	50% petal drop + 100 % petal drop
Tebuconazole @ 80 ml/100L + Breakthru	Full Bloom
2,4-D @ 10 ppm + Tebuconazole @ 80 ml/100L + Breakthru	Full Bloom

Data collection: At the time of commercial maturity, 20 fruit (160 per treatment) were randomly collected from each replicate tree. The size of the navel-end opening of each sampled fruit was measured using an electronic calliper and the percentage fruit with closed navel-ends was calculated by dividing the number of fruit with closed navel-ends by the total number of fruit sampled per replicate tree.

To identify any core rot or other symptoms of *Alternaria* black core rot infection, fruit halves were individually photographed. Tissue from each fruit was removed from visually symptomatic areas of infection in the core or calyx with a sterilised scalpel as aseptically as possible in a laminar flow cabinet. Symptomatic tissue was subsequently placed onto potato dextrose agar plates and incubated at 27°C for 7 to 14 days or until any fungal spore structures were visible under stereomicroscope inspection. As soon as spore structures were visible, cultures were single-spored onto PDA plates and incubated a further 7 to 14 days until cultures could be identified using conidium morphology.

Statistical analysis: Analysis of variance (ANOVA) was performed using Statistica software (version 13, Dell Statistica Inc.). Mean separations were carried out using the least significant difference (LSD) test, where applicable, at $P \leq 0.05$.

Results and discussion

All the treatments significantly reduced the percentage of *Alternaria* black core rot infected fruit compared to untreated control fruit. The combination treatment of 2 ml/100L 2,4-D amine and 80 ml/100L tebuconazole applied at FB was most successful, followed by the application of 80 ml/100L tebuconazole at 50% and 100% PD, and 4 ml/100L 2,4-D at FB, respectively (Tables 4.3.2.1, 2 and 3).

The treatment of 80 ml/100L tebuconazole applied at FB was effective in significantly increasing the percentage of fruit with closed navel-ends, as well as reducing the size of the navel-end opening of remaining fruit which did not have a fully closed navel-end opening, compared to the untreated control (Tables 4.3.2.1, 2 and 3). When 2,4-D and tebuconazole were applied as a combination treatment during FB, a synergistic response was obtained which resulted in a significantly higher percentage of fruit with closed navel-ends, as well as a reduced navel-end opening compared to the untreated control fruit and the FB 2,4-D treatment applied alone.

No phytotoxicity was observed on any of the treatments. Analyses of the treated fruit for the presence of 2,4-D and tebuconazole traces at time of commercial harvest detected little to no residues (Tables 4.3.2.4 and 5).

The combination treatment of 2 ml/100L 2,4-D amine and 80 ml/100L tebuconazole applied at FB provides the best control method for Alternaria black core rot infection and the subsequent development of navel-end rot. The effect of 2,4-D on the navel-end characteristics provides a physical barrier against fungi penetrating the fruit, while the addition of tebuconazole also provides a systemic control action.

Objective / Milestone	Achievement
Objective A-B	Foliar treatments of 2,4-D as well as 2,4-D + tebuconazole at full bloom, significantly increased the percentage of fruit with closed navel-ends and reduced the size of the navel-end opening. All treatments reduced the percentage of *Alternaria* infected fruit significantly, with the treatment of 2,4-D + tebuconazole at full bloom, being most effective. Treatment with 2,4-D significantly reduced the percentage of mealybug-infected fruit. No residue-levels were detected for any of the treatments at time of commercial harvest.

Technology transfer

CRI annual extension workshops in 2015.
Stander, O.P.J., Gilbert, M.J., Moore, S.D., Kirkman, W. and Schutte, G.C. 2017. Benefits of reducing the size of the navel-end opening in 'navel' sweet oranges [*Citrus sinensis* (Osbeck)]**.** Crop Protection. 96: 123–129.

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Goren, R. 1993. Anatomical, physiological, and hormonal aspects of abscission in citrus. Hort. Rev. 15:145–182.

Mupambi, G. 2010. Studies to reduce the size of the Navel-end opening of Navel oranges. MSc Thesis, University of Stellenbosch, South Africa.

Schutte, G.C., K.V. Beeton, P. Du T. Pelser, and K. Lesar. 1994. Post-harvest control of *Alternaria* Navel-end rot with pre-harvest chemical sprays. Sitrusjoernaal (4)1:26-28.

Stander, O.P.J. 2013. Fruit split and fruit size studies on citrus. MSc Thesis, University of Stellenbosch, South Africa.

Table 1. The effects of foliar applications of 2,4-D at full bloom (FB), applied alone or in combination with tebuconazole, on fruit diameter, the prevalence and characteristics of the navel-end opening, and Alternaria black core rot in 'Lane Late' Navel sweet orange fruit, at Kirkwood during the 2014/15 growing season.

Treatments (n=8)	Fruit diameter (mm)	Closed navel-ends (% fruit)	Navel-end opening (mm)	Navel size (mm)	Alternaria black rot (% fruit)
Control	74.61 bc[z]	19.38 d	7.50 a	13.93 ns[y]	20 a
20 ppm 2,4-D amine at FB	76.54 a	55.63 b	4.16 b	14.86	13 b
80 ml/100L tebuconazole at FB	74.22 cd	29.38 c	6.34 ab	14.30	14 b
10 ppm 2,4-D amine + 80 ml/100L tebuconazole at FB	75.61 ab	69.38 a	2.82 c	14.59	5 d
80 ml/100L tebuconazole at 50% PD + 100% PD	74.20 d	21.88 d	6.52 ab	14.07	9 c

[Z] Different letters in the same column denote significant differences between values (P>0.05, LSD multiple range test).

[Y] No significant differences.

Table 2. The effects of foliar applications of 2,4-D at full bloom (FB), applied alone or in combination with tebuconazole, on fruit diameter, the prevalence and characteristics of the navel-end opening, and Alternaria black core rot in 'Lane Late' Navel sweet orange fruit, at Kirkwood during the 2015/16 growing season.

Treatments (n=8)	Fruit diameter (mm)	Closed navel-ends (% fruit)	Navel-end opening (mm)	Navel size (mm)	Alternaria black rot (% fruit)
Control	75.91 ns	24.56 d	6.97 a	14.15 ns	12 a
20 ppm 2,4-D amine at FB	74.23	48.11 b	3.98 bc	13.98	6 b
80 ml/100L tebuconazole at FB	74.11	34.25 c	4.55 b	14.25	7 b
10 ppm 2,4-D amine + 80 ml/100L tebuconazole at FB	75.93	68.39 a	3.11 c	14.68	3 c
80 ml/100L tebuconazole at 50% PD + 100% PD	74.01	28.87 cd	5.98 a	1.88	5 bc

[Z] Different letters in the same column denote significant differences between values (P>0.05, LSD multiple range test).

[Y] No significant differences.

Table 3. The effects of foliar applications of 2,4-D at full bloom (FB), applied alone or in combination with tebuconazole, on fruit diameter, the prevalence and characteristics of the navel-end opening, and Alternaria black core rot in 'Robyn' Navel sweet orange fruit, at Kleinvlei, Citrusdal, during the 2015/16 growing season.

Treatments (n=8)	Fruit diameter (mm)	Closed navel-ends (% fruit)	Navel-end opening (mm)	Navel size (mm)	Alternaria black rot (% fruit)
Control	78.91 ns	24.56 d	6.57 a	13.05 ns	23 a
20 ppm 2,4-D amine at FB	79.23	48.11 b	2.08 b	13.28	16 b
80 ml/100L tebuconazole at FB	77.11	34.25 c	7.05 a	14.11	14 bc
10 ppm 2,4-D amine + 80 ml/100L tebuconazole at FB	79.93	68.39 a	2.01 b	14.01	8 c
80 ml/100L tebuconazole at 50% PD + 100% PD	78.01	28.87 cd	5.98 a	13.88	`16 b

[Z] Different letters in the same column denote significant differences between values (P>0.05, LSD multiple range test).

[Y] No significant differences.

Table 4. The effects of foliar applications of the amine formulation of 2,4-D at full bloom (FB), applied alone or in combination with tebuconazole during the 2014/15 growing season, on the residue levels of 2,4-D in fruit of 'Lane late' Navel sweet orange fruit at time of commercial harvest.

Treatments (n=8)	**Residue level[Z] (mg·kg^{-1}) in the fruit pulp**		
2014/15	**Average**	**Highest**	**Lowest**
20 ppm 2,4-D amine at FB	n.d.	n.d.	n.d.
10 ppm 2,4-D amine + 80 ml/100L tebuconazole at FB	n.d.	n.d.	n.d.

[n.d.] Non detected

[Z] Samples were analyzed in accordance with analytical method(s) LCMS, for 2,4-D and tebuconazole.

Table 5. A summary of the effects of foliar applications of the amine formulation of 2,4-D at full bloom (FB), applied alone or in combination with tebuconazole during the 2015/16 growing season, on the residue levels of 2,4-D in fruit of 'Robyn' Navel sweet orange fruit at time of commercial harvest.

Treatments (n=8)	**Residue level[Z] (mg·kg^{-1}) in the fruit pulp**					
2015/16	**Average 2,4-D**	**Average Tebuconazole**	**Highest 2,4-D**	**Highest Tebuconazole**	**Lowest 2,4-D**	**Lowest Tebuconazole**
20 ppm 2,4-D amine /100L water at FB	0.02	n.d.	0.02	n.d.	0.01	n.d.
10 ppm 2,4-D amine and 80 ml tebuconazole /100L water at FB	0.03	<0.01	0.05	<0.01	0.02	<0.01

[Z] Samples were analyzed in accordance with analytical method(s) LCMS and AH (LCMS), for 2,4-D and LCMS for tebuconazole.

4.3.3 PROGRESS REPORT: Studies on the reproductive development of 'Nadorcott' mandarin (C. reticulata Blanco)

Project 1131 (Apr 2015 - Mar 2018) by Jakkie Stander and Paul Cronje (CRI), and G.H. Barry (XLnT Citrus)

Summary

The objective of this PhD research project is to study the reproductive development of "on" and "off" 'nadorcott' mandarin (C. reticulata Blanco) trees throughout fruit development. Data will be collected throughout the production season to determine possible correlations between various horticultural responses such as root growth, flowering and fruit set, vegetative growth, fruit load and fruit quality, to measurements of physiological parameters such as leaf and root carbohydrate levels, leaf endogenous hormone content, leaf nutrient content and photosynthesis. The overall aim of this multi-season research project is to identify measure and integrate the various aspects that could influence yield, i.e. carbohydrates, phytohormones, and photosynthesis capacity into a crop model. Such a model would potentially be used to identify opportune timing of a horticulture manipulation technique during the various tree phenological stages to obtain a consistent yield. The best possible predictors of return bloom and yield in alternate bearing 'nadorcott' mandarin trees appear to be spring root carbohydrates in season 1, fruit load in season 1, summer vegetative flush in season 1, and winter leaf carbohydrates in season 1. Roots appear to play an important role in alternate bearing tendencies of 'nadorcott' mandarin in winter rainfall areas (Mediterranean climate). The study provides new insights into how fruit load influences vegetative shoot development in alternate bearing citrus trees. These results affirm that fruit are the major carbohydrate sink, and most probably disturb the balance between root growth and vegetative shoot development.

Opsomming

Die objektief van hierdie PhD navorsingsprojek is om die reproduktiewe ontwikkeling van 'nadorcott' mandaryn (C. reticulata Blanco) te bestudeer. Data word deurgaans versamel om moontlike korrelasies te vind tussen

verskeie hortologiese verskynsels soos wortelgroei, blom-ontwikkeling, vrugset, vegetatiewe groei, vruglading en –kwaliteit, en fisiologiese parameters soos blaar- en wortel koolhidraatvlakke, mineraal vlakke, interne planthormone en fotosintese tempo. Die oorhoofse doel van hierdie multi-seisoenale projek is om na die eerste seisoen, die verskeie faktore wat vruglading beïnvloed te identifiseer, kwantifiseer en uiteindelik te manipuleer om konstante oeste van goeie kwaliteit vrugte te verseker. Die beste moontlike voorspellers van opvolgblom en opbrengs in alternerende drag ' Nadorcott ' mandaryn bome blyk lente wortel koolhidrate in die seisoen 1, vruglading in seisoen 1, somer vegetatiewe groei in seisoen 1 en die winter blaar koolhidrate in seisoen 1. Die data dui op 'n belangrike vir wortels in alternerende drag neigings van ' Nadorcott ' mandaryne in winterreënvalgebiede (Mediterreense klimaat).

4.3.4 PROGRESS REPORT: Determining the time and duration of flower induction in early vs late mandarin cultivars and evaluating the effect of hand thinning, pruning and girdling on leaf and root carbohydrate levels, fruit size, vegetative regrowth and alternate bearing in 'nadorcott' mandarin.

Project 1106 (Apr 2014-Mar 2018) by Jakkie Stander, Paul Cronje (CRI at SU) and Graham Barry (Xclnt citrus)

Summary

The objective of this project is to pinpoint and compare the time and duration of flower induction in mandarins, by measuring the flowering inhibition response to GA3 applications at different times throughout the expected flower induction period. The project also evaluates manipulations of vegetative and reproductive growth to change carbohydrate allocation and/or restore carbohydrate levels and reduce the effect of endogenous gibberellins on flower induction. 'nadorcott' mandarin trees are used in experiments to establish whether there are significant treatment effects on carbohydrate availability correlation with the following season's fruit load and quality. Treatment effects on problems such as small fruit size and vigorous vegetative regrowth will be quantified throughout.

Opsomming

Die doel van hierdie projek is om die tyd en lengte van blominduksie in mandaryne te bepaal en te vergelyk, deur die blomreaksie op verskillende GA3 toedienings tydens die verwagte blominduksie periode te meet. Daarna sal verskillende manipulasies van vegetatiewe, sowel as reproduktiewe groei vanaf Januarie tot April evalueer word, met die doel om koolhidraat allokasie tussen sinkorgane te manipuleer en/of om koolhidraatvlakke te herstel en die inhiberende effek van interne gibberelliene op blominduksie vanaf Mei tot Augustus te verminder. 'nadorcott' mandaryn bome sal in eksperimente gebruik word om vas te stel of daar enige betekenisvolle effek van behandelings op blaar- en wortel koolhidraat-vlakke is en dit moontlik korreleer met vruglading en kwaliteit. Behandelingseffekte sal ook addisioneel evalueer word op probleme soos klein vrugte en aggressiewe vegetatiewe groei.

4.3.5 PROGRESS REPORT: Effect of pruning on fruit production of Nadorcott mandarin

Project number 000190 (2014/15 – 2017/18) R.B. Cronje, C.F. Human and I.M. Ratlapane (ARC-ITSC)

Summary

A project on pruning strategies for 'nadorcott' mandarin for newly established, young and old trees was initiated in 2014. The aim of the project was to determine the effect of various pruning methods on flowering, yield, fruit quality, starch reserves and alternate bearing pattern as well as to develop a practical and economical pruning practice for 'nadorcott' mandarin. The trial on newly established and young trees (2 and 5-year old, respectively) included three treatments, namely two selective hand-pruning treatments (pyramid and open vase shape) and a control (untreated until trees touched each other). The trial on older trees (11-year) consisted of six treatments including selective pruning by hand (light and severe after harvest or just after fruit drop), mechanical pruning after harvest, a combination of hand and mechanical pruning in alternate years and a control (farm practice). Pruning was carried out in July/August 2016 and January 2017 (shoot control). All removed branches were weighed at all pruning times to determine the amount of plant material removed from each treatment and pruning time. Tree height was measured before and after pruning. Leaf samples were taken at harvest (Jun/Jul), fruit set (Sep), after fruit drop (Nov) and at flower initiation (April) to determine changes in starch levels of the trees.

The 2-year-old trees were only lightly pruned in 2015 and 2016 to correct branching angles and shape them into the specific tree structures. In both years, tree height was reduced on average by about 10-20 cm (16%

of original height). The trees responded more vigorously to the open vase shaping, and removal of water shoots was more than 50% higher compared to the pyramid shaped trees. The pyramid shape was easier to establish and maintain for such young trees. First yield and starch data will be taken in the 2017 season.

In the 5-year old trees, tree height was adjusted to 2 m for all data trees in 2016. For this reason, amount of plant material removed after harvest was highest in the control as this treatment was previously pruned the least. The amount of water shoots was highest in the open vase trees, followed by the pyramid shaped trees. However, over the three years, most plant material was removed in the pyramid shaped trees. In 2016, flower intensity was high overall with 87% for the open vase and pyramid shaped trees, and 85% for the control trees. Alternate bearing caused a reverse trend in yield for the pyramid and control trees compared to the previous season. The pyramid treatment showed the highest yield (39.3 kg/tree), followed by the control treatments (33.4 kg/tree). Yield between the open vase treatment stayed the same for the two years (mean of 31 kg/tree). Unlike in the 2015 season, no significant differences in fruit size were observed between the treatments in 2016. The highest percentage of fruit for all treatments was found in the categories 1XX and 1XXX. An influence of crop load on starch reserves was observed for two seasons in a row. The higher the yield was, the lower were the starch reserves at harvest time. So far, it can be concluded that pruning of trees older than 3 years is necessary to improve light and fruit quality inside the tree. This was more easily achieved with the pyramid shape.

In the 10-year old trees, tree height was reduced to 2.7 m for all treatments in August 2016. This caused a higher amount of plant removal after harvest for the control trees, similar to the 5-year old trees. The severe selective pruning treatments (after harvest and after every second year) also had one of the highest plant removals after harvest in 2016. Overall, selective pruning after fruit drop, light selective and mechanical pruning removed the least plant material. Year-on-year, less pruning was necessary to achieve the desired tree shape and height. While there were no differences between treatments in flower intensity during an on-year (August 2015), flower intensity in an off-year (August 2016) was high for the severe selective pruning treatments despite the repeatedly severe pruning. These treatments also had the highest amount of green blossom. A clear alternate bearing pattern was visible overall independent of treatment. Yield data corresponded with the amount of cut-off branches of the previous year's pruning. Severe pruning reduced yield more than light pruning. The control and light selective pruning treatments showed high yields two seasons in a row compared to the other treatments. Severe selective pruning treatments showed the lowest yields over two seasons. Mechanical pruning, which was combined with some selective pruning in the first year to accommodate the pruning machine, had a low yield after the first year (2015), but one of the highest yields in the following year (2016) because only light mechanical pruning was carried out after the first harvest. While there were no big differences in fruit size in 2015, the severe selective pruning after harvest treatment had significantly bigger fruit compared to the other treatments due to a lower yield in 2016 (50% in categories 1XX and 1XXX). Starch reserves were higher during an off-year compared to an on-year, especially during flower initiation (April) and at harvest (June/July). No clear differences in starch reserves between treatments has been observed yet, but an in-depth analysis of the data is planned. As with the younger trees, pruning of old trees is necessary to control tree shape and height, reduce canopy density and improve fruit quality. From the current results is appears that a light to medium selective hand-pruning is preferred. Mechanical pruning creates a dense canopy, which influences light interception. It also reduces the number of bearing shoots for the next season. The project will finish in August 2017 and the final report will be submitted before March 2018.

Opsomming

'n Projek oor snoeistrategieë vir 'nadorcott' mandaryne vir nuut gevestigde, jong en ou bome is in 2014 begin. Die doel van die projek was om die effek van verskeie snoeimetodes op blom, opbrengs, vruggehalte, styselreserwes en alternatiewe drag te bepaal, sowel as om 'n praktiese en ekonomiese snoeipraktyk vir 'Nadorcott' mandaryn te ontwikkel. Die proef op nuut gevestigde en jong bome (onderskeidelik 2 en 5 jaar oud) het drie behandelings ingesluit, naamlik twee selektiewe handsnoeibehandelings (piramide en oop vaasvorm) en 'n kontrole (onbehandeld tot bome mekaar raak). Die proef op ou bome (11 jaar oud) bestaan uit ses behandelings, wat selektiewe snoei met die hand (lig en erg na oes of net na vrugval), meganiese snoei na oes, 'n kombinasie van hand- en meganiese snoei in alternatiewe jare en 'n kontrole (plaaspraktyk) ingesluit het. Snoei het plaasgevind in Julie / Augustus 2016 en Januarie 2017 (lootbeheer). Alle verwyderde takke is op alle snoeitye geweeg om die hoeveelheid plantmateriaal wat van elke behandeling en snoeityd verwyder is te bepaal. Boomhoogte is voor en na snoei gemeet. Blaarmonsters is met oes (Jun/Jul), vrugset (Sep), na vrugval (Nov) en by blom-inisiasie (Apr) geneem om veranderings in styselvlakke van die bome te bepaal.

Die 2-jarige bome is in 2015 en 2016 net liggies gesnoei om takke te korrigeer en in die spesifieke boomstrukture te vorm. In albei jare is die gemiddelde boomhoogte met ongeveer 10-20 cm verminder (16% van die oorspronklike hoogte). Die bome het sterker op die oop vaasvorming gereageer, en die verwydering

van waterlote was meer as 50% hoër as die piramiedvormige bome. Die piramide vorm was makliker om te bereik en in stand te hou vir sulke jong bome. Eerste opbrengs en styseldata sal in die 2017 seisoen geneem word.

In die 5-jarige bome is boomhoogte in 2016 aangepas tot 2 m vir alle databome. Om hierdie rede was die hoeveelheid plantmateriaal wat na oes verwyder is, die hoogste in die kontrole aangesien hierdie behandeling voorheen die minste gesnoei is. Die hoeveelheid waterlote was die hoogste in die oop vaasbome, gevolg deur die piramiedvormige bome. Oor die drie jaar is die meeste plantmateriaal egter in die piramiedvormige bome verwyder. In 2016 was blomintensiteit hoog met 87% vir die oop vaas- en piramiedvormige bome, onderskeidelik, en 85% vir die kontrolebome. Alternatiewe drag het 'n omgekeerde neiging in opbrengs vir die piramide en kontrolebome veroorsaak in vergelyking met die vorige seisoen. Die piramiedbehandeling het die hoogste opbrengs (39.3 kg/boom) getoon, gevolg deur die kontrolebehandeling (33.4 kg/boom). Opbrengs tussen die oop vaasbehandeling het dieselfde vir die twee jaar gebly (gemiddeld 31 kg/boom). Anders as in die 2015 seisoen, was daar geen beduidende verskille in vruggrootte tussen die behandelings in 2016 waargeneem nie. Die hoogste persentasie vrugte vir alle behandelings is in die kategorieë 1XX en 1XXX gevind. 'n Invloed van vruglading op styselreserwes is vir twee seisoene in 'n ry waargeneem. Hoe hoër die opbrengs was, hoe laer was die styselreserwes tydens oes. Tot dusver kan tot die gevolgtrekking gekom word dat snoei van bome ouer as 3 jaar nodig is om lig- en vrugkwaliteit binne die boom te verbeter. Dit was makliker haalbaar met die piramiede vorm.

In die 10-jarige bome is boomhoogte verminder tot 2,7 m vir alle behandelings in Augustus 2016. Dit het na die oes 'n hoër hoeveelheid plantverwydering vir die kontrolebome veroorsaak, soortgelyk aan die 5-jarige bome. Die ernstige selektiewe snoeibehandelings (na oes en na elke tweede jaar) het ook in 2016 een van die hoogste verliese aan plantmateriaal na oes gehad. Oor die algemeen het selektiewe snoei na vrugval, lig selektiewe en meganiese snoei die minste plantmateriaal verwyder. Op ʼn jaar-tot-jaar basis was minder snoei nodig om die gewenste boomvorm en -hoogte te bereik. Terwyl daar gedurende 'n aan-jaar (Augustus 2015) geen verskille was tussen behandelings in blomintensiteit nie, was blomintensiteit in ʼn af-jaar (Augustus 2016) hoog vir die ernstige selektiewe snoeibehandeling ten spyte van die herhaaldelike ernstige snoei. Hierdie behandelings het ook die hoogste hoeveelheid groenbloeisels gehad. 'n Duidelike alternatiewe drag patroon was sigbaar alhoewel onafhanklik van behandeling. Opbrengsdata het met die hoeveelheid afgesnyde takke van die vorige jaar se snoei gekorrespondeer. Strawwe snoei het opbrengs meer verminder as ligte snoei. Die kontrole en lig selektiewe snoeibehandelings het hoë opbrengste twee seisoene in 'n ry behaal in vergelyking met die ander behandelings. Strawwe selektiewe snoeibehandelings het die laagste opbrengs oor twee seisoen getoon. Meganiese snoei, wat gekombineer is met selektiewe snoei in die eerste jaar om die snoeimasjien te akkommodeer, het 'n lae opbrengs ná die eerste jaar (2015) gehad, maar een van die hoogste opbrengste in die volgende jaar (2016) aangesien slegs ligte meganiese snoei na die eerste oes gedoen is. Terwyl daar in 2015 geen groot verskille in vruggrootte was nie, het die strawwe selektiewe snoei na oes aansienlik groter vrugte getoon in vergelyking met die ander behandelings weens 'n laer opbrengs in 2016 (50% in kategorieë 1XX en 1XXX). Styselreserwes was hoër in 'n af-jaar in vergelyking met 'n aan-jaar, veral tydens blominisiasie (April) en met oes (Junie / Julie). Geen duidelike verskille in styselreserwes tussen behandelings kon nog waargeneem word nie, maar ʼn in-diepte analise van die data sal volg. Soos met die jonger bome, is snoei van ou bome absoluut noodsaaklik om boomvorm en hoogte te beheer, blaardak digtheid te verminder en vruggehalte te verbeter. Uit die huidige resultate blyk dit dat 'n ligte tot medium selektiewe hand snoei verkies word. Meganiese snoei skep 'n digte blaardak, wat ligpenetrasie beïnvloed. Dit verminder ook die aantal dratakke vir die volgende seisoen. Die projek eindig in Augustus 2017 en die finale verslag sal voor Maart 2018 ingedien word.

4.3.6 PROGRESS REPORT: Effect of shade net on fruit production of mandarin citrus

Project 000191 (2014/15 – 2016/17) by N.J.R. Roets, R.B. Cronje (ARC-ITSC) and I.F. Ngwamba (UKZN)

Summary

As an increasing number of mandarin growers consider covering their orchards with shade nets, this study became important in addressing gaps in knowledge on whether there are any real benefits in using shade nets. This study investigated the effect of low-density white shade nets (as mostly used by growers) on tree performance (physiology), phenology, production and post-harvest storage potential and fruit quality of ʼnadorcott' mandarin. In general, air temperatures were lower during the day and higher during the night under the shade net, while daily relative humidity was increased. Shade created by the net resulted in some plant morphological adaptations, such as larger leaves and more vigorous vegetative growth. For this reporting season, ʼnadorcott' was in an "on-year" with yields significantly higher than the 2015 season. Yield was significantly lower for the covered orchard during this season and it would appear as if the net reduced the

severity of alternate bearing. However, this needs further investigation for confirmation. Fruit from the covered orchard were larger with higher juice potassium levels and lower TA levels but were slightly greener at harvest. The shade net had no effect on the post-harvest storage potential of 'nadorcott' mandarin.

Opsomming

Die gebruik van skadu-nette oor mandaryn boorde word toenemend oorweeg deur produsente. Hierdie studie is dus belangrik om onsekerheid uit die weg te ruim of daar enige voordeel bestaan om skadunette te gebruik. Met hierdie studie was die effek van 'n lae-digtheid wit skadunet soos meestal gebruik deur produsente op die plant se fisiologie, fenologie, produksie en na-oes opbergingspotensiaal van 'nadorcott' mandaryn ondersoek. Oor die algemeen, het die net laer dagtemperature en hoër nagtemperature tot gevolg gehad, sowel as hoër relatiewe humiditeit indie dag. Die skadu wat deur die net teweegg ebring was tot gevolg gehad dat sekere morfologiese aanpassings plaasgevind het, soos grootter blare en groeikragtiger vegetatiewe groei. Vir hierdie oesseisoen was opbrengs betekenisvol hoër ("aan-jaar") as vir die 2015 seisoen en is 'nadorcott' dus 'n duidelike alternerende draer. Die opbrengs was egter betekenisvol laer in die boord wat met die skadunet bedek was, wat dit wil laat voorkom asof die net die graad van alternerende drag verlaag. Dit moet egter verder ondersoek word om dit te bevestig. Vrugte van die boord wat bedek was met die skadunet was grootter, met hoër sap kalium en laer TS vlakke. Die vrugte van die skadenet bedekte boord was egter effens groener gedurende oes. Die skadunet het geen beduidende effek gehad op opbergingspotensiaal van 'nadorcott' mandaryn nie.

4.3.7 PROGRESS REPORT: The benefits of shade netting for citrus fruit quality

Project RCE4 (Exp: 1125) (2015/6 – 2017/8) by Paul Cronje, Jakkie Stander, Teunis Vahrmeijer, Jade North, Martin Gilbert, Jan van Niekerk (CRI), Graham Barry (XLnt citrus) Remy Rosalie, Robert Brown, Johane Botes and Du Toit Prins (SU)

Summary

This project was in its development phase during 2015, the first data collection season started at fruit set in 2015. All aspects regarding the development of the experimental site were successfully completed and a 2 ha Nadorcott orchard (3-year old) was covered with 20% shade netting. After completion of the structure and redesign of the irrigation system, various instrumentations were installed to the site to measure environmental and soil conditions. Subsequently data on various aspects which includes microclimate, insect species present, fruit quality, horticultural practises and water use efficacy was gathered over the 2016/17 season. The aim of the project is to determine if shade netting either improve or reduce the export volume and quality of citrus fruit. After the first season the data indicate that the shade netting in general improve fruit number, size and reduced the superficial blemishes. The carbon accumulation and water use was also improved in the hot summer months (Dec-Feb). The negative aspect was a reduction in fruit rind strength and rind carbohydrates. The second season will shed additional light on this information and if there are negative impacts on rind condition. The results and conclusions in this report should only be seen as preliminary.

Opsomming

Gedurende 2015 was hierdie projek in die ontwikkelingsfase, waarna die eerste data seisoen in aanvang geneem het in 2015 na vrugset. Al die aspekte met betrekking tot die ontwikkeling van die proef-preseel is suksesvol afgehandel en 'n 2 ha Nadorcott boord (3 jaar oud) was met 'n 20% wit skadunet bedek. Na afhandeling van die strukture en besproeiings sisteem was verskei instrumente geïnstalleer om die mikroklimaat verandering en die grond water kontinu te meet. Daarna is data op verskeie aspekte wat insluit die mikroklimaat, insek spesie teenwoordigheid, vrug kwaliteit, hortologiese bestuursaspekte en waterverbruik ingewin gedurende die 2016/17 seisoen. Die doel van die projek is om te bepaal of skadunet die volume en kwaliteite van sitrusvrugte vir die uitvoermark verhoog. Na die eerste seisoen dui die data voorlopig daarop dat die skadunette wel lei tot 'n verhoogde opbrengs, vruggrotte en verlaging van oppervlakletsels. Die koolhidrate akkumulasie en water gebruik was beter gedurende die somer maande (Des-Feb). 'n Negatiewe aspek was die verlaging in skilsterkte as ook die skil koolhidrate van vrugte onder die nette. Die twee seisoen se data sal egter die prentjie verder belig en aandui of die aspek kan lei tot 'n probleem in die skilkondisie. Dei resultate en afleidings in die verslag moet egter slegs gesine word as voorlopig.

4.4 PROGRAMME: COLD CHAIN & PACKAGING

Programme coordinator: Paul Cronjé (CRI-SU)

4.4.1 Programme summary

The increase in export volumes in addition to the long and complex logistic of SA citrus export make the constant improvement of the cold chain important. During the 2016 season a successful effort was made to document those vital stage involved in cooling fruit destined for a cold sterilisation markets. The result, published as a guideline with the help of John McGlashan has resulted in all parties in the cold chain i.e. producers, exporters, cold room operators, shipping lines and PPECB being aware of potential pitfalls. The document is available on request from CRI or CGA. Forced-air precooling is a widely applied postharvest technology to remove field heat quickly out of packed fresh fruit however those aspects affecting it efficacy in the citrus cold chain has not received attention. Wrapping was found to induce slower cooling rates and larger cooling heterogeneity in the downstream cartons. The fruit in Opentop cartons were cooled faster compared to the Supervent cartons. This study will be instrumental to improve understanding of the cooling rate and uniformity in forced-air precooling of packed fresh fruit. This program will continue with projects in the commercial cold chain, as data from simulated conditions do not translate well into the actual cold chain involved in the export of SA citrus fruit to cold sterilization and conventional markets.

Programopsomming

Die styging in die uitvoer volumes asook die komplekse en lang logistieke ketting betrokke in die SA sitrus uitvoere maak die konstante verbetering koueketting belangrik. Gedurige 2016 was daar ŉ geslaagde poging gewees om al die belangrike aspekte rondom verkoeling van sitrus vir ŉ kouesterilisasie mark saam te vat. Die resultaat hiervan was gepubliseer met die medewerking van John MacGlashan en het teweeg gebring dat alle persone betrokke in die koue-ketting nl. Produsent, uitvoerder, koelkamer operateurs, verskepings rederye as ook PPECB bewus is van moontlike slaggate. Hierdie dokument is beskikbaar van CRI of CGA op aanvraag. Geforseerde lug verkoeling word algemene in die vrugte industrie gebruik om so vinnig moontlik die veldhitte uit vrugte te verwyder. Die aspekte wat die effektiwiteit beïnvloed is egter nog nie ondersoek vir sitrus vrugte nie. In die projek is bevind dat “wrapping” van vrugte die tempo van verkoeling benadeel asook die heterogeniteit. Daar was ook gevind dat vrugte in “open top” vinniger verkoel as Supervent kartonne. Hierdie studie was belangrik om die verkoelingstempo en uniformiteite daarvan in geforseerde lug verkoeling van verpakte sitrus te verbeter. Hierdie program gaan voortgaan om projekte in die kommersieel koueketting te onderneem omrede data gegenereer uit gesimuleerde toestande nie die totale prentjie skets van die werklike koueketting betrokke in uitvoer van SA Sitrus na kouesterilisasie en gewone markte nie.

4.4.2 FINAL REPORT: Precooling of citrus fruit – manual and practical guidelines

Project 1154: (2016/17) by Paul Cronje (CRI) and John McGlashan

Summary

The increased exposure of SA citrus exports to cold sterilisation markets demands a re-look at the in-depth knowledge of personnel involved in this process. Inadequate or erroneous pre-cooling have resulted in significant quality losses as well as failure to initiate cold sterilisation during shipment of containers. In order to address this industry need, John McGlashan, a cooling engineer from the citrus industry, has been asked to write a complete manual to be used by all parties in the citrus cold chain.

Opsomming

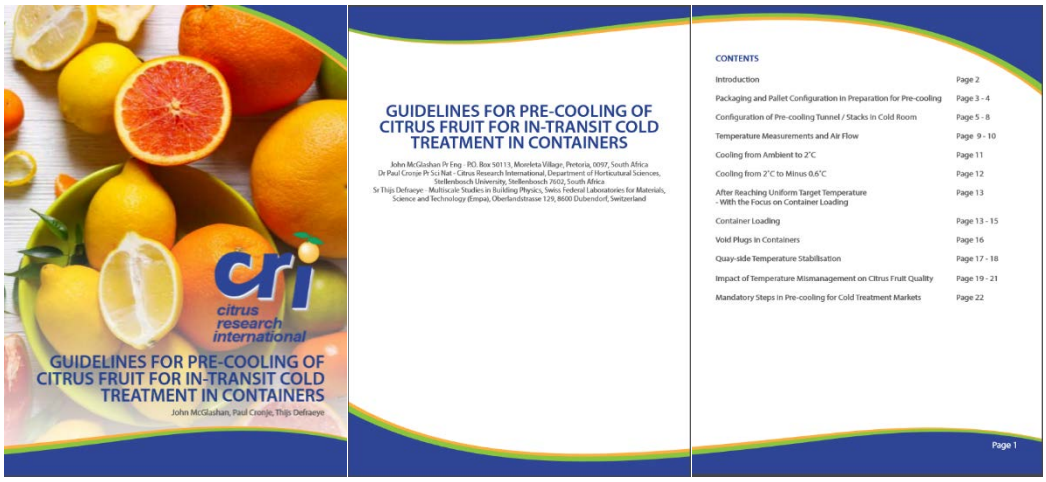
Die toenemende blootstelling van SA sitrus-uitvoere aan koue-sterilisasie markte vereis dat daar weer gekyk moet word na die in-diepte kennis van personeel wat by hierdie proses betrokke is. Onvoldoende of foutiewe vóór-verkoeling het tot betekenisvolle verliese in kwaliteit gelei, asook mislukte pogings om koue-sterilisasie gedurende verskeping van houers te inisieer. Ten einde hierdie behoefte van die industrie aan te spreek, is John McGlashan, ŉ verkoelingsingenieur van die sitrus-industrie, gevra om ŉ volledige handleiding op te stel wat deur alle partye in die sitrus koue-ketting gebruik moet word.

Introduction

The main aim of the document, is not to address pre-cooling *per se* but also looking at critical aspects that may be overlooked in the successful cold treatment of citrus for any particular market. The real secret lies in doing everything correctly, without rushing the process and by paying great attention to detail. This cannot be over-emphasized. There are no shortcuts, and if shortcuts are taken a problem will arise that will be transferred further and further down the line where it will be impossible to eliminate. The industry has to work within the infrastructure that exists. Very few, if any of the facilities were designed for the very strict temperature protocols required. The document would therefore focus on pre-cooling; on the steps that should always be followed, from start to completion, while at the same time draw attention to procedures that will precede pre-cooling and procedures that must be followed after the completion of pre-cooling. For example, how the stabilization process should be carried out and monitored

Objectives

Compiled a guideline, which could be available to all in citrus industry on correct pre-cooling of citrus for Markets that require fruit to undergo the process of cold treatment**.**



Technology transfer

- The booklet is available in e-format from Paul Cronje from CRI (paulcronje@sun.ac.za) and CGA (Mitchell Brooke, mitchell@cga.co.za).
- CRI packhouse workshops during Jan-Feb 2017.

4.4.3 **PROGRESS REPORT: Precooling: ambient loading and forced air cooling of citrus for cold sterilisation markets**

Project 1125 (2014/15 – 2017/18) by Paul Cronje, Jade North and Thijs Defraeye (EMPA-Zurich)

Summary

Forced-air precooling is a widely applied postharvest technology to remove field heat quickly out of packed fresh fruit. The cooling uniformity of the fruit in different pallets and cartons remains unknown during forced-air precooling in commercial operations. The objective of this study was to investigate the cooling rate and heterogeneity of packed citrus fruit in a full-scale and forced-air precooler in South Africa. The influence of package design (package type and wrapping) and fruit size on precooling performance was assessed. Results showed that the cooling heterogeneity mainly occurred along the flow direction in the whole precooling room and also in a single pallet. The influence of fruit size on cooling time can be considered insignificant without appreciable error in this study. Wrapped 'navel' orange fruit were cooled slower than wrapped 'Eureka' lemon fruit with similar size. Fruit wrapping induced slower cooling rate and larger cooling heterogeneity in the downstream cartons. The 'nova' mandarin fruit in Opentop cartons were cooled 24 - 42% faster ($p<0.05$) than the 'Eureka' lemon fruit with similar fruit size in the Supervent cartons. This study will be instrumental to understand the cooling rate and uniformity in forced-air precooling of packed fresh fruit.

Opsomming

Gedurende die na-oes hantering van vrugte word geforseerde lugverkoeling (FAC) algemeen gebruik om die veld-hitte uit die vrugte te haal. Die verkoelings-eenvormigheid in verskillende palette en kartonne is egter nog onbekend. Die doel van die studie was onder verkoelingtempo en heterogeniteit in gepaletiseerder sitrus vrugte te ondersoek in 'n kommersieel FAC fasiliteit in SA. Verder meer is die invloed van impak van die ontwerp van dei karton (tipe en "wrapping") asook vrugrootte op verkoelingstempo ondersoek. Die resultate toon dat die beste verkoelings heterogeniteit hoofsaaklik in die rigting van lugvloei voorkom in die hele verkoelingskamer asook in palette. In die studie is gevind dat vruggrootte nie 'n bydrae maak toet verkoelingstempo nie. 'navel' lemoen vrugte wat "ge-wrap" was verkole betekenisvol stadiger as Eureka' suurlemoene wat ook "gewrap" was. Die gebruik van vrug "wrapping" lei tot 'n stadiger verkoelingtempo en groter verkoling heterogeniteit in die kartonne. Die 'nova' mandaryn vrugte in Opentop kartonne verkoel 24-42% vinniger as suurlemoen van die selfde gewig en grotte verpak in Supervent kartonne. Hierdie studie is instrumenteel om FAC sisteme beter te verstaan en verkoelingstempo en uniformiteit te verbeter in die citrus koueketting.

4.5 PROGRAMME: NUTRITION AND WATER MANAGEMENT

Programme coordinator: Teunis Vahrmeijer

4.5.1 Programme summary

Modelling of water use efficiency of the citrus trees have been an overarching aim in this program. Significant progress has been made in the modelling of citrus tree transpiration by using a canopy conductance models. In the next season renewed efforts will focus on measuring transpiration data from all the instrumented orchards and capturing the driving variables for water use in citrus in order to determine water used efficiency. In this nutrition management section of the program the improvement of the physical properties of soils such as the water holding capacity, aggregate stability and soil porosity via organic material was researched. For the duration of the field trials, an increase in the soluble carbon percentage in the soil profile was observed in all applications.

Programopsomming

Die modulering van die water-gebruik effektiwiteit in die sitrus boom is die oorkoepelende doelwit in die program. Daar is goeie vordering gemaak met die modulering van die boom se transpirasie stroom deur gebruik te maak van geleidings modelle. In die volgende seisoen sal die fokus wees op die transpirasie data van al die boorde waar instrumente geplaas is om die faktore wat verantwoordelik is vir water gebruik in sitrus te bepaal en om dan water gebruis effektiwiteit te bereken. In die bemestingsbestuur afdeling was gekyk na die verbetering van die grondeienskappe soos water hou vermoei, aggregaat stabiliteit en gronddeurlugting duur die gebruik van organiese materiaal. Gedurende die proef was daar ŉ verhoging in die oplosbare fraksie koolstof in die profil vir alle behandelings.

4.5.2 PROGRESS REPORT: A novel approach to water and nutrient management in citrus

Project 986 (2013/4 – 2016/7) by JT Vahrmeijer (CRI) and M Banda (UP)

Summary

The first phase of measuring citrus water use in winter rainfall area was completed in May 2017. However, measurements continue in the 'McLean' Valencia orchard, to ascertain if there will be any changes in water use with change in the irrigation regime. Measuring of the citrus water use in the summer rainfall area (Letsitele) is still in progress and will be completed by the end of February 2018. Full season data for citrus water use has been collected from 'Star Ruby' orchards, which were planted in 2006, 2010 and 2011 and from 'Midknight' Valencia orchards, which were planted in 1995 and 2008. However, due to heavy rainfall, data loggers from the 'Midknight' Valencias orchard (planted in 2014) and two 'Valley Gold' Mandarin orchards (planted in 2015 and 2013) were damaged, which resulted in some data loss. These data loggers were repaired and reinstalled and a full season of data will have been collected by the end of February 2018. Information on transpiration, weather, evapotranspiration, soil evaporation and radiation interception by the canopy, stomatal conductance and water potential (pre-dawn, leaf and stem) is being collected at regular time intervals. Progress has been made with the water stress trial in Groblersdal. Water stress has been applied at the flowering phase, fruit enlargement phase and fruit colouring phase. Preliminary results indicated clear physical differences between the treatments and the control. Weekly measurements of the leaf water potentials, fruit size and stomatal conductance were taken throughout the trial period. Progress has also been made in the modelling of citrus tree transpiration, using a canopy conductance model (Villalobos et al. 2013), which requires information on the radiation interception by the canopy and vapour pressure deficit as the main input variables. Future efforts will continue to focus on obtaining good transpiration data from all the instrumented orchards and capturing the driving variables for water use in citrus.

Opsomming

Die eerste fase van die projek, om die watergebruik van sitrusbome in ŉ winterreënval gebied te bepaal, is in Mei 2017 afgehandel. Watergebruik word egter nog in die "McLean' Valencia gemeet om te bepaal of daar enige veranderinge in die watergebruik van die sitrusbome is, indien die besproeiingskedulering verander word. Die meet van watergebruik in sitrus in die somerreënval area (Letsitele) gaan nog voort. ŉ Volle seisoen se watergebruik data vir 'Star Ruby' boorde (geplant in 2006, 2010 en 2011) en vir 'Midknight' Valencia boorde (geplant in 1995 en 2008) is ingesamel. As gevolg van swaar reën is van die instrumente, wat in die 'Midknight' Valencias boord (geplant in 2014) en twee 'Valley Gold' Mandaryn boorde (geplant in 2013 en 2015) geïnstalleer was, beskadig en is van die data verloor. Die instrumente is egter herstel en weer geïnstalleer en ŉ vol seisoen se data sal teen Februarie 2018 ingesamel wees. Inligting ten opsigte van transpirasie, weerveranderlikes, evapotranspirasie, grond evaporasie en radiasie-onderskepping deur die blaardak,

huidmondjiegeleiding en waterpotensiaal (voor dagbreek, blaar en stam) word gereeld ingesamel. Goeie vordering is met die waterstres eksperiment in Groblersdal gemaak. Waterstres is tydens blomstadium, vrugvergrotingstadium en vrugverkleuringstadium geïnduseer. Voorlopige resultate dui op merkbare verskille tussen die waterstresbehandelings en die kontrole waar geen water tekorte geïnduseer is nie. Weeklikse metings van blaarwaterpotensiale, vruggrootte en huidmondjie geleiding is tydens die studie geneem. Vordering is ook gemaak met die modellering van transpirasie van sitrusbome deur gebruik te maak van die blaardakgeleidingsmodel (Villalobos et al. 2013). Hierdie model maak gebruik van radiasie-onderskepping deur die blaardak en dampdruktekort as primêre veranderlikes. Met die studie verder sal daar gepoog word om goeie en betroubare inligting ten opsigte van transpirasie en die veranderlikes wat sitruswatergebruik bepaal, in te samel

4.5.3 PROGRESS REPORT: Nitrogen and Potassium release from organic soil amendments over time

Project 1113 (2015/6 – 2016/7) by J.T. Vahrmeijer (CRI), CM van Heerden (UP) and E Tesfamarium (UP)

Summary

Soil organic matter is a major source of plant nutrients and improves the physical properties of soil such as water holding capacity, aggregate stability and soil porosity. However, there is some concern that nitrogen (N) may be released from the organic material late during the season that will influence the colouring and quality of citrus fruits. Incubation studies to determine the release patterns of N, in the form of NO_3^- and NH^{4+}, and potassium (K) released from four different organic materials have been done. The incubation studies proved that manure based composts (vermicompost (VC) and cattle manure (CM)) have high NH^{4+} and K contributions during the first 28 days of incubation, where 82.69% of the total extracted K in the CM and 73.00% of the total extracted K in the VC was released during the first 28 days the incubation period. At the end of the incubation trial (after 150 days) the N and K content of the different organic soil amendments were determined.

The incubation showed the change in the total N and total K percentage of the organic soil amendments as follows, in VC, N decreased with a 4.17% and K decreased with 74.42%, in CM, N increased with 13.69% and K decreased with 58.30%, in wood based composts (WBC), N increased with 16.21% and K decreased with 36.66%, and in citrus waste composts (CWC), N decreased with 16.50% and K decreased with 26.59%. Results from field trails at Letaba Estates, that commenced in September 2015, indicated an initial increase in total N percentage in the leaves after fertiliser and organic material application for the first and second year of the field trials. CM and VC resulted in the higher increase in total N percentage in the leaves during the first and second year than WBC and CWC. The soil samples also had a significant increase in mg/kg of NO_3^- along with an increase in total N percentage in leaves, VC and CM measured higher in NO^{3-} mg/kg than WBC and CWC after application, suggesting that manure based compost has a higher initial NO_3^- contribution to the soil profile during the first two months after application. of the higher K in the soil profile showed an increase with VC suggesting that VC has the highest contribution of K to the soil profile.

Opsomming

Grond organiese materiaal is 'n belangrike bron van plantvoedingstowwe en verbeter die fisiese eienskappe van grond soos waterhouvermoë, aggregaatstabiliteit en grondporositeit. Daar is egter 'n mate van kommer dat stikstof (N) van die organiese materiaal laat vrygelaat kan word tydens die seisoen wat die kleur en kwaliteit van sitrusvrugte sal beïnvloed. Die inkubasie studies het bewys dat die mis gebaseerde komposte (Vermicompost (VC) en Beesmis (CM)) 'n hoë NH^{4+} en K bydrae gedurende die eerste 28 dae van inkubasie het. 82,69% van CM en 73,00% van VC van die totale K wat onttrek is, is vrygestel gedurende die eerste 28 dae van die 150 dae inkubasie. Die inkubasie het die verandering in die totale N en die totale K persentasie van die organiese grond wysigings wat geïnkubeer is as volg uitgewys, in VC, N het afgeneem met 4,17% en K het met 74,42% afgeneem, in CM, N het met 13.69% toegeneem en K het verminder met 58,30%, in houtgebaseerde kompos (WBC), N het met 16,21% toegeneem en K het met 36,66% afgeneem, en in sitrus-afvalkompos (CWC) het N met 16.50% afgeneem en K het met 26,59% afgeneem. Uitslae van die veldproewe by Letaba Estates, wat in September 2015 begin het, was 'n aanvanklike toename in die totale N persentasie in die blare opgemerk, na bemesting en organiese materiaal toegedien is vir die eerste en tweede jaar van die veldproewe. CM en VC het gelei tot die hoër toename in totale N persentasie in die blare gedurende die eerste en tweede jaar as wat WBC en CWC bygedra het. Die grondmonsters het ook 'n beduidende toename in mg/kg NO^{3-} gehad, tesame met 'n toename in totale N persentasie in blare, VC en CM, het hoër gemeet in NO^{3-} mg/kg as WBC en CWC na toediening, wat daarop dui dat misgebaseerde kompos 'n hoër aanvanklike NO^{3-} bydrae tot die grondprofiel gedurende die eerste twee maande na toediening maak. K in die grondprofiel toon 'n toename na VC toeding wat daarop dui dat VC die hoogste bydrae van K tot die grondprofiel het.

4.5.4 **PROGRESS REPORT: Foliar uptake of urea and micronutrients in mandarins grown under shade net in different climatic regions**
Project 1167 (2017/8 – 2018/19) by JT Vahrmeijer (CRI) and C Botha (UP)

Summary

This study aims to evaluate the ability of urea foliar sprays to manipulate flowering intensity, as well as to assess the effect of shade netting on leaf surface morphology. Urea with micronutrients will be applied as pre-bloom foliar sprays 6 - 8 weeks before flowering at each of the sites respectively. The flowering intensity, flower quality and inflorescence types will be assessed. Since the sprays only commence in the late winter season of 2017 and measurements will start two weeks after spraying, no data is available yet. This study will cover both 2017 and 2018 seasons. However, some progress was made. The literature review was completed and the protocol was presented to the post graduate evaluation committee at the Department of Plant and Soil Sciences at the University of Pretoria. The trial sites were also identified and the necessary arrangements for the trials were made with the farm owners. The summer rainfall trials will be conducted at Roslè Boerdery (25.2026° S, 29.4163° E), close to Groblersdal on 'nadorcott' Mandarin, planted in 2009. The winter rainfall trials will be conducted at Mouton Citrus (32.542º S, 19.0110º E), in Citrusdal on 'nadorcott' Mandarin, planted in 2013.

Preliminary microscope analyses were done on sample leaves from trees grown under shade netting and trees grown in the open. Leaves were prepared and analysed with a scanning electron microscope (SEM). The surface micromorphology was assessed and was attempted to measure the cuticle thickness through fracturing the leaf. However, the cuticle structure was unclear, therefore transmission electron microscopy (TEM) will be used in future to determine the difference in leaf cuticle thickness. Apart from TEM, light microscopy can also be used to measure the thickness of the cuticle. However, due to the waxy consistency of the leaf cuticle, difficulty was experienced with embedding the material in an epoxy resin. Hence, appropriate alterations will be made to the infiltration steps during the preparation procedure. Results from the microscopy study will depend on the clarity of the cuticle structure to detect a difference in thickness between the leaves of the trees grown under shade netting and those grown in the open.

Opsomming

Die doelwitte van hierdie studie is om te bepaal of ureum blaarbespuitings gebruik kan word om die blomintensiteit van sitrusbome te manipuleer. 'n Tweede doelwit is om die verskil in blaarmorfologie van bome onder skadunet en bome wat in boorde sonder skadunet groei, te evalueer. Ureum met mikro-elemente sal 6 – 8 weke voor blom, as 'n blaarbespuiting toegedien word. Blomintensiteit, blomkwaliteit en blomtipe sal geëvalueer word. Omdat die blaarbespuitings laat in die winterseisoen toegedien word en metings eers twee weke later begin, is daar nog geen resultate beskikbaar nie. Hierdie studie sal strek oor beide die 2017 en 2018 seisoene. Daar is egter vordering met die studie gemaak. Die literatuur studie is afgehandel en die projek is aan die nagraadse evalueringskomitee van die Departement van Plant- en Grondwetenskappe aan die Universiteit van Pretoria voor gelê. Die eksperimentele persele is geïdentifiseer en die nodige reëlings is met die plaaseienaars getref. Die veldproewe in die somer reënval streek, sal uitgevoer word op Nadorcott Mandaryne by Roslè Boerdery (25.2026° S, 29.4163° O), naby Groblersdal. In die winter reënvalstreek, sal die veldproewe uitgevoer word op 'nadorcott' mandaryn by Mouton Citrus, (32.542º S, 19.0110º O), naby Citrusdal.

Voorlopige mikroskoopanalises is op blare van bome wat onder skadunet groei en bome wat nie onder skadunet groei, uitgevoer. Die blare is voorberei en die verskille in blaaroppervlakmorfologie is met behulp van 'n skanderings elektronmikroskoop (SEM) geëvalueer. Daar is ook gepoog om die dikte van die kutikula te meet deur die blaar te breek, maar die struktuur was te onduidelik. Daarom sal 'n transmissie elektronmikroskoop (TEM) in die toekoms gebruik word om die kutikula-dikte te meet. Ligmikroskopie kan ook gebruik word om die dikte van die kutikula te meet, maar daar is probleme ondervind om die blaar in die epoksie-hars te set. Die nodige aanpassing in die infiltrasie stappe tydens die voorbereidingsproses sal gedoen word. Die resultate van die mikroskopie studie sal afhang van die duidelikheid van die kutikula om sodoende die kutikula-dikte verskil te meet van die blare van bome wat onder skadunette groei en die wat nie onder skadunette groei nie.

4.6 **PROGRAMME: CULTIVAR EVALUATION**
Programme coordinator: Johan Joubert (CRI)

4.6.1 **PROGRESS REPORT: Cultivar characteristics and climatic suitability of Satsuma mandarins in a cold production region (East Cape Midlands)**
Project 57A by W. Swiegers and Z. Zondi (CRI)

Summary

This specific Satsuma trial is a commercial planting. The trial location is in an area well suited for Satsuma production. The orchards were planted between 1991 and 2000. The trees are mature with large tree canopies. The order of ripening was as follows; Primosole, Ueno, Miyagawa Wase, and Dobashi-Beni

Picking periods for Satsumas should be limited to 2-3 weeks to ensure good internal quality and avoid puffiness. Satsuma selections need degreening after harvest as the internal quality is ahead of the colour development.

Opsomming

Hierdie spesifieke Satsuma proef is kommersiële aanplanting. Die proef se ligging is goed geskik vir Satsuma produksie. Die boorde is op verskillende tye aangeplant van 1991 tot 2000. Die bome is volwasse met groot boom volume. Die orde van rypwording was as volg: Primosole, Ueno, Miyagawa Wase, en Dobashi-Beni.

Pluk periodes vir Satsumas sal strek oor 2-3 weke aangesien vrugte se suur vinnig daal en die skil powwerig raak. Vrugkleur is laat teenoor die interne kwaliteit en ontgroening sal moet gedoen word.

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 early to late Satsuma selections from the East Cape Midlands part of the Eastern Cape. The following selections were evaluated: Primosole, Miyagawa Wase, Dobashi Beni and Ueno.

Table 4.6.1. List of Satsuma selections evaluated at Saxfold Park (Adelaide) during 2016.

Selection	Rootstock	Planted
Ueno	Carrizo	1997
Dobashi Beni	Unknown	Unknown
Miyagawa Wase	Swingle	1997
Primosole	Unknown	Unknown

Results and discussion

Primosole
Primosole was the first selection to mature this season. It developed a very good fruit size (count 1xxx). Primosole was the only selection with a seed count and at an average of 0.9 seeds per fruit. It developed the second best fruit colour on the colour plate range (T4 – T5). The juice percentage increased with maturity and peaked at 50.1 %. Primosole had the lowest Brix with maturity at 8,9 as well as the lowest Acid % (0.82%).

Dobashi Beni
Dobashi Beni were the late maturing Satsuma selection, with maturity being in the beginning of May. The fruit size was good and peaked at count 1x. The juice percentage of the Dobashi Beni were the lowest of all the selections evaluated. The juice percentage decreased by the timeof maturity to 46.4%. Dobashi Beni had the second highest Brix° and Acid % with maturity (9,9° and 0.98%). Dobashi Beni had no seeds and the fruit colour on the colour plate range peaked at T5.

Miyagawa Wase
The fruit size development of Miyagawa Wase ranged from count 1 to 1x. There was a slight decrease in juice % by the time of maturity, but still good (56.3%). The Brix and acid levels were very good at maturity, being

9.7 and 0.97% respectively. There were no seeds in Miyagawa Wase. The colour on the colour plate at peak maturity were T5.

Ueno

Ueno had an above average fruit size count with with large fruit size (count 1x). Ueno's juice % increased with maturity and it also had the highest juice % of the 4 selections with 57.5% at peak maturity. The Brix° were the highest at maturity being 10.8 with very good acids of 1.04%. The acid % of Ueno were also the highest at maturity. The fruit were completely seedless and Ueno's colour development on the colour plate range with maturity were the best at T4.

For Satsuma mandarins, 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 the fruit is considered over mature. 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.

Conclusion

Primosole had the best fruit size count ranging from 1xx to 1xxx, while Dobashi Beni, Ueno and Miyagawa Wase peaked at a size count of 1x. Ueno and Miyagawa Wase had the best juice percentages: 57.5 % and 56.3 % respectively. Ueno had the highest Brix° of all the Satsuma selections (10.8°). Ueno also had the highest acid percentage of all the Satsuma selections (1.04%). Primosole had the highest seed count with 0.9 seeds per fruit. Ueno had the best colour development with T4, while Primosole were between T4 and T5.

Table 4.6.1.2. Internal fruit quality data for Satsuma selections in the Adelaide region (Saxfold) of the East Cape Midlands during the 2016 season.

Date	Cultivar	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2016/03/29	Dobashi Beni	Unknown	2	49,9	9,2	1,45	6,3	0,0	T7
2016/05/03	Dobashi Beni	Unknown	1x	46,4	9,9	0,98	10,1	0,0	T5
2016/03/14	Miyagawa Wase	SC	1x	57,9	8,9	1,33	6,7	0,0	T7
2016/03/29	Miyagawa Wase	SC	1	56,3	9,7	0,97	10,0	0,0	T5
2016/03/14	Primosole	Unknown	1xx	49,7	8,7	1,16	7,5	0,3	T7
2016/03/29	Primosole	Unknown	1xxx	50,1	8,9	0,82	10,9	0,9	T4-5
2016/03/14	Ueno	CC	1x	55,5	8,6	1,15	7,5	0,0	T7
2016/03/29	Ueno	CC	1x	57,5	10,8	1,04	10,4	0,0	T4

4.6.2 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Satsuma mandarins in a cold production region (Sundays River Valley)

Project 57B by W. Swiegers and Z. Zondi (CRI)

Summary

This specific Satsuma trial is new and 2016 were the first crop on the trees. The trees were topworked to the following selections; Aoshima, Miho Wase, Miyagawa Wase, Sugiyama en Ueno. The trees were still small with limited crop. The order of ripening was as follows: Miho Wase, Miyagawa Wase, Ueno, Aoshima en Sugiyama.

Picking periods for Satsumas should be limited to 2-3 weeks to ensure good internal quality and avoid puffiness. Satsuma selections need degreening after harvest as the internal quality is ahead of the colour development.

Opsomming

Hierdie spesifieke Satsuma proef is ŉuut en 2016 was die bome se eerste drag. Die bome was in 2012 getopwerk na die volgende seleksies; Aoshima, Miho Wase, Miyagawa Wase, Sugiyama en Ueno. Die bome was nog jonk met beperkte drag. Die orde van rypwording was as volg: Miho Wase, Miyagawa Wase, Ueno, Aoshima en Sugiyama.

Pluk periodes vir Satsumas sal strek van 2-3 weke aangesien vrugte se sure vinnig daal en die skil powwerig raak. Vrugte se kleur is laat teenoor die interne kwaliteit en ontgroening sal moet gedoen word.

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 early to late Satsuma selections from the Sundays River Valley part of the Eastern Cape. The following selections were evaluated: Miho Wase, Miyagawa Wase, Ueno, Aoshima en Sugiyama

Table 4.6.2.1. List of Satsuma selections evaluated at Invercloy (Kirkwood) during 2016.

Selection	Rootstock	Planted
Aoshima	Carrizo	2012
Miho Wase	Carrizo	2012
Miyagawa Wase	Carrizo	2012
Sugiyama	Carrizo	2012
Ueno	Carrizo	2012

Results and discussion

Aoshima
Aoshima were overripe at the beginning of May with a Ratio of 11,9 and the Acid % being at 0.64%. The Brix was at 7.6 when overripe, and 7.4 with an acid content of 1.18 % towards peak maturity. Aoshima did have a very good fruit size count with the count being 1xxx. The juice % did decrease from 50.6% to 42.4% from build up to peak maturity to being over mature. There were no seeds in the fruit. The external colour development of the Aoshima was not very good and peaked between T6 and T7 on the colour plate.

Miho Wase
Miho Wase were the first selection to mature to peak maturity this year. Miho Wase are also the control for this site. They had a good fruit size count of 1xx. Miho Wase had the highest juice % of all the Satsuma selections, although the juice % did decrease towards peak maturity from 66.9% to 58.6%. It decreased even futher towards over maturity. Miho Wase had the second highest Brix° and acid % at peak maturity (8° and 0.83% respectively). The fruit had no seeds and the external colour development were again behind the internal quality development. The colour for Miho Wase on the colour plate were T6.

Miyagawa Wase
Miyagawa Wase were the second selection to mature (peak maturity). The fruit size count was very good with a count of 1xxx. The juice % did increase towards peak maturity, but did decrease as the fruit start to over mature. The Brix° decreased with the acid % towards peak maturity. It does look if the Brix stabilized at peak maturity (Table 4.6.2.2). Miyagawa Wase had no seeds in the fruit. The colour on the colour plate were T7 and it stayed there even when the fruit were over mature.

Sugiyama
Sugiyama were the last selection to mature at this trial site. Sugiyama had a very good fruit size count and peaked at 1xxx. The juice % increased towards peak maturity and Sugiyama had the second highest juice % (58.2 %). Brix levels decreased with the acid % towards peak maturity. The Brix of Sugiyama were the lowest at 6.9 of all the selections evaluated. Sugiyama were the only one with a seed count, it was a very low count of 0.3 seeds per fruit. This was only found once during the fruit sampeling. The colour on the colour plate were T6 and T7.

Ueno
Ueno's fruit size count was very good ranging from 1xx to 1xxx. The juice percentage decreased towards peak maturity to quite a low % (48.2%). There was also a slight decrease in Brix towards peak maturity. Ueno had

no seeds and the colour development of Ueno peaked at T6. This was one of the better external colour developments compared to the other selections.

For Satsuma mandarins, 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 the fruit is considered over mature. 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.

Conclusion

Miyagawa Wase had the best fruit size and peaked at count 1xxx. Aoshima, Miho Wase, Sugiyama and Ueno also had a good fruit size count ranging from 1xx to 1xxx. Miho Wase and Sugiyama had the highest juice content, being 58.6 % and 58.2 % respectively. Miho Wase had the highest Brix at peak maturity of all the Satsuma selections being (average 8). Sugiyama developed the highest seed count with 0.3 seeds per fruit.

Table 4.6.2.2. Internal fruit quality data for Satsuma selections in the Kirkwood region (Invercloy) of the Eastern Cape during the 2016 season.

Date	Cultivar	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2016/03/10	Aoshima	CC	1xx	50,6	7,4	1,18	6,3	0,0	T8
2016/05/05	Aoshima	CC	1xxx	42,4	7,6	0,64	11,9	0,0	T6-7
2016/03/10	Miho Wase	CC	1xx	66,9	7,4	0,88	8,4	0,0	T7
2016/03/23	Miho Wase	CC	1xx	58,6	8,0	0,83	9,6	0,0	T6
2016/04/07	Miho Wase	CC	1xx	54,2	7,8	0,60	13,0	0,0	T4
2016/03/10	Miyagawa Wase	CC	1xxx	55,0	7,7	0,96	8,0	0,0	T7
2016/03/23	Miyagawa Wase	CC	1xxx	57,1	7,2	0,81	8,9	0,0	T7
2016/04/07	Miyagawa Wase	CC	1xx	47,2	7,3	0,67	10,9	0,0	T6
2016/03/23	Sugiyama	CC	1xxx	53,2	7,2	0,98	7,3	0,3	T8
2016/04/19	Sugiyama	CC	1xxx	58,2	6,9	0,78	8,8	0,0	T6-7
2016/05/05	Sugiyama	CC	1xx	33,6	6,7	0,64	10,5	0,0	T6-7
2016/03/10	Ueno	CC	1	54,5	8,0	1,17	6,8	0,0	T8
2016/03/23	Ueno	CC	1xxx	51,4	8,2	1,00	8,2	0,0	T7
2016/04/07	Ueno	CC	1xx	48,2	7,7	0,76	10,1	0,0	T6

4.6.3 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (East Cape Midlands)

Project 997A by W Swiegers and Z. Zondi (CRI)

Summary

The 2016 season was the second last harvest for this Mandarin trial in the East Cape Midlands area. A range of new mandarin hybrids have been added to this area and should be bearing fruit in the 2022 season. Nadorcott is the control selection for this trial site. The order of ripening was as follows: Nadorcott, Tango, Golden Nugget, Tahoe Gold, Yosemite Gold en Shasta Gold.

Opsomming

Die 2016 seisoen was die 2de laaste oes jaar vir hierdie mandaryn proef in die Oos-Kaap Middelande. Daar is opwinde nuwe seleksies getopwerk op die perseel. Die nuwe seleksies behoort vrugte te dra teen 2022. Nadorcott dien as kontrole vir hierdie proef perseel. Die volgorde van rypwording was as volg: Nadorcott, Tango, Golden Nugget, Tahoe Gold, Yosemite Gold en Shasta Gold.

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 region of the East Cape Midlands. The following varieties were evaluated: Gold Nugget, Nadorcott, Shasta Gold, Tahoe Gold, Tango and Yosemite Gold.

A ratio of 11:1 for mandarin hybrids 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 over mature. 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.

Table 4.6.3.1. List of mandarin hybrid selections in the Cookhouse (J&B) region of the East Cape Midlands during the 2016 season.

Selection	Rootstock	Topwork
Gold Nugget	CC	2010
Nadorcott	CC	2010
Shasta Gold	CC	2010
Tahoe Gold	CC	2010
Tango	CC	2010
Yosemite Gold	CC	2010

Results and discussion

Gold Nugget
Gold Nugget was the third selection to mature with a Brix of 13.8° and the Acid still being at 1.28%. This indicates that the fruit will likely have an extended shelf life. The selection had a few seeds but it was low counts ranging from 0.2 to 0.4 seeds per fruit. There were also a couple of times were no seeds were counted. Gold Nugget has a very upright growth habbit. The selection had good fruit size with peaks from 1 to 1x. The juice % of the Gold Nugget also increased as the selection gets to peak maturity, with the highest juice content at 61 %. Gold Nugget had a good colour development at the trial site and peaked at colour plate T1 before peak maturity was reached.

Nadorcott
Nadorcott is used as a control for the mandarin varieties, and more specifically Tango. Nadorcott were the first selection to reach peak maturity. Nadorcott developed the second smallest fruit size (count 1) for this trial site for a second year in a row. The selection was not seedless with a seed count of 0.3 seeds per fruit. The colour development was good (T1 colour) at peak maturity. The selection also had no problem with acids remaining high when Brix levels dropped.

Shasta Gold
Shasta Gold were the last selection to mature. The selection had high Brix levels of 12.6° with acids still at 1.5% (in the beginning of August). Shasta Gold had the best colour development of all the selections at the trial site and peaked at colour plate T1 well before peak maturity was reached for the second year. The selection had the lowest internal juice content of 46.9% in 2015. While in 2016 it have some of the highest internal juice content at 60.8%. Shasta Gold also had the highest seed count at 1.8 seeds per fruit.

Tahoe Gold
Tahoe Gold was the first selection to mature in 2015, but in 2016 it was fourth towards peak maturity. The selection had the second highest seed count with an average of 1.4 seeds per fruit for the second year running. Tahoe Gold had a very high juice content (above 58%) in 2015 and 2016. Tahoe Gold had a slightly smaller fruit size in 2016 with the fruit size count between 1 and 1x. The selection was still progressing to peak maturity and the fruit size could have been a little bit bigger at peak maturity. Tahoe Gold also had no problem with reaching T1 colour on the colour plate before peak maturity.

Tango

Tango were the second selection to mature in 2016, although the last selection to mature in 2015, due to a high acid percentage. The selections had the smallest fruit size and peaked between count 2 and count 1. External colour development was very good with a T1 on the colour plate before peak maturity. Tango had the highest juice levels above 60% in 2015 and in 2016 it was just below 60% on its way towards peak maturity. Tango also kept its acids high as the Brix were increasing, resulting in a very good eating fruit.

Yosemite Gold

Yosemite Gold was the second last selection to mature with very good external colour development, reaching T1 on the colour plate, before peak maturity. Yosemite Gold developed a good fruit size count ranging from 1x to 1xx towards peak maturity. The selection was completely seedless for 3 evaluations, but devepoled 1.4 seeds per fruit with the 4th round. The juice percentages tested higher in 2016.

Conclusion

Yosemite- and Shasta Gold is the two selections with the largest fruit size ranging from count 1x to 1xx. None of the selections struggled with colour development as all of them reach T1 on the colour plate before peak maturity. All six selections are likely to have a good shelf life due to the high acid levels. Gold Nugget, Shasta Gold and Tahoe Gold had the best juice percentages (61%, 60.8% and 60.9%). Shasta Gold had the highest number of seeds per fruit (1.8).

Table 4.6.3.2. Internal fruit quality data for Mandarin hybrid selections from the Cookhouse (J&B) region of the East Cape Midlands during the 2016 season.

Date	Selection	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2016/05/23	Gold Nugget	CC	2	54.3	11.3	1.85	6.1	0,4	T6 & 7
2016/06/07	Gold Nugget	CC	1	51.9	12.9	1.7	7.6	0	T1
2016/06/27	Gold Nugget	CC	1x	51.4	13.5	1.72	7.8	0	T4
2016/07/22	Gold Nugget	CC	1x	56,6	14,5	1,56	9,3	0,3	T1
2016/08/05	Gold Nugget	CC	1x	61,0	13,8	1,28	10,8	0,4	T1
2016/08/26	Gold Nugget	CC	1	56,7	15,4	1,23	12,5	0,2	T1
2016/09/09	Gold Nugget	CC	1	56,0	16,1	1,11	14,5	0	T1
2016/05/23	Nadorcott	CC	2	46.4	10.5	1.26	8.3	0	T6
2016/06/07	Nadorcott	CC	1	53.5	11	1.23	8.9	0	T6
2016/06/27	Nadorcott	CC	1	52.8	12.2	1.36	9	0	T2
2016/07/22	Nadorcott	CC	2	59,8	13,8	1,10	12,5	0	T1
2016/08/05	Nadorcott	CC	1	53,1	14,4	1,31	11,0	0,3	T1
2016/05/23	Shasta Gold	CC	1	58.1	11.2	2.33	4.8	0	T6 & 7
2016/06/07	Shasta Gold	CC	1xx	55.9	10.8	1.72	6.3	0	T3 & 4
2016/06/27	Shasta Gold	CC	1xx	54.9	11.8	1.69	7	0.3	T3
2016/07/22	Shasta Gold	CC	1x	60,8	12,9	1,47	8,8	1,8	T1
2016/08/05	Shasta Gold	CC	1xx	59,4	12,6	1,50	8,4	1,6	T1
2016/05/23	Tahoe Gold	CC	1	61.5	11	1.75	6.3	1.3	T 6 & 7
2016/06/07	Tahoe Gold	CC	1	62.6	11.8	1.6	7.4	0	T5
2016/06/27	Tahoe Gold	CC	1x	60.2	11.5	1.33	8.6	0	T2
2016/08/05	Tahoe Gold	CC	1	60,9	13,0	1,23	10,6	1.4	T1
2016/05/23	Tango	CC	2	55.6	10.2	1.32	7.7	0	T6
2016/06/07	Tango	CC	1	50.3	11.7	1.53	7.6	0	T4
2016/06/27	Tango	CC	2	51.9	12.3	1.41	8.7	0	T1 & 2
2016/07/22	Tango	CC	2	59,3	14,6	1,42	10,3	0	T1
2016/05/23	Yosemite Gold	CC	1xx	53.7	10.3	1,81	5.7	0	T6 & 7
2016/06/07	Yosemite Gold	CC	1xx	57	11	1.72	6.4	0	T3 & 4

2016/06/27	Yosemite Gold	CC	1xx	53.2	11.8	1.73	6.8	0	T2
2016/07/22	Yosemite Gold	CC	1x	59,6	12,8	1,41	9,1	1,4	T1

4.6.4 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (Sundays River Valley)

Project 997B by W. Swiegers and Z. Zondi (CRI)

Summary

The mandarin trial is divided into two different trial sites. The one trial site is in Kirkwood and is a semi commercial planting. The following selections were planted in the order of ripening: Saint Andre, African Sunset (B24), Gold Nugget, Tango, Valley Gold (B17), Tahoe Gold, Yosemite Gold and Shasta Gold. The other site is in Addo about 20 km from Kirkwood, also part of the Sundays River Valley. This site has the following selections and in the order of ripening: Sonet 2, Sonet, Michal 6/47, Michal 89/64, Edit x Nova, Merav 119, Merav 63, Nova (kontrole), Nova ARC, B24, 2 PH Phoenix, IRM 2, Nadorcott SL, IRM 1, Shani and Nadorcott ARC.

Opsomming

Die mandaryn proef is opgedeel in twee verskillende proef persele. Die een proef perseel bestaan uit die volgende seleksies wat ook die volgorde van rypwording was: Saint Andre, African Sunset (B24), Gold Nugget, Tango, Valley Gold (B17), Tahoe Gold, Yosemite Gold en Shasta Gold. Die proef perseel is in Kirkwood en is ŉ semi kommersiële aanplanting. Die ander perseel is in Addo so 20 km vanaf Kirkwood, maar maak nogsteeds deel uit van die Sondagsrivier Vallei. Die perseel bestaan uit die volgende seleksies wat ook die volgorde van rypwording was: Sonet 2, Sonet, Michal 6/47, Michal 89/64, Edit x Nova, Merav 119, Merav 63, Nova (kontrole), Nova ARC, B24, 2 PH Phoenix, IRM 2, Nadorcott SL, IRM 1, Shani en Nadorcott ARC.

Objectives

- To select mandarin hybrid cultivars with 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. The following varieties were evaluated: Saint Andre, African Sunset (B24), Gold Nugget, Tango, Valley Gold (B17), Tahoe Gold, Yosemite Gold, Shasta Gold, Sonet 2, Sonet, Michal 6/47, Michal 89/64, Edit x Nova, Merav 119, Merav 63, Nova (kontrole), Nova ARC, 2 PH Phoenix, IRM 2, Nadorcott SL, IRM 1, Shani SL and Nadorcott ARC.

A ratio of 11:1 for mandarin hybrids 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 over mature. 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.

Table 4.6.4.1. List of Mandarin hybrid selections from Kirkwood region of the Sundays River Valley (Dunbrody) during the 2016 season.

Selection	Rootstock	Topwork
Saint Andre	Carrizo	2013
African Sunset (B24)	Carrizo	Unknown
Valley Gold (B17)	Carrizo	Unknown
Gold Nugget	Carrizo	2013
Shasta Gold	Carrizo	2013
Tahoe Gold	Carrizo	2013
Yosemite Gold	Carrizo	2013
Tango	Carrizo	2013

Table 4.6.4.2. List of Mandarin hybrid selections evaluated in the Sundays River Valley (Penhill) region during the 2016 season.

Selection	Rootstock	Topwork
African Sunset (B24)	Carrizo	2011
Nova	Carrizo	2011
Nova ARC	Carrizo	2011
Sonet	Carrizo	2011
Sonet 2	Carrizo	2011
Michal 6/47	Carrizo	2011
Michal 89/64	Carrizo	2011
Edit x Nova	Carrizo	2011
Merav 119	Carrizo	2011
Merav 63	Carrizo	2011
Shani SL	Carrizo	2011
Nadorcott ARC	Carrizo	2011
Nadorcott SL	Carrizo	2011
2PH Phoenix	Carrizo	2011
IRM 1	Carrizo	2011
IRM 2	Carrizo	2011

Results and discussion

African Sunset (B24)
The fruit size at both sites were very large and peaked at count 1xxx. For the second year B24 was completely seedless. The colour development of B24 was very good with T1 on the colour plate before peak maturity. African Sunset had a good juice content being above 56% in Addo and above 60% in Kirkwood. In 2015 the B24 did not even reach Brix above 10, while in 2016 it peaked above 12 at the Kirkwood site.

Nova
Nova was used as a control in this mandarin trial site. Nova's fruit size count have ranged from 2 to 1x. The juice level increased towards peak maturity to 59.1 %. The fruit kept its acid content quite well even when it was over mature. Nova peaked at 1.8 seeds per fruit for this trial (crosspollination). The colour development of the Nova fruit ranged from T1 to T2 on the colour plate by the time of peak maturity.

Nova ARC
Towards peak maturity, Nova ARC had a smaller fruit size (count 3) compared to Nova (count 1x). Nova ARC had a higher juice content (62.5%) towards peak maturity, as well as Brix and acid levels towards peak maturity compared to Nova. The fruit was completely seedless without any colour development problems and developed a T1 on the colour plate well before peak maturity.

Saint André
At peak maturity the Saint Andre have a very good juice content and peaked at 63%, decreasing as the fruit get over mature. The fruit size of the Saint Andre was good and ranged from count 1x to 1xx. Brix increased slightly towards peak maturity with the acid level stabilizing aroud 0.8%. Two of the three fruit samples had a zero seed count and the one had 0.4 seeds per fruit. The Saint Andre had an external colour of T6 to T7 on the colour plate. Before peak maturity colour development peaked between T1 and T2 on the colour plate.

Gold Nugget
Gold Nugget developed a good fruit size and peaked at count 1xx. The juice cotent of the Gold Nugget fruit were a bit low towards peak maturity ranging around 50%. At peak maturity, Gold Nugget peaked at T1 colour on the colour plate as well as good acid levels indicating the fruit will have a good self life. There were no seeds in the Gold Nugget fruit.

Shasta Gold
The Shasta fruit peaked at count 1xxx. Shasta Gold had a very good external colour development on the fruit before (T1) peak maturity. The selection had very good juice percentages, well over 55% and the fruit was completely seedless this season.

Tahoe Gold

Tahoe Gold's fruit size count ranged from 1x to 1xx. At a Brix: Acid ratio of 10 Tahoe Gold had a T2 toT3 colour development on the colour plate range. The juice level of Tahoe Gold did decrease towards peak maturity from 62.8% to 58.7%, at a Brix: acid ratio of 10. The selection had a fairly low seed count of just 0.3 seeds per fruit on average.

Tango
The selection was seedless with low juice percentages (around 50%) towards peak maturity. The fruit size peaked at count 1 to 1x. Tango had no problems with external colour development having reach a T1 on the colour plate range before peak maturity. Tango had good Brix and acid % just before peak maturity.

Yosemite Gold
The fruit developed a very good external colour and was fully coloured before peak maturity was reached. The selection had a good juice percentage of over 55% towards peak maturity (increased towards peak maturity). Yosemite Gold produced large fruit towards peak maturity and averaged with count 1xxx fruit size.

Edit x Nova
Edit x Nova had a good fruit size count starting at count 1x and just before peak maturity having reached a count of 1xxx. Edit x Nova remained for more or less a month just below peak maturity ratio. In that time the juice content decreased, but the Brix and acid levels stabilized around 11 to 12 Brix; acids around 1%. When the juice level was high Edit x Nova developed a T4 to T5 colour on the colour plate. As soon as the juice % dropped the colour development progressed to T1 on the colour plate.

Sonet and Sonet 2
Sonet 2 were the second selection to reach peak maturity, while Sonet was the third selection to reach peak maturity. At peak maturity Sonet had a fruit size count of 1, smaller compared to Sonet 2 with a 1xxx count. The selection had a very good juice content (60.2%), higher than Sonet 2 at 55.6%. Both these selections did not reach Brix above 10°.with Sonet having a slighty higher Brix. The acid levels of both selections were not very high at peak maturity. Both selections had seeds; Sonet with 0.7 seeds per fruit and Sonet 2 with 1.1 seeds per fruit. Sonet developed a better colour (T3 to T4) compared to Sonet 2 (T5 to T6).

Michal 6/47
Michal 6/47 developed a small fruit size (count 3) towards peak maturity and improved to count 1 when the fruit was over mature. The selection had a very good juice content that increase towards peak maturity. Colour development peaked from T5 to T6. The selection did have seeds with a maximum of 0.8 seeds per fruit.

Michal 89/64
The Michal 89/64 fruit size ranged from count 1 to 2. The juice levels increased as the fruit reach maturity and peaked at 64% when the ratio was above 13. With one of the evaluations, there were 0.7 seeds per fruit; the rest had no seeds. The external colour was delayed compared to the internal quality with a colour plate reading of T6 when the fruit was over mature.

Merav 119
Merav 119 produced a large fruit size on the trees and peaked between count 1 and 1xx. The juice % were above 60 towards peak maturity but decreased as the selection reached over maturity. The selection has a low seed count; completely seedless fruit with the first evalaution and 0.7 seeds per fruit with the second. Merav 119 were a T6 to T7 on the colour plate towards peak maturity, and only advanced to T2 on the colour plate at over maturity. This selection matures before Merav 63.

Merav 63
The fruit size count on this selection were the same as on Merav 119 with a count 1 to 1xx range. Merav 63 had a slightly higher juice % towards peak maturity at 62.8%, but the juice levels also decrease as the selection became over mature. Merav 63 produced slightly more seeds; 0.3 to 0.7 seeds per fruit. Towards peak maturity, the external colour on the colour plate were at T7 but the fruit was fully coloured at a ratio of 13.

Shani SL
The selection was completely seedless. The colour development of the Shani SL fruit was very good. The selection was at T1 on the colour plate long before peak maturity. The fruit size count range from 1x to 1xx before peak maturity with a juice content of 53%.

Nadorcott ARC
The fruit size count of the Nadorcott ARC fruit ranged from count 1 to 1x. Nadorcott SL had the same count. The juice percentage of the Nadorcott ARC selection were lower (53.3%). This selection had a very good

colour development, being fully coloured long before peak maturity. Nadotcott ARC had a very low seed count with 2 of the evaluations being seedless and one having 0.1 seeds per fruit. Nadorcott SL was completely seedless. The Nadorcott ARC had good Brix (12) and acids of 1.07% close to peak maturity.

Nadorcott SL
The fruit size count of the Nadorcott SL range peaked from count 1 to 1x. This selection were completely seedless with all the evaluations completed. Nadorcott SL also had a higher juice % compared to Nadorcott ARC with a juice content of 59.5% close to peak maturity. Nadorcott SL developed a T1 on the colour plate long before peak maturity. This selection had good Brix and acid levels before peak maturity which indicate the fruit will have a good shelf life.

Valley Gold (B17)
The selection Valley Gold (B17) had a fruit size count of 1x. The juice % of this selection was 60.9% before peak maturity. The selection had no problem with external colour development and peaked at T1 on the colour plate before peak maturity. One of the evaluations had a seed count of 0.4 seeds per fruit and the rest was completely seedless.

Phoenix
The fruit size of this selection varied between count 1x and 1xx. Just before peak maturity the selection had a T2 colour development on the colour plate range and with peak maturity it had a T1 colour on the colour plate. This selection has a very good juice % that increased towards peak maturity. Juice levels averaged 55.8% just before peak maturity and increased to 63.5% during peak maturity. The seed count with all thee evaluations completed ranged from 0.6 to 3.2 seeds per fruit.

IRM 1
The IRM 1 selection reached peak maturity after the IRM 2. The IRM 1 fruit size count were 1xx, the same as IRM 2. IRM 1 had a very good juice % which increased with maturation. The selection had a juice content of 63.3% just before peak maturity with the Brix: acid ratio at 11.4. By the time of peak maturity Brix was above 13 and acids above 1%. The selection was not seedless with seed counts between 2.6 and 5.6 seeds per fruit. IRM 1 also had no problem with its external colour development reaching T1 colour on the colour plate before peak maturity.

IRM 2
IRM 2 selection have coloured up completely before peak maturity (T1 on the colour plate). IRM 2 had a good fruit size development and peaked at count 1xx. The juice levels of the IRM 2 increased with maturity up to 64.5 % (peak maturity), slightly higher than IRM 1. IRM 2 had seeds with the evaluation ranging from 0.8 to 6.1 seeds per fruit. The Brix and acid levels were slightly lower compared to the IRM 1 selection.

Conclusion

The following selections had the largest fruit size count (count 1 xxx): Sonet 2, Edit x Nova, African Sunset, Shasta Gold and Yosemite Gold. The selections with the highest juice % were: Michal 6/47 (64.7%), Michal 89/64 (64%), Phoenix (63.5%), IRM 1 (63.3%) and IRM 2 (64.5%). The selections with the highest Brix were Phoenix, IRM 1 and IRM 2. Phoenix, IRM 1 and IRM 2 also developed the most seeds per fruit; 3.2, 5.6 and 6.1 respectively. Most of the selections had a colour development of T1 on the colour plate range by the time of peak maturity

Table 4.6.4.3. Internal fruit quality data for Mandarin hybrid selections from Addo region of the Sundays River Valley (Penhill) during the 2016 season.

Date	Selection	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2016-04-07	Sonet	CC	1	60,2	9,1	0,75	12,0	0,7	T3-4
2016-04-07	Sonet 2	CC	1xxx	55,6	7,5	0,63	11,9	1,1	T5-6
2016-04-07	Michal 6/47	CC	3	60,5	9,7	0,93	10,4	0,8	T6
2016-04-20	Michal 6/47	CC	1	64,7	9,8	0,70	14,0	0,5	T5
2016-04-07	Michal 89/64	CC	2	56,7	8,8	0,91	9,7	0,7	T7
2016-04-20	Michal 89/64	CC	1	64,0	9,4	0,71	13,2	0,0	T6
2016-04-20	Edit x Nova	CC	1x	65,3	10,9	0,98	11,1	0,0	T6

2016-05-05	Edit x Nova	CC	1x	60,8	11,3	0,97	11,6	0,0	T4-5
2016-05-25	Edit x Nova	CC	1xx	52,2	12,1	1,07	11,7	0,0	T1
2016-06-08	Edit x Nova	CC	1xxx	53,9	11,8	1,02	11,6	0,0	T1
2016-04-20	Nova	CC	1x	62,2	10,4	1,02	10,2	1,8	T6-7
2016-05-05	Nova	CC	1x	55,6	10,4	0,98	10,6	1,3	T4-5
2016-05-25	Nova	CC	2	59,1	11,5	0,91	12,6	1,2	T1-2
2016-06-08	Nova	CC	1	56,0	11,8	0,88	13,4	0,0	T1
2016-05-05	Nova ARC	CC	3	62,5	11,4	1,22	9,3	0,0	T1
2016-04-20	Merav 119	CC	1	60,7	9,6	0,93	10,3	0,0	T6-7
2016-05-25	Merav 119	CC	1xx	48,3	10,6	0,72	14,7	0,7	T2
2016-04-20	Merav 63	CC	1	62,8	10,4	1,17	8,9	0,7	T7
2016-05-25	Merav 63	CC	1xx	56,7	11,1	0,84	13,2	0,3	T1
2016-05-25	Shani SL	CC	1	51,8	11,1	1,45	7,7	0,0	T6
2016-06-08	Shani SL	CC	1xx	53,1	11,5	1,29	8,9	0,0	T5-6
2016-06-22	Shani SL	CC	1xx	53,2	11,7	1,34	8,7	0,0	T3-4
2016-07-06	Shani SL	CC	1x	53,3	12,4	1,33	9,3	0,0	T1
2016-05-25	African Sunset (B24)	CC	1xxx	56,9	10,2	0,95	10,7	0,0	T6
2016-06-08	African Sunset (B24)	CC	1xxx	56,0	9,7	0,89	10,9	0,0	T2-3
2016-06-22	African Sunset (B24)	CC	1xxx	57,4	10,3	0,95	10,8	0,0	T1
2016-07-06	African Sunset (B24)	CC	1xxx	56,4	10,3	0,90	11,4	0,0	T1
2016-06-22	Nadorcott ARC	CC	1x	52,5	11,1	1,33	8,3	0,0	T3-4
2016-07-06	Nadorcott ARC	CC	1	50,4	11,1	1,22	9,1	0,0	T1
2016-07-19	Nadorcott ARC	CC	1	53,3	12,0	1,07	11,2	0,1	T1
2016-06-22	Nadorcott SL	CC	1	52,1	10,4	1,25	8,3	0,0	T2
2016-07-06	Nadorcott SL	CC	1x	54,2	11,1	1,09	10,2	0,0	T1
2016-07-19	Nadorcott SL	CC	1	59,5	11,7	1,02	11,5	0,0	T1
2016-06-22	Phoenix	CC	1xx	55,8	12,0	1,07	11,2	0,6	T2
2016-07-19	Phoenix	CC	1xx	62,0	13,0	1,06	12,3	2,3	T1
2016-08-02	Phoenix	CC	1x	63,5	14,3	1,19	12,0	3,2	T1
2016-06-22	IRM1	CC	1xx	60,8	11,8	1,44	8,2	3,3	T3-4
2016-07-06	IRM1	CC	1xx	62,3	12,6	1,69	7,5	2,6	T1
2016-07-19	IRM1	CC	1xx	63,3	13,3	1,17	11,4	5,6	T1
2016-06-22	IRM2	CC	1xx	60,8	11,3	1,26	9,0	4,4	T3
2016-07-06	IRM2	CC	1xx	62,7	11,6	1,28	9,1	0,8	T1
2016-07-19	IRM2	CC	1xx	64,5	12,6	1,05	12,0	6,1	T1

Table 4.6.4.4. Internal fruit quality data for Mandarin hybrid selections from Kirkwood region of the Sundays River Valley (Dunbrody) during the 2016 season.

Date	Selection	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2016-04-20	Saint Andre	CC	1x	63,0	10,2	0,88	11,6	0,4	T6-7
2016-05-25	Saint Andre	CC	1xx	49,4	10,8	0,87	12,4	0,0	T1-2
2016-06-08	Saint Andre	CC	1xx	50,3	10,6	0,81	13,1	0,0	T1
2016-05-25	Valley Gold	Unknown	2	61,9	11,3	1,59	7,1	0,0	T5
2016-06-08	Valley Gold	Unknown	1	63,3	12,1	1,62	7,5	0,0	T1-2
2016-06-22	Valley Gold	Unknown	1x	60,9	12,2	1,37	8,9	0,4	T1

2016-05-25	African Sunset	Unknown	1xx	61,7	11,7	1,33	8,8	0,0	T5
2016-06-08	African Sunset	Unknown	1xxx	60,2	12,0	1,10	10,9	0,0	T1
2016-06-22	African Sunset	Unknown	1xxx	61,8	12,4	1,14	10,9	0,0	T1
2016-05-25	Gold Nugget	CC	1xx	50,6	11,1	1,16	9,6	0	T6-7
2016-06-08	Gold Nugget	CC	1xx	54,1	12,3	1,37	9,0	0	T4
2016-06-22	Gold Nugget	CC	1xx	49,3	11,0	1,04	10,6	0	T4
2016-07-06	Gold Nugget	CC	1xx	49,3	11,4	0,90	12,7	0	T1
2016-07-19	Gold Nugget	CC	1xx	51,7	12,4	1,01	12,3	0	T1
2016-05-25	Shasta Gold	CC	1xxx	55,7	9,3	1,86	5,0	0	T5-6
2016-06-08	Shasta Gold	CC	1xxx	57,7	9,4	1,62	5,8	0	T5-6
2016-06-22	Shasta Gold	CC	1xxx	55,9	11,0	1,57	7,0	0	T3-4
2016-07-06	Shasta Gold	CC	1xxx	57,1	10,7	1,63	6,6	0	T1
2016-05-25	Tahoe Gold	CC	1xx	62,8	10,4	1,26	8,3	0	T5-6
2016-06-08	Tahoe Gold	CC	1x	62,4	10,6	1,51	7,0	0	T4
2016-06-22	Tahoe Gold	CC	1xx	60,6	10,8	1,31	8,2	0.3	T2
2016-07-06	Tahoe Gold	CC	1xx	58,7	10,7	1,07	10,0	0	T2-3
2016-05-25	Tango	CC	1	45,3	10,9	1,30	8,4	0	T6
2016-06-08	Tango	CC	1	51,8	11,2	1,07	10,5	0	T2-3
2016-06-22	Tango	CC	1x	49,5	10,8	1,02	10,6	0	T2
2016-07-06	Tango	CC	1	51,5	11,2	1,09	10,3	0	T1
2016-05-25	Yosemite Gold	CC	1xx	56,0	10,0	1,15	6,7	0	T6-7
2016-06-08	Yosemite Gold	CC	1xxx	54,8	10,7	1,47	7,3	0	T5
2016-06-22	Yosemite Gold	CC	1xxx	55,1	10,5	1,40	7,5	0	T3-4
2016-07-06	Yosemite Gold	CC	1xxx	55,5	9,5	1,25	7,6	0	T1

4.6.5 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (Gamtoos River Valley)

Project 997C by W. Swiegers and Z. Zondi (CRI)

Summary

There is a main trial site in the Patensie area, as well as a back-up site in the Hankey area. Both trial sites form part of the Gamtoos River Valley. Both sites have all the UCR 5 selections planted. In Patensie the season started with Gold Nugget followed by Tango, Yosemite Gold, Nadorcott, Tahoe Gold and the season ended off with Shasta Gold. A new main trial site was planted in the Patensie area this year with a wide variety of selections. There is another expanded trial site in the Loerie area that also forms part of the Gamtoos River Valley. At Loerie the season started off with Nova followed by Saint Andre, Etna, Nova ARC, Sirio, Tasty 1, Phoenix, Tahoe Gold, Gold Nugget, Nadorcott, Tango, Yosemite Gold, Shasta Gold and the season ended with Tanor Late.

Opsomming

Die hoof proefperseël is in die Patensie area en dan is daar ook ŉ bystand perseël in die Hankey area. Beide die proef persele maak deel uit van die Gamtoosrivier Vallei. In beide persele is die URC 5 seleksies. In Patensie het die seisoen begin met Gold Nugget gevolg deur Tango, Yosemite Gold, Nadorcott, Tahoe Gold en die seisoen word afgesluit met Shasta Gold. Daar is ŉ nuwe hoof proefperseël met ŉ wye verskeidenheid van seleksies vanjaar aangeplant in die Patensie area. Daar is ook nog ŉ addisionele en baie goeie proefperseël in die Loerie area en dit maak ook deel uit van die Gamtoosrivier Vallei. By Loerie het die seisoen begin met Nova gevolg deur Saint Andre, Etna, Nova ARC, Sirio, Tasty 1, Phoenix, Tahoe Gold, Gold Nugget, Nadorcott, Tango, Yosemite Gold, Shasta Gold en die seisoen word afgesluit deur Tanor Late.

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. A range of new mandarin hybrids had been added to this area. The following varieties were evaluated: Nova, Saint Andre, Etna, Nova ARC, Sirio, Tasty 1, Phoenix, Tahoe Gold, Gold Nugget, Nadorcott, Tango, Yosemite Gold, Shasta Gold as well as Tanor Late.

A ratio of 11:1 for mandarin hybrids 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 over mature. 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.

Table 4.6.5.1. List of experimental mandarin hybrid selections evaluated in the Patensie (L. Ferreira) region of the Gamtoos River Valley during the 2016 season.

Selection	Rootstock	Topwork
Gold Nugget	Carrizo	2011
Shasta Gold	Carrizo	2011
Tahoe Gold	Carrizo	2011
Tango	Carrizo	2011
Nadorcott	Carrizo	2011
Yosemite Gold	Carrizo	2011

Table 4.6.5.2. List of experimental mandarin hybrid selections evaluated in the Loerie (N. Ferreira) region of the Gamtoos River Valley during the 2016 season.

Selection	Rootstock	Topwork
Etna	Carrizo	2012
Sirio	Carrizo	2012
Saint Andre	Carrizo	2012
Nova	Carrizo	2012
Tasty 1	Carrizo	2012
Nadorcott	Carrizo	2012
Phoenix	Carrizo	2012
Nova ARC	Carrizo	2012
Tanor Late	Carrizo	2012
Gold Nugget	Carrizo	2012
Shasta Gold	Carrizo	2012
Tahoe Gold	Carrizo	2012
Tango	Carrizo	2012
Yosemite Gold	Carrizo	2012

Results and discussion

<u>Gold Nugget</u>
Of the UCR 5 selection Gold Nugget were first to reach peak maturity in Patensie, but second in Loerie. There were roughly a 3 week difference in maturity timing between the two areas. Gold Nugget reached peak maturity at the end of June, beginning of July in Patensie. In Loerie the selection reached peak maturity about the third week in July. The selection had good Brix and acid ratios with good colour development (T1 colour on the colour plate range). The selection was very low seeded; being seedless in Patensie and in Loerie the the seed count on one occation were 0.1 seeds per fruit. Gold Nugget fruit size count were 1xx. The internal juice percentages were around 53%.

Shasta Gold
Shasta Gold ended off the UCR 5 mandarin selection season. The fruit was seedless in 2015, and in 2016 the highest seed count were 0.8 seeds per fruit for this selection. Shasta Gold had excellent colour development (colour plate T1) long before peak maturity. Shasta Gold developed a very good juice percentage, well over 57%. The selection had extra large fruit size and peaked at count 1xxx. Shasta Gold also had good Brix° and acid ratios towards peak maturity.

Tahoe Gold
Tahoe Gold were first to reach peak maturity at Loerie for the UCR 5 selections, but second last for the Patensie area. The difference in ripening periods for the two sites were about 3 to 4 weeks. The fruit size count ranged between 1 and 1xx at the Patensie site, having slightly smaller fruit. Tahoe Gold had no problem with external colour development as it was fully coloured (T1) long before peak maturity. Tahoe Gold had some of the best juice levels of the UCR 5 selections, with a juice percentage around 59%. Tahoe Gold had a very low seed count of 0.1 seeds per fruit for Patensie area and in the Loerie area the seed count per fruit were 0.6.

Tango
Tango had a good fruit size range from count 1 to 1x and were seedless at both sites. The selection had very good external colour development and the fruit was fully coloured before peak maturity. Tango along with Gold Nugget had the lowest juice percentage of the UCR 5 selections, ranging around 53%. Tango's Brix and acid ratios were good, indicating that the fruit will have a good shelf life. Tango were close to peak maturity by the beginning of July in the Patensie area, but at the same time it was still on its way to peak maturity in Loerie with a 10 ratio.

Yosemite Gold
Yosemite Gold along with Shasta Gold were the only two UCR 5 selections to reach peak maturity more or less the same time in Patensie as well as Loerie. Yosemite Gold had big fruit this year and peaked at count 1xxx for the second year in a row. The juice levels increased towards peak maturity reaching a high of 59%. The juice content was slightly higher in the Loerie area, as well as the seed count with the highest seed count being 0.9 seeds per fruit. Yosemite Gold had no problem with colour development reaching T1 on the colour plate before peak maturity.

Nadorcott
Nadorcott peaked between count 1 and 1x this season. There were roughly a 5% difference in the juice percentages towards peak maturity, between Patensie and Loerie for the Nadorcott selection (Patensie being around 55% and Loerie around 50%). Ripening periods for Nadorcott seems to be more or less the same time between Patensie and Loerie and the selection was seedless at both trial sites. Nadorcott achieved a T1 on the colour plate range long before peak maturity. Nadorcott's Brix and acid ratios were good towards peak maturity.

Nova
Nova was the the first selection to mature for these cool production areas. Nova developed a 2 count on the fruit size range. The juice percentage of the Nova were good being 55.8%. The Nova selection was completely seedless and the colour development ranged from T3 to T4 by the time of peak maturity.

Nova ARC
Nova ARC had bigger fruit than the Nova selection and peaked at count 1xx. Nova ARC did not have any seeds. The selection had no problem with external colour development, being T1 on the colour plate range by peak maturity. The juice content of Nova ARC was lower compared to Nova (50.6%). Nova ARC reached peak maturity about 6 weeks after the Nova (control) selection.

Saint Andre
Saint Andre were the second selection to mature in these production regions. The fruit size count was between 1x and 1xx. The selection was close to peak maturity by the 3rd week in April. The juice percentage increased from 52.5% to 56.3%, as well as the colour development improved from T6/T7 to T4 on the colour plate range by the time of peak maturity. The acids remained stable during the production season for Saint Andre. The highest seed count noted for the selection were 1.8 seeds per fruit.

Etna
The fruit size count for the Etna ranged from 1x to 1xxx. The juice percentage for Etna were good, at 59.4 % juice at peak maturity and better compared to Sirio. The Brix and Acid levels of Etna were slightly lower than Sirio towards peak maturity. Etna reached peak maturity before Sirio in the manadarin range of new

experimental cultivars. Etna had a T1 on the colour plate range at peak maturity. On all three evaluations there was a seed count 0.9 seeds per fruit, being the lowest for the season and the highest 3.4 seeds per fruit.

Sirio
Sirio had a good fruit size count and peaked at 1x. Sirio developed a lower juice percentage than Etna at 47%. The juice % decreased towards peak maturity. The Brix and Acid levels of Sirio were slightly higher than Etna at peak maturity. Sirio had no external colour development problems, being fully coloured before peak maturity. During the first evaluation there were no seeds, but with the other 2 evaluations we did find seeds; the highest being 3.5 seeds per fruit

Tasty 1
Tasty 1 developed a large/xtra-large fruit size and peaked at count 1xxx. The external colour development were very good with a T1 on the colour plate before peak maturity. The juice percentages increased towards peak maturity, but decreased after peak maturity. On all 3 evaluations there were a seed count ranging between 1.4 and 3.8 seeds per fruit.

Phoenix
Phoenix bore a good fruit size on the trees with a 1xx count at peak maturity. The juice percentage of the Phoenix were at 55.6 % and it had a T1 on the colour plate range at peak maturity. The average seed count for Phoenix were 0.8 seeds per fruit. Peak maturity was reached at the beginning of July. The acid percentage were still above 1% at peak maturity.

Tanor Late
Tanor Late is an ultra late maturing mandarin hybrid selection. This selection was last to reach peak maturity, being fully ripen from middle August onwards at the Loerie trial site. The fruit size of Tanor Late was good and peaked at count 1xxx (large fruit). The external colour development of this selection was very good, being a T1 on the colour plate long before peak maturity. Tanor Late had a good juice percentage (around 56%). The average seed count for this selection were about 0.9 seeds per fruit. The Brix and Acid ratio of Tanor Late were very good with an average Brix and Acid content being 13.9°and 1.17%. The fruit will have a good shelf life.

Conclusion

Most of the selections had a very good external colour development (T1) at peak maturity. Tango and Gold Nugget matured later in Loerie (cooler area) compared to Patensie. Yosemite Gold and Shasta Gold matured the same time at Loerie and Patensie. Tahoe Gold reached peak maturity earlier in Loerie than Patensie. The following selections had the largest fruit size (count 1xxx); Shasta Gold, Yosemite Gold, Tasty 1 and Tanor Late. Tango, Nadorcott and Nova croppped the smallest fruit size (count 1 to 2). Shasta Gold, Tahoe Gold, Yosemite Gold and Etna developed percentages above 58%. Sirio and Tasty 1 had juice percentages below 50%. Tanor Late, Tahoe Gold, Gold Nugget and Sirio had the highest Brix level above 14°. The UCR 5, Tanor Late and Nadorcott performed well with good Brix: acid ratios, improving shelf life and fruit quality. The selection with the highest seed count were Tasty 1; average 3.8 seeds per fruit.

Table 4.6.5.3. Internal fruit quality data for experimental mandarin hybrid selections from the Patensie (L. Ferreira) region of the Gamtoos River Valley region during the 2016 season.

Date	Selection	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2016-06-14	Gold Nugget	CC	1x	50,0	12,0	1,16	10,3	0,0	T4
2016-06-21	Gold Nugget	CC	1xx	52,4	12,4	1,12	11,0	0,0	T4
2016-07-05	Gold Nugget	CC	1xx	53,0	12,2	0,94	13,0	0,0	T1
2016-07-18	Shasta Gold	CC	1xx	58,9	11,1	1,32	8,4	0,1	T1
2016-08-11	Shasta Gold	CC	1xxx	57,1	12,5	1,29	9,7	0,2	T1
2016-08-18	Shasta Gold	CC	1xxx	58,7	12,7	1,20	10,6	0,0	T1
2016-06-21	Tahoe Gold	CC	1x	56,6	12,3	1,48	8,3	0,0	T2-3
2016-07-05	Tahoe Gold	CC	1x	59,8	12,2	1,37	8,9	0,0	T1
2016-08-11	Tahoe Gold	CC	1	59,3	13,3	1,22	10,9	0,1	T1
2016-06-14	Tango	CC	1	55,3	12,5	1,12	11,2	0,0	T3-4

2016-06-21	Tango	CC	1	53,3	12,3	1,11	11,1	0,0	T2-3
2016-07-05	Tango	CC	1x	52,7	11,5	0,97	11,9	0,0	T1
2016-07-18	Tango	CC	1	55,5	13,7	1,10	12,5	0,0	T1
2016-08-11	Tango	CC	1	62,9	12,1	0,88	13,8	0,0	T1
2016-06-14	Nadorcott	CC	1x	52,6	12,1	1,20	10,1	0,0	T3-4
2016-06-21	Nadorcott	CC	1	55,8	11,4	1,22	9,3	0,0	T3
2016-07-05	Nadorcott	CC	1x	54,8	12,5	1,26	9,9	0,0	T1
2016-06-21	Yosemite Gold	CC	1xxx	53,9	12,8	1,43	9,0	0,0	T4
2016-07-05	Yosemite Gold	CC	1xxx	56,5	12,4	1,12	11,1	0,8	T1
2016-07-18	Yosemite Gold	CC	1xxx	58,1	11,7	1,10	10,6	0,5	T1
2016-08-11	Yosemite Gold	CC	1xxx	57,5	12,8	1,15	11,1	0,6	T1
2016-08-18	Yosemite Gold	CC	1xx	58,4	14,1	1,03	13,7	0,3	T1

Table 4.6.5.4. Internal fruit quality data for experimental mandarin hybrid selections from the Loerie (N. Ferreira) region of the Gamtoos River Valley region during the 2016 season.

Date	Selection	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2016-04-19	Etna	CC	1xxx	58,9	10,4	1,00	10,4	0,9	T5-6
2016-05-04	Etna	CC	1xx	58,2	11,1	1,08	10,3	3,4	T2-3
2016-05-24	Etna	CC	1x	59,4	11,3	0,95	11,9	1,5	T1
2016-05-04	Sirio	CC	1x	50,5	12,8	1,28	10,0	0,0	T1-2
2016-05-24	Sirio	CC	1x	49,1	12,8	1,12	11,0	3,5	T1
2016-06-21	Sirio	CC	1x	47,0	14,4	1,28	11,3	1,2	T1
2016-04-19	Saint Andre	CC	1x	52,5	10,5	0,92	11,4	1,4	T6-7
2016-05-04	Saint Andre	CC	1x	56,3	10,6	0,93	11,4	1,4	T4
2016-05-24	Saint Andre	CC	1xx	50,8	11,0	0,79	13,9	1,8	T1
2016-05-04	Nova	CC	2	55,8	12,1	0,96	12,6	0,0	T3-4
2016-05-24	Tasty 1	CC	1xxx	42,7	12,3	1,21	10,2	3,8	T5
2016-06-21	Tasty 1	CC	1xxx	47,1	12,9	1,23	10,5	3,8	T1
2016-07-05	Tasty 1	CC	1xxx	40,5	12,5	0,99	12,7	1,4	T1
2016-05-24	Nadorcott	CC	1	48,2	10,2	1,10	9,3	0,0	T6
2016-06-21	Nadorcott	CC	1x	50,6	11,0	1,02	10,8	0,0	T2-3
2016-07-05	Nadorcott	CC	1	50,4	11,2	1,09	10,3	0,0	T1
2016-07-05	Phoenix	CC	1xx	55,6	12,4	1,05	11,8	0,8	T1
2016-06-21	Nova ARC	CC	1xx	50,6	10,6	0,85	12,5	0,0	T1
2016-07-05	Gold Nugget	CC	1xx	49,3	12,8	1,20	10,7	0,0	T1
2016-07-18	Gold Nugget	CC	1xx	51,6	13,6	1,15	11,8	0,1	T1
2016-08-11	Gold Nugget	CC	1xx	55,5	14,6	1,17	12,5	0,0	T1
2016-07-05	Yosemite Gold	CC	1xxx	53,4	11,7	1,22	9,6	0,0	T1-2
2016-07-18	Yosemite Gold	CC	1xxx	57,3	12,3	1,10	11,2	0,4	T1
2016-08-11	Yosemite Gold	CC	1xxx	59,5	12,4	1,15	10,8	0,9	T1
2016-08-25	Yosemite Gold	CC	1xxx	56,7	12,5	0,83	15,1	0,5	T1
2016-07-18	Shasta Gold	CC	1xxx	58,9	13,7	1,67	8,2	0,8	T1
2016-08-25	Shasta Gold	CC	1xxx	61,8	13,5	1,27	10,6	0,5	T1
2016-06-21	Tahoe Gold	CC	1x	55,7	12,5	1,36	9,2	0,0	T3
2016-07-05	Tahoe Gold	CC	1xx	56,3	13,3	1,05	12,6	0,0	T1

2016-07-18	Tahoe Gold	CC	1xx	58,2	14,2	1,22	11,6	0,6	T1
2016-08-11	Tahoe Gold	CC	1x	60,0	14,3	1,06	13,5	0,5	T1
2016-05-24	Tango	CC	1	50,8	10,3	1,19	8,7	0,0	T6
2016-06-21	Tango	CC	1	53,6	11,5	1,16	9,9	0,0	T2
2016-07-05	Tango	CC	1x	48,8	11,5	1,15	10,0	0,0	T1
2016-07-18	Tanor Late	CC	1xxx	56,1	12,8	1,44	8,9	0,9	T1
2016-08-11	Tanor Late	CC	1xxx	54,2	13,4	1,20	11,2	0,8	T1
2016-08-25	Tanor Late	CC	1xxx	57,4	14,4	1,15	12,5	0,9	T1

4.6.6 PROGRESS REPORT: Cultivar characteristics and climatic suitability of mandarin hybrids in a cold production region (Western Cape)

Project 997D by W. Swiegers (CRI)

Summary

The trial site in Citrusdal consists of a variety block with a selection of all the new experimental cultivars from early maturing to late maturing selections. The cross pollination is high in this block due to all the different selections that are present. Nova ARC was first to reach peak maturity and it was followed by Edit x Nova, Furr (Clemcott), Meirav 63, Nadorcott ARC and African Sunset (B24); maturing at the same time. They were followed by Tahoe Gold, Gold Nugget, Tango, Nadorcott LS, Shani SL and Nadorcott. The mandarin season ended off with Tanor Late, one of the new ultra late selections.

Opsomming

Die proef perseël in Citrusdal bevat meeste van die nuwe eksperimentele seleksies van vroeg tot laat rypwordend. Die kruisbestuiwing in hierdie proef perseël is baie hoog weens al die verskillende seleksies teenwoordig. Die orde van rypwording was as volg gewees: Nova ARC, Edit x Nova, Furr (Clemcott), Meirav 63, Nadorcott ARC en African Sunset (B24) het saam ryp gewording. Hulle is verder opgevolg deur Tahoe Gold, Gold Nugget, Tango, Nadorcott LS, Shani SL, Nadorcott en Tanor Late aan die einde van die mandarin seisoen.

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 Citrusdal region of the Western Cape. The following selections were evaluated: Nova ARC, Edit x Nova, Furr (Clemcott), Meirav 63, Nadorcott ARC, African Sunset (B24), Tahoe Gold, Gold Nugget, Tango, Nadorcott LS, Shani SL, Nadorcott and Tanor Late.

A ratio of 11:1 for mandarin hybrids 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 over mature. 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.

Table 4.6.6.1. List of experimental mandarin hybrid selections evaluated in the Citrusdal region of the Western Cape during the 2016 season.

Selection	Rootstock	Topwork	Planted
African Sunset (B24)	CC		2009
Furr (Clemcott)	CC	2011	
Gold Nugget	CC	2010	
Meirav 63	CC		

Nadorcott	CC		2009
Nadorcott ARC	CC	2010	
Nadorcott LS	CC	2010	
Edit x Nova	CC		
Nova ARC	CC		Unsure
Tahoe Gold	CC	2010	
Tango	CC	2010	
Shani SL	CC		
Tanor late	CC	2012	

Results and discussion

African Sunset (B24)
African Sunset bore extra large fruit on the trees (count 1xxx). Colour development was very good (T1) before peak maturity. When evaluated in 2015, the internal juice percentages were low (44.9%) closer to peak maturity and the selection was completely seedless. During the 2016 season, the internal juice percentage were better (54.9%) close to peak maturity and low average seed counts of 0.4 seeds per fruit.

Furr (Clemcott)
Furr is used as a control for the mid-maturing mandarin selections. The juice content was 53.6 %. The fruit size range from count 1xx to 1xxx. Furr peels easily and has a very good eating quality. Due to the high cross pollination in the mixed trial block, Furr produced the highest number of seeds per fruit; 9.3. Furr's external colour development were slower than the internal development; T3 to T4 on the colour plate range at peak maturity.

Gold Nugget
Tree manipulation is necessary to control the strong vegetative and upright growth habit. Gold Nugget developed one of the best tasting fruit with a high Brix:acid ratio. The fruit peaked internally with Brix of 14.3°. Due to the good quality of the fruit it will be possible to hang the fruit longer on the trees with an extended shelf life. Gold Nugget's fruit size were at count 1 before peak maturity and fully coloured (T1). The juice percentage for this selection was 52.6%. Average seed counts were very low with 0.1 seeds per fruit.

Nadorcott & Nadorcott ARC & Nadorcott SL
Nadorcott ARC and Nadorcott SL are induced Nadorcott selections to minimise the average seeds per fruit. Both selections have the same growth habit and charactaristics as the Nadorcott. Fruit size for these selections peaked at count 1. The internal juice percentages were below 55% for Nadorcott and Nadorcott SL towards peak maturity, while Nadorcott ARC had a 55.4% juice percentage at peak maturity. The three Nadorcott selections developed good Brix above 13°) with acids of 1.1%, ensuring a good balance and eating quality. The fruit was fully coloured (T1) before peak maturity. The highest seed count during the three evaluations for Nadorcott was 2.4 seeds per fruit, for Nadorcott ARC it was 0.1 seeds per fruit and for Nadorcott LS 0.3 seeds per fruit. This is in a high cross pollination trial site.

Nova ARC
Nova ARC is a selection from the ARC that was irradiated to improve the selection (completely seedless). The fruit had a very good external colour development (T1) before peak maturity was reached and fruit size for Nova ARC was medium (count 1). The selection was low seeded with 0.2 seeds per fruit and the internal juice percentages were below 50%. Nova ARC had a high Brix level of 14.2°.

Edit x Nova
Edit x Nova cropped a medium fruit size on the trees (count 1) with a juice percentage of 51.9% close to peak maturity. Degreening might be neccesary for Edit x Nova because of its external colour delay; T6 on the colour plate range close to peak maturity. The average seed count for Edit x Nova were 0.3 seeds per fruit.

Tahoe Gold
Tahoe Gold struggled with high acids in the 2014 season, and similar levels in the 2015 season. The acid levels did not drop low enough for the fruit to reach the peak maturity ratio of 12:1. In 2016 the acid levels dropped to 1.18% before peak maturity. The fruit had a very good colour development and peaked at colour plate T1 before the fruit were fully matured. Tahoe Gold developed low seed counts up to 0.3 seeds per fruit. The juice percentage has decreased towards peak maturity to 53.4%.

Meirav 63

Merav 63 bore a smaller fruit size on the trees (count 2). The juice percentage of Merav 63 were 57.9% towards peak maturity. External colour development for Merav 63 were very good with a T1 colour on the colour plate before peak maturity. The average seed count for the selection were 1.2 seeds per fruit.

Tango
Tango developed a very smooth rind texture (similar to Nadorcott) with a natural shine. The fruit had a very good colour development in the cooler areas (colour plate T1) at peak maturity. Tango were seedless at the Citrusdal trial site. The fruit size peaked at count 1. Internally the juice percentage for Tango was 48.1% at a Brix: acid ratio of 10.8 (Brix 10.6°).

Shani SL
In this high pollination site Shani SL had an average seed count of 1.3 seeds per fruit. The fruit size was small and peaked at count 3. Internal quality was good with a juice percentage 52.8% and the fruit was fully coloured before peak maturity. Shani SL had the highest Brix: acid ratio; Brix of 16.1° and acid of 1.68% towards peak maturity at a 9.6 ratio.

Tanor late
Tanor late is an ultra-late experimental mandarin selection maturing end of August to middle September. The selection is very thorny but on the oldest trees in South Africa the thorns tend to disappear or become fairly small. The external colour development is very good (T1) well before peak maturity. Tanor late bore fruit with a juice content of 47.2% towards peak maturity. The variety peels easily and clean, the fruit size peaked at count 1xxx (extra large). The selection had a low seed count of 0.3 seeds per fruit.

Conclusion

African Sunset, Furr and Tanor late had the largest fruit size (1xxx). Shani SL had the smallest fruit size with a count 3 followed by Meirav 63 at a count 2. Furr had the most seeds per fruit on average (9.3 seeds per fruit). Tango were to only selection that was completely seedless. Nadorcott ARC had a juice percentage over 55% at peak maturity. It seems like most of the juice percentages of the selections evaluated decreased towards peak maturity. All the selections were a T1 on the colour plate range (good colour development) before or at peak maturity except Edit x Nova (T6 close to peak maturity).

Table 4.6.6.2. Internal fruit quality data for experimental mandarin hybrid selections from the Citrusdal region of the Western Cape during the 2016 season.

Date	Cultivar	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2016-06-03	African Sunset (B24)	1xxx	52,9%	12,3	1,28	9,6	0,3	T4
2016-07-07	African Sunset (B24)	1xxx	54,9%	13,0	1,09	11,9	0,0	T1
2016-07-25	African Sunset (B24)	1xxx	50,7%	13,2	1,04	12,7	0,5	T1
2016-06-03	Clemcott	1xx	53,6%	13,1	1,06	12,3	9,3	T3-4
2016-07-07	Clemcott	1xxx	42,9%	13,4	0,95	14,1	7,1	T1
2016-05-11	Edit x Nova	1	51,9%	12,8	1,12	11,4	0,3	T6
2016-07-14	Gold Nugget	1	52,6%	14,3	1,30	11,0	0,1	T1
2016-06-03	Meirav 63	2	57,9%	14,0	1,31	10,7	1,2	T1
2016-06-03	Nadorcott	1	57,0%	13,0	1,75	7,4	2,4	T3
2016-07-07	Nadorcott	1	54,8%	14,0	1,67	8,4	2,3	T1
2016-07-25	Nadorcott	2	52,4%	14,7	1,50	9,8	1,1	T1
2016-06-03	Nadorcott ARC	1	58,4%	12,9	1,28	10,1	0,1	T3
2016-07-07	Nadorcott ARC	1	55,4%	13,2	1,10	12,0	0,0	T1
2016-07-25	Nadorcott ARC	1	54,3%	14,5	1,18	12,3	0,0	T1
2016-06-03	Nadorcott LS	2	57,5%	12,4	1,53	8,1	0,1	T1-3
2016-07-07	Nadorcott LS	1	53,9%	12,8	1,32	9,7	0,0	T1
2016-07-25	Nadorcott LS	2	53,8%	13,4	1,41	9,5	0,3	T1
2016-05-11	Nova ARC	1	49,2%	14,2	1,20	11,8	0,2	T1

2016-07-07	Shani SL	3	52,8%	16,1	1,68	9,6	1,3	T1
2016-06-03	Tahoe Gold	1x	58,6%	12,4	1,38	9,0	0,1	T5
2016-07-14	Tahoe Gold	1xx	53,4%	13,4	1,18	11,4	0,3	T1
2016-07-14	Tango	1	48,1%	10,6	0,99	10,8	0,0	T1
2016-07-07	Tanor late	1xxx	47,2%	12,6	1,45	8,7	0,3	T1

4.6.7 PROGRESS REPORT: Cultivar characteristics and climatic suitability of navel oranges in a cold production region (Sundays River Valley)

Project 998B by W. Swiegers and Z. Zondi (CRI)

Summary

The early to late maturing navel selection trial is based in the Addo area of the Sundays River Valley. Most of the selections were topworked in the 2007 season. The rootstocks Swingle citrumelo and Troyer citrange were used for the trial. For the navel selections in this trial site the season started with Newhall on Troyer Citrange, Fukumoto, Tule Gold, Lina, Newhall on Carrizo Citrange, Addo Early, Clarke, Chislett, Lane Late, Barnfield Summer, Powell Summer, Autumn Gold and the season ended with Gloudi, Glen Ora Late and Witkrans.

Opsomming

Die vroeë tot laat rypwording nawel seleksie proef is gevestig in die Addo area van die Sondagsrivier vallei. Die meeste van hierdie seleksies is gedurende die 2007 seisoen getopwerk. Swingle citrumelo, Troyer citrange en Carrizo word as onderstamme vir hierdie proef gebruik. Die volgorde van rypwording vir die nawel seleksie wat ge-evalueer was, is as volg: Newhall op Troyer Citrange, Fukumoto, Tule Gold, Lina, Newhall op Carrizo Citrange, Addo Early, Clarke, Chislett, Lane Late, Barnfield Summer, Powell Summer, Autumn Gold, Gloudi, Glen Ora Late en Witkrans.

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 late maturing selections were evaluated: Fukumoto, Lina, Newhall, Tulegold, Addo Early, Autumn Gold, Barnfield Summer, Chislett, Clarke, Powell Summer, Glen Ora Late, Gloudi, Lane Late and Witkrans.

For navels, 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 over mature. 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.

Table 4.6.7.1. List of navel selections evaluated at Sundays River Valley (Penhill) during 2016.

Selection	Rootstock	Planted
Addo early	TC/SC	2007
Fukumoto	TC/CC	2007
Lina	TC/CC	2007
Newhall	TC/CC	2007
Autumn Gold	CC	2007
Tulegold	TC	2007
Barnfield Summer	CC	2007
Chislett	CC	

Clarke	CC	
Powell Summer	CC	
Glen Ora Late	CC	
Gloudi	CC	
Lane Late	CC	
Witkrans	CC	

Results and discussion

Addo Early
The selection had a smaller fruit size compared to 2015 being at count 48, while in 2016 it peaked at count 56. Internal quality was good with a juice percentage between 50% and 53,5%. Swingle citrange rootstock had the higher juice percentage for this trial. Addo early's external colour development on the colour plate range were T4 at peak maturity. The selection developed a protruding navel-end on the fruit. Addo Early on Troyer citrange reached peak maturity slightly earlier than on Swingle citrange. Addo Early on Swingle citrange had a slightly higher Brix and acid content compared to Troyer citrange.

Fukumoto
Fukumoto was one of the selection with the highest Brix for this trial site (above 10°). The colour development was delayed, with colour plate T5 to T6 at peak maturity. Fukumoto produced a good fruit size and peaked at count 56. The navel-end on the fruit was fairly open and protruding, one of the characteristics of the selection. The juice percentage of Fukomoto were around 52%. Fukomoto was second selection to reach peak maturity.

Lina
Lina had a delayed colour development with a colour plate T6 to T7 at peak maturity. The selection had a very good fruit size and peaked at count 56; Lina Navel is a selection that has a problem with smaller fruit size in general. The fruit shape was more elongated with a large navel-end (fairly open). Lina developed a higher juice content (55%) compared to the 2015 season. Lina on Troyer citrange had a slightly higher juice level and lower acids; Lina on Troyer reached peak maturity slightly earlier than on Carrizo.

Newhall
The fruit size peaked at count 56 for this season which is very favorable for navel production and export. Newhall had a delayed colour development (colour plate T6) when the fruit was at peak maturity. The selection's juice percentage increased (above 50%) for the second year in a row. Newhall on Troyer citrange rootstock were the first to reach peak maturity. Newhall on Troyer had a lower juice, Brix and acid content compared to Carrizo citrange.

Autumn Gold
The external colour development of the selection was one of the better ones (colour plate T3) at peak maturity. The selection bore large fruit and peaked at count 48 with a low juice percentage of 46.1 %.

Tulegold
Tulegold had a delayed colour development with colour plate T6 at peak maturity. Tulegold had a very good fruit size which peaked at count 56. The juice percentage increase towards peak maturity to above 55%. The Brix for this selection were below 10° with acids around 0.9% at peak maturity.

Barnfield Summer
The fruit size count at peak maturity for Barnfield Summer were 36 at the first evaluation and at the second one count 56. The juice percentages were below 55% for this selection. Barnfield Summer have a delayed external colour development, being a T5 to T6 at peak maturity. The acid remained fairly good up until peak maturity.

Chislett
Chislett cropped a good yield on the trees and peaked at count 56. Chislett were one of the first selections from the mid to late navel selections in this trial to reach peak maturity with a delayed external colour development (T4 to T6). The juice percentage of Chislett ranged from 48.8% to 53.6%.

Clarke
Clarke were first to reach peak maturity of the mid to late navel selections. Clarke's fruit size count was between count 36 and 56, with the bigger fruit having a lower juice percentage. The smaller fruit had a good juice percentage, above 55%. The external colour development of Clarke were delayed compared to the internal quality. The external colour were T5 to T6 on the colour plate.

Powell Summer
Just before peak maturity the fruit size count of Powell was good (count 56) with very promising juice levels above 54.8%. This was one of the higher juice percentages of all the selection in this trial. The external colour of Powell were T6 on the colour plate range (delayed). Powell cropped fruit with one of the lowest Brix levels for the navel trial (Brix 8.9°).

Glen Ora Late
Glen Ora Late were the second last selection to reach peak maturity in this navel trial site. The fruit size count of Glen Ora Late was good and it peaked at a 56 count. The juice percentage for this selection was around 53%. The external colour development of Glen Ora late was also delayed like most of the other navel selections with a T4 to T5 on the colour plate. Close to peak maturity the Brix levels were good with high acids (1.05%), also indicating that the fruit will be able to hang slightly longer on the trees.

Gloudi
Gloudi had the best external colour development of all the navel selections in this trial site (T2 on the colour plate). The juice percentage of Gloudi was high at peak maturity, being 55.8%. Gloudi had a good fruit size count for navel export markets and peaking at 56 count. Brix levels decreased slightly towards peak maturity. Gloudi's acid levels remained between 0.9 and 1.0 % for four weeks (good shelf life).

Lane Late
Lane Late produced slightly bigger fruit with a fruit size count of 48 at peak maturity. Lane Late had the highest juice percentage (58.3%) of all the navel selections at peak maturity. The external colour development was also better on Lane Late, but still delayed (T3 to T5) by peak maturity. Lane Late also kept its acids quite well (good shelf life).

Witkrans
Witkrans were the last selection to reach peak maturity in this trial site, but the external colour development were slow with a T5 on the colour plate close to peak maturity and only reaching T1 when it was over mature. The fruit size count for Witkrans were good peaking at a count of 56. The juice percentage for Witkrans were 54.4% and acids were still above 1% close to peak maturity.

Conclusion

The Addo area is well suited for navel production in South Africa. Most of the selections had a very good fruit size and peaked at count 56, with Autumn Gold and Lane Late peaking at count 48. All of the selections had delayed external colour development. The delayed external colour development, as well as high acid percentages in some of the selections could be due to the Swingle rootstock that is 2 to 3 weeks later in maturity compared to Carizzo- and Troyer citrange with delayed external colour development and higher acid levels. Lane Late, Gloudi and Lina had the highest juice percentage at peak maturity with 58.3%, 55.8% and 55.4% respectivly. Fukumoto and Newhall had the highest Brix above 10.0° for this trial at peak maturity. Gloudi had the best external colour development with a T2 at peak maturity. All the navel selections were seedless.

Table 4.6.7.2. Internal fruit quality data for early to late Navel selections from the Addo (Penhill) region of the Sundays River Valley during the 2016 season.

Date	Selection	Rootstock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Colour	Avg. Seed
2016-04-20	Addo Early	SC	56	51,9	8,6	0,98	8,8	T6-7	0,0
2016-04-20	Addo Early	TC	56	51,0	8,2	1,07	7,7	T6	0,0
2016-05-05	Addo Early	SC	56	55,5	9,0	1,18	7,6	T6	0,0
2016-05-05	Addo Early	TC	56	51,9	8,4	0,92	9,1	T6	0,0
2016-05-18	Addo Early	SC	56	53,5	9,3	0,99	9,4	T4	0,0
2016-05-18	Addo Early	TC	56	50,8	8,7	0,83	10,5	T3-4	0,0
2016-04-20	Fukumoto	CC	56	52,1	10,6	0,99	10,7	T5-6	0,0
2016-04-20	Fukumoto	TC	56	52,5	10,4	1,01	10,3	T6	0,0
2016-05-18	Fukumoto	CC	56	52,2	11,2	0,98	11,4	T3-4	0,0
2016-05-18	Fukumoto	TC	56	52,5	10,4	0,85	12,2	T2-3	0,0

2016-04-07	Tulegold	TC	56	53,6	8,9	0,94	9,4	T7	0,0
2016-04-20	Tulegold	TC	56	55,1	9,3	0,91	10,2	T6	0,0
2016-05-05	Tulegold	TC	56	56,2	10,3	0,78	13,2	T5-6	0,0
2016-04-20	Lina	CC	56	55,4	9,9	1,00	9,9	T6	0,0
2016-04-20	Lina	TC	56	56,2	9,9	0,92	10,8	T6-7	0,0
2016-05-18	Lina	CC	56	56,4	10,1	0,93	10,9	T4-5	0,0
2016-05-18	Lina	TC	56	54,8	10,5	0,93	11,3	T3-4	0,0
2016-04-20	Newhall	CC	56	53,8	10,4	1,16	9,0	T6	0,0
2016-04-20	Newhall	TC	56	50,0	9,8	0,85	11,5	T6	0,0
2016-05-18	Newhall	CC	56	54,3	10,5	0,90	11,7	T4-5	0,0
2016-05-18	Newhall	TC	56	55,2	10,9	0,87	12,5	T3-4	0,0
2016-06-29	Autumn Gold	CC	48	46,1	9,6	0,90	10,7	T3	0,0
2016-07-12	Autumn Gold	CC	48	50,8	9,4	0,71	13,2	T1-2	0,0
2016-06-15	Barnfield	CC	36	48,9	8,7	0,87	10,0	T6	0,0
2016-06-29	Barnfield	CC	56	53,1	9,4	0,96	9,8	T5	0,0
2016-07-12	Barnfield	CC	48	51,5	9,8	0,88	11,1	T5	0,0
2016-06-15	Chislett	CC	56	48,8	8,9	0,83	10,7	T6	0,0
2016-06-29	Chislett	CC	36	53,6	9,6	0,88	10,9	T4	0,0
2016-07-12	Chislett	CC	48	45,5	9,2	0,75	12,3	T3	0,0
2016-06-15	Clarke	CC	36	48,6	8,6	0,78	11,0	T6	0,0
2016-06-29	Clarke	CC	56	55,1	9,1	0,87	10,5	T5	0,0
2016-07-12	Clarke	CC	48	54,7	9,5	0,75	12,7	T3	0,0
2016-06-15	Powell Summer	CC	56	54,8	8,9	0,94	9,5	T6	0,0
2016-06-29	Powell Summer	CC	56	52,4	8,9	0,81	11,0	T3-4	0,0
2016-07-12	Powell Summer	CC	48	54,1	9,3	0,72	12,9	T2	0,0
2016-06-15	Glen Ora Late	CC	56	53,1	8,6	1,02	8,4	T6	0,0
2016-06-29	Glen Ora Late	CC	56	53,3	9,8	1,05	9,3	T4-5	0,0
2016-07-12	Glen Ora Late	CC	56	55,0	9,8	0,87	11,3	T5-6	0,0
2016-06-15	Gloudi	CC	56	54,7	9,4	0,97	9,7	T6	0,0
2016-06-29	Gloudi	CC	56	52,2	9,3	0,95	9,8	T5	0,0
2016-07-12	Gloudi	CC	56	55,8	9,1	0,89	10,2	T2	0,0
2016-08-02	Gloudi	CC	56	55,4	10,5	0,94	11,2	T1	0,0
2016-06-15	Lane Late	CC	48	54,7	9,3	0,91	10,2	T4-5	0,0
2016-06-29	Lane Late	CC	48	58,3	9,3	0,92	10,1	T3-4	0,0
2016-07-12	Lane Late	CC	56	55,4	10,1	0,88	11,5	T2-3	0,0
2016-06-15	Witkrans	CC	56	54,4	8,8	1,12	7,9	T7	0,0
2016-07-12	Witkrans	CC	56	54,4	9,4	1,00	9,4	T5	0,0
2016-08-02	Witkrans	CC	56	52,7	10,6	0,80	13,3	T1	0,0

4.6.8 PROGRESS REPORT: Cultivar characteristics and climatic suitability of experimental navel oranges in a cold production region (Gamtoos River Valley)

Project 1001B by W. Swiegers and Z. Zondi (CRI)

Summary

The trial consists of a few experimental early, mid and late navel selections. Cambria were used as a control at the trial site. Ryan and Early Lina started the season as the two early navel selections for evaluation. De Wet 1 is a mid-maturing navel producing a round fruit shape. The fruit developed a closed navel end. The mid navel selections that was evaluated with they order of ripening were as follows: Washington and De Wet 1 that

were very close to each other followed by Caloma. The late maturing selection evaluated consist of Cambria and Kakamas Laat.

Opsomming

Hierdie proef bestaan uit ŉ paar eksperimentele vroeë-, middel- en laat nawel seleksies. Cambria is as kontrole gebruik in die proefperseël. Early Lina en Ryan is die 2 vroeë seleksies wat geevalueer was. De Wet 1 is ŉ mid-rypwordende nawel met ŉ ronde vrugvorm. Die vrugte het ŉ toe nawel-ent. Die ander mid-rypwordende kultivars wat ge-evalueer was bestaan uit Caloma en Washington. Die laat nawel seleksie wat ge-evalueer was bestaan uit Cambria en Kakamas laat. Die volgorde van rypwording was as volg: Ryan was eerste gevolg deur Early Lina, Washington, Cambria en De Wet 1 wat baie dieselfde was, gevolg deur Caloma en Kakamas Laat.

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 regions of the Gamtoos River Valley. The following selections were evaluated: Early Lina, Ryan, De Wet 1, Caloma, Kakamas Laat, Washington, with Cambria as a control.

A ratio of 9:1 is considered to be the build-up towards peak maturity for selections. When the ratio between sugar and acid is 10:1, the fruit is considered to be at peak maturity. After reaching the peak, the ratio increases to 11:1, after which it is considered over mature. 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.

Table 4.6.8.1. List of navel selections evaluated at various sites in the Gamtoos River Valley, Eastern Cape during the 2016 season.

Selection	Rootstock	Topworked
De Wet 1	Carrizo	2012
Cambria	Carrizo	2012
KS Navel	Carrizo	2012
Early Lina	Carrizo	2012
Ryan	Carrizo	2012
Kakamas Laat	Carrizo	2012
Washington	Carrizo	2012

Results and discussion

Cambria
Cambria is a well-known mid-late navel selection with very good internal quality. The selection was used as control for the mid to late maturing navel trial in the Gamtoos River Valley. The fruit shape was more elongated compared to the other navel selections. Cambria had a good fruit size and peaked at count 56. The selection had a delayed colour development being at colour plate T6 at peak maturity. The juice was above 55% at peak maturity and Brix was 12° with acids above 1.2%.

De Wet 1
De Wet 1 is a mid-maturing navel that has produced a good crop consistently every year. Manipulation is necessary to control fruit size because over cropping results in smaller fruit. The selection developed a fairly soft rind, one of the characteristics of the De Wet selection. De Wet 1 had a closed navel end on the fruit without having to spray 2,4-D; and developed a small internal navel. The selection had good fruit size and peaked at count 56. Fruit shape was round. The internal quality was good with low juice content of 46.7%. At

peak maturity, the external colour peaked at colour plate T5. The Brix remained around 10° and acid % around 1 %.

Caloma
Caloma bore fruit with a large fruit size and peaked at count 56. The fruit shape appeared more round compared to the standard Cambria selection. The selection had a juice percentage of 53.6%. The external colour development of Caloma were delayed with a T6 on the colour plate before peak maturity. Similar to Cambria, Caloma produced a good Brix and acid content close to peak maturity.

Early Lina
Early Lina were the second selection at peak maturity in this trial site. This selection had a good fruit size count that peaked at count 56. The juice percentage for Early Lina were around 52%. The external colour development range were between T3 and T5. Early Lina had a good Brix and acid at peak maturity.

Ryan
Ryan was the earliest selection to mature for this navel trial. The fruit size for the selection averaged count 56. Ryan were a T6 - T7 on the colour plate at peak maturity. The juice percentage of Ryan were 48.3%. Ryan performed average with the lowest Brix and acid level at peak maturity compared to the other selections.

Washington
The external colour development were behind the internal quality of the fruit (T5) on the colour plate standards. Washington fruit size count peaked at count 56. The juice content of Washington tested around 52% juice. High Brix levels above 11° with acids of 1.09%, assured good tasting fruit with good flavour.

Kakamas Laat
Kakamas Laat was one of the late maturing navel selections evaluated, with external colour development between T3 and T4. Kakamas Laat peaked at count 56. The selection produced a high juice percentage of 57.2% juice this season. High Brix levels of nearly 12° with acids of 1.14% assure a good internal quality and compare well with Cambria.

Conclusion

The fruit size of all the navel selections peaked at count 56. The Navel selection with the highest juice percentage were Kakamas Laat (57.2%) and Cambria at (55.3%). The best colour development on the fruit remained Kakamas Laat (T3 to T4). Cambria, Kakamas Laat and Washington respectively developed the highest Brix values for this trial.

Table 4.6.8.2. Internal fruit quality data for Experimental Navel selections from the Gamtoos River Valley region of the Eastern Cape during the 2016 season.

Date	Selection	Rootstock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Colour	Avg. Seed
2016-05-04	Early Lina	CC	56	52,3	10,9	1,11	9,8	T5	0,0
2016-05-17	Early Lina	CC	56	52,8	11,0	1,02	10,8	T3-4	0,0
2016-05-31	Early Lina	CC	56	52,4	10,4	1,01	10,3	T4	0,0
2016-06-28	Early Lina	CC	56	53,1	11,3	0,91	12,4	T1	0,0
2016-04-19	Ryan	CC	56	48,3	9,5	0,90	10,5	T6-7	0,0
2016-05-04	Ryan	CC	56	46,8	9,6	0,75	12,8	T6	0,0
2016-05-04	De Wet 1	CC	56	49,3	10,7	1,16	9,2	T6-7	0,0
2016-05-17	De Wet 1	CC	56	45,3	10,2	1,08	9,4	T6-7	0,0
2016-05-31	De Wet 1	CC	56	46,7	10,3	1,03	10,0	T5	0,0
2016-06-28	De Wet 1	CC	56	48,4	10,9	0,97	11,2	T1	0,0
2016-06-28	Kakamas Late	CC	56	57,2	11,9	1,14	10,4	T3-4	0,0
2016-07-13	Kakamas Late	CC	56	55,7	12,1	1,04	11,6	T1-2	0,0
2016-05-31	Caloma	CC	56	53,6	10,5	1,11	9,5	T6	0,0
2016-07-13	Caloma	CC	56	55,2	11,5	1,02	11,3	T3	0,0
2016-05-17	Washington	CC	56	52,2	11,4	1,09	10,5	T5	0,0

2016-05-31	Washington	CC	56	52,3	10,9	0,97	11,2	T4	0,0
2016-05-31	Cambria	CC	56	55,3	12,0	1,19	10,1	T6	0,0
2016-06-28	Cambria	CC	56	54,0	12,4	1,10	11,3	T3-4	0,0

4.6.9 **PROGRESS REPORT: Cultivar characteristics and climatic suitability of navel oranges in a cold production region (East Cape Midlands)**
Project 998A by W. Swiegers and Z. Zondi (CRI)

Summary

The late maturing navel trial is based in the Baddaford area of the East Cape Midlands. This is one of the best Navel production areas in South Africa due to the cooler tempretures. Unfortunatly the original site does not exist anymore, but we have started with a new site at Cat River Valley that will bear fruit in 2019. The evaluations included four selections and their order of reaching peak maturity were as follows: Autumn Gold, Powell Summer, Chislett and Barnfield Summer.

Opsomming

Die laat nawel proef is gevestig in die Baddaford area van die Oos-Kaap Middelande. Dit is een van die beste Nawel produksie areas in die land a.g.v. hul koeler temperature. Ongelukkig bestaan die oorspronklike perseel nie meer nie, maar daar is ŉ nuwe perseel in die Kat rivier vallei gevestig wat teen 2019 vrugte sal dra. Die volgende kultivars is ge-evalueer en hul orde van rypwording was soos volg: Autumn Gold, Powell Summer, Chislett en Barnfield Summer.

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 (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 East Cape Midlands region of the Eastern Cape. The following late maturing selections were evaluated: Autumn Gold, Barnfield Summer, Chislett, Powell Summer.

For navels, 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 over mature. 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.

Table 4.6.9.1. List of navel selections evaluated at East Cape Midlands (Baddaford) during 2016.

Selection	Rootstock	Planted
Autumn Gold	Troyer	2007
Barnfield Summer	Troyer	2007
Chislett	Troyer	2007
Powell Summer	Troyer	2007

Results and discussion

Autumn Gold
The selection cropped fruit with a good fruit size and peaked at count 56. Internal quality was good with a high juice percentage of 54.8%. Autumn Gold developed a delayed external colour on the colour plate (T4 to T5) at peak maturity. Autumn Gold developed a good internal quality by the time of peak maturity.

Barnfield Summer
Barnfield Summer were the last selection to reach peak maturity at this cool inland trial site. The external colour development on this selection were very good, reacheing a T1 on the colour plate before maturity. The juice

percentage were high and peaked above 56.5%. Barnfield bore large fruit on the trees at the trial site and averaged fruit size of count 56. This selection had the highest Brix of all the selections evaluated (14°) and a acid percentage of 1.44% close to peak maturity.

Chislett
Chislett performed well and had a good juice percentage above 55% towards peak maturity. The fruit size count for Chislett remained on the large side (count 56). The external colour development advanced slowly towards peak maturity, having a T4 on the colour plate and still a long way to peak matrurity (delayed).

Powell Summer
The fruit size peaked at count 56 for this season which is very favorable for navel production and export. The juice percentage for Powell were just below 55% (meeting export standards). Powell also had good Brix and acid percentage this season for the cool production area. The external colour development was similar to Chislett; T5 on the colour plate range. When completing the last evaluation, there were an average seed count of 0.3 seeds per fruit.

Conclusion

The East Cape Midlands area is well suited for navel production in South Africa. The internal quality were very good with high Brix (above 11°) and good acids, a indication that the fruit will be able to hang a bit longer on the trees with a good shelf life. The fruit quality was good; very good flavour. All the selections had a large fruit size and peaked at count 56. Autumn Gold, Chislett and Powell Summmer had a delayed external colour development; peaked from T4 to T5 on the colour plate range. Barnfield Summer had no problem with external colour development with a T1 on the colour plate at peak maturity. Barnfield Summer developed the highest Brix content of 14°, as well as the highest juice percentage with 56.5%. Powell Summer were the only selection with seeds when the final evaluation was completed; low seed count (averaged 0.3 seeds per fruit).

Table 4.6.9.2. Internal fruit quality data for early to late Navel selections from the Beddaford region of the East Cape Midlands during the 2016 season.

Date	Selection	Rootstock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Colour	Avg. Seed
2016-06-27	Autumn Gold	TC	56	54,8	12,3	1,15	10,7	T4-5	0,0
2016-08-05	Barnfield	TC	56	56,5	14,0	1,44	9,7	T1	0,0
2016-08-26	Barnfield	TC	56	61,2	13,9	1,00	13,9	T1	0,0
2016-06-27	Chislett	TC	56	55,4	10,0	1,37	7,3	T4	0,0
2016-08-05	Chislett	TC	56	56,9	11,3	0,93	12,2	T1	0,0
2016-06-27	Powell Summer	TC	56	54,7	11,8	1,21	9,8	T5	0,0
2016-08-05	Powell Summer	TC	56	57,5	12,9	1,01	12,8	T1	0,3

4.6.10 **PROGRESS REPORT: Cultivar characteristics and climatic suitability of Valencia oranges in a cold production region (Sundays River Valley)**
Project 1097A by W. Swiegers and Z. Zondi (CRI)

Summary

The Valencias discussed in this trial were top worked in the 2011 season. The trees produced their first crop in the 2015 season. There is a possibility to expand the trial site further. The early maturing selection for the trial site is Bennie 2. Die mid maturing Valencia selections is Alpha, Gusocora, Henrietta, Midknight 1 and Midknight as control. The late maturing Valencia selections will be Louisa, McClean SL, Lavalle and Lavalle 2. At this trial site the season started with Midknight 1, followed by Ruby Red, Mc Clean SL, Alpha, Gusocora, Midknight, Henrietta, Bennie 2, Louisa and the season ended off with Lavalle and Lavalle 2. The differences in the maturing times of these Valencia selections could be due to the youth of the trees.

Opsomming

Die Valencias wat bespreek word in hierdie proef was in die 2011 seisoen getopwerk. Die bome het hulle eerste drag in die 2015 seisoen gehad. Daar is ŉ moontlikheid om die perseel verder uit te brei. Die vroeë seleksie vir die proef perseel bestaan uit Bennie 2. Die mid seleksies is Alpha, Gusocora, Henrietta, Midknight 1 en Midknight wat as kontrole dien. Die laat rypwordende Valencia seleksies was as volg; Louisa, McClean

SL, Lavalle en Lavalle 2. Die proef perseel se seisoen het begin met Midknight 1, gevolg deur Ruby Red, Mc Clean SL, Alpha, Gusocora, Midknight, Henrietta, Bennie 2, Louisa en die seisoen word afgesluit deur Lavalle en Lavalle 2. Verskille in rypwording kan toegeskryf word aan die bome se jong ouderdom.

Objective

To select Valencia cultivars with improved and consistent productivity, fruit size, rind colour, peelability, and internal fruit quality (seedlessness, ratio), and extended harvest period (both earlier and later maturity).
To describe the cultivar characteristics of new Valencia cultivars and to determine the climatic suitability of these cultivars in a cold production region.

Materials and methods

Field evaluations and laboratory analyses were conducted on Alpha, Bennie 2, Gusocora (G5), Henrietta, Lavalle, Lavalle 2, Louisa, McClean SL, Midknight (control), Midknight 1, Ruby Red.

Table 4.6.10.1. 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
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

*Interim internal fruit quality standards.

Table 4.6.10.2. List of Valencia selections evaluated at Panzi (Kirkwood) during 2016.

Selection	Rootstock	Topwork
Alpha	CC	2011
Bennie 2	CC	2011
Henrietta	CC	2011
Lavalle	CC	2011
Gusocora	CC	2011
Lavalle 2	CC	2011
Louisa	CC	2011
McClean SL	CC	2011
Midknight 1	CC	2011
Midknight	CC	2011
Ruby Red	CC	2011

Results and discussion

Alpha
Alpha bore medium size fruit this season on the trees, count 64. The tree condition on Carrizo rootstock were good. Alpha Valencia were completely seedless and the fruit shape remained fairly round with smooth skin texture. The external colour development peaked at T1 with good internal quality by the time of maturity. Juice content were above 56% and time of maturity seems to be end of July to beginning of August.

Bennie 2
The fibre strength were soft compared to the other Valencia selections. The fruit size peaked from count 64 to 56 this season, a good Valencia export fruit size. Bennie 2 have good acids for the fruit to hang on the trees longer to harvest at a later time, resulting in fewer rind problems (pitting). Average seed count peaked at 2 seeds per fruit. There was no delay in external colour development (T1) before peak maturity. Bennie 2 developed a good juice content around 59% towards peak maturity.

Gusocora
There was no delay in external colour development on the fruit (T1) when compared to the internal quality by the time of peak maturity, with an average Brix of 9.9 and acid of 0.96%. Gusocora was completely seedless and will be regarded as a seedless selection. The juice content of Gusocora was 56.9% and the fruit size peaked at count 72.

Henrietta
The fruit shape of Henrietta remained round and the rind texture fairly smooth. Fruit peeled easily and contained a medium amount of rind oil. The seed count peaked at 0.4 seeds per fruit, with 2 evaluations being seedless. Fruit size range from medium to large fruit and peaked at from count 64 to 56. This specific size range was more favourable for Valencia exports. Henrietta had no problem with external colour development with T1 on the colour plate range by maturity time. Henrietta produced a good juice content of 56% this season.

Lavalle 2
One of Lavalle 2's qualities is large fruit size for a Valencia selection and the fruit size peaked at count 56, excellent for Valencia production. The higher acid level (1.27%) indicated that this selection was late maturing and ended of the Valencia season at this trial site. Lavalle was completely seedless and the juice content of this selection increase towards peak maturity (58%) with aBrix: acid ratio of 8.6. There was no problem with the external colour development when Lavalle developed a T1 on the colour plate range.

Louisa
Three evaluations were completed for Louisa; the first evaluation had the lowest seed count (completely seedless) and the highest seed count with the other evaluations were 3 seeds per fruit. The fruit size ranged from count 72 to 64. The fruit shape was round with a fairly smooth rind texture and the fruit peeled easily. The juice content of Louisa decrease towards peak maturity from 57% to around 52%. Louisa were fully coloured before peak maturity (T1).

McClean SL
McClean SL produced fairly round fruit with a soft fibre strength that peeled easily, containing low rind oil levels. All the fruit evaluated remained completely seedless. Many of the seedless selections have fruit set problems and bear poor crops, but this does not seem to be the case with this cultivar (good production). The trees bore medium fruit on the trees (count 72 to 64). The internal quality was good with high juice levels for this trial site (60%). There was no delay in external colour development being a T1.

Midknight 1
Midknight 1 cropped medium size fruit (count 64) on the trees this season and the fruit shape is fairly round, rind texture was medium to coarse, fibre strength fairly soft and the fruit peels easily. The juice levels of Midknight 1 were the highest for the trial (average 59%). The Midknight 1 selection were seedless during all evaluations completed. Before peak maturity the external colour development were between T2 and T3 which is fine for export standards. Over matured fruit peaked at a T1 on the colour plate range.

Midknight
Midknight were used as control in this trial site. The fruit size for the Midknight selection ranged between count 72 and count 56 (very variable fruit size for Valencia export). The juice content of Midknight peaked at 57.5%. The external colour development of Midknight were very good with a T1 on the colour plate range before peak maturity.

Lavalle
Lavalle produced 0.3 seeds per fruit during the first evaluation, all the other evaluations were seedless. Lavalle is a late Valencia selection with good shelf life and the optimal harvest time will be in August/September. The juice content decreased towards peak maturity, but still remained good for export standards at peak maturity (57.7%). The fruit size count for Lavalle peaked at from count 64 to 56. The external colour of this selection were T1 at peak maturity with a good internal quality.

Ruby Red
Ruby Red bore medium to large fruit and peaked at count 72 to 64. The juice content of Ruby Red increased towards peak maturity above 60.9%. Ruby Red also had the highest juice percentage of all the Valencia selections at this trial site. At peak maturity Ruby's external colour development were T1 on the colour plate range and the fruit were completely seedless during all evaluations completed.

Conclusions

None of the Valencia selections had a problem with external colour development; all of them were T1 on the colour plate range. All of the selection's internal and external qualities complied with the minimum export requirement for Valencia types. The following selections had a seed count; Louisa with the highest count of 3 seeds per fruit and Bennie 2 with an average seed count of 2 seeds per fruit. Henrietta and Lavalle had very low seed count (virtually seedless) and all the other selections were completely seedless. All the selections

had a good fruit size for export requirements. The following selections developed a juice content above 57% at peak maturity; Alpha, Bennie 2, Lavalle, McClean SL, Midknight, Midknight 1 and Ruby Red.

Table 4.6.10.3. Internal fruit quality data for Valencia selections at Panzi (Sundays River Valley) during the 2016 season.

Date	Cultivar	Root stock	Count	Juice %	Brix°	Acid %	Ratio	Colour	Avg. Seed
2016-07-12	Alpha	CC	64	54,2	8,8	0,92	9,6	T2	0,0
2016-08-02	Alpha	CC	64	57,4	10,0	1,00	10,0	T1	0,0
2016-08-17	Alpha	CC	64	56,6	9,8	0,94	10,4	T1	0,0
2016-09-01	Alpha	CC	64	55,3	10,1	0,87	11,6	T1	0,0
2016-07-12	Benny 2	CC	56	58,7	9,4	1,10	8,5	T1	2,0
2016-08-02	Benny 2	CC	64	59,2	10,1	1,10	9,2	T1	1,5
2016-08-17	Benny 2	CC	64	59,4	10,3	1,13	9,1	T1	1,0
2016-09-01	Benny 2	CC	56	58,2	10,5	0,91	11,5	T1	1,8
2016-08-02	Gusocora	CC	72	60,4	10,0	1,14	8,8	T1	0,0
2016-08-17	Gusocora	CC	72	56,9	9,9	0,96	10,3	T1	0,0
2016-09-01	Gusocora	CC	72	59,8	10,5	0,91	11,5	T1	0,0
2016-08-02	Henrietta	CC	64	56,7	9,8	1,03	9,5	T1	0,1
2016-08-17	Henrietta	CC	64	56,0	9,7	0,97	10,0	T1	0,0
2016-09-01	Henrietta	CC	56	55,6	9,6	0,93	10,3	T1	0,0
2016-09-13	Henrietta	CC	56	54,5	9,9	0,71	13,9	T1	0,4
2016-08-02	Lavalle	CC	64	61,1	10,4	1,23	8,5	T1	0,3
2016-09-01	Lavalle	CC	56	59,2	10,3	1,11	9,3	T1	0,0
2016-09-13	Lavalle	CC	56	57,7	10,7	1,09	9,8	T1	0,0
2016-09-20	Lavalle	CC	56	56,0	10,5	0,91	11,5	T1	0,3
2016-09-01	Lavalle 2	CC	56	57,7	10,7	1,37	7,8	T1	0,0
2016-09-20	Lavalle 2	CC	56	58,1	10,7	1,27	8,6	T1	0
2016-08-02	Louisa	CC	72	57,2	10,4	1,30	8,0	T1	3,0
2016-08-17	Louisa	CC	64	51,2	10,1	1,11	9,1	T1	0,5
2016-09-01	Louisa	CC	64	52,4	10,4	0,96	10,8	T1	0,0
2016-07-12	McClean SL	CC	72	60,0	9,2	1,15	8,0	T4	0,0
2016-08-02	McClean SL	CC	72	60,8	10,0	0,90	11,1	T1	0,0
2016-08-17	McClean SL	CC	64	58,8	10,3	0,78	13,2	T1	0,0
2016-07-12	Midknight	CC	56	60,2	9,6	1,04	9,2	T1	0,0
2016-08-02	Midknight	CC	56	54,2	9,9	1,03	9,6	T1	0,0
2016-08-17	Midknight	CC	64	56,8	10,2	1,00	10,2	T1	0,0
2016-09-01	Midknight	CC	72	57,5	9,9	0,91	10,9	T1	0,0
2016-09-13	Midknight	CC	56	54,6	10	0,87	11,5	T1	0,0
2016-06-15	Midknight 1	CC	64	56,7	8,5	0,97	8,8	T6	0,0
2016-06-29	Midknight 1	CC	64	58,8	8,9	0,95	9,4	T2-3	0,0
2016-07-12	Midknight 1	CC	64	59,7	9,7	0,89	10,9	T1	0,0
2016-08-02	Midknight 1	CC	72	57,8	9,9	0,87	11,4	T1	0,0
2016-06-15	Ruby Red	CC	64	56,4	8,6	1,05	8,2	T6	0,0
2016-06-29	Ruby Red	CC	72	56,5	9,4	1,17	8,0	T4-5	0,0
2016-07-12	Ruby Red	CC	64	60,9	9,2	0,87	10,6	T1	0,0

4.6.11 **PROGRESS REPORT: Evaluation of Valencia selections in a semi-desert production area (Kakamas)**

Project 964B by W. Swiegers and J. Joubert (CRI)

Summary

The Valencias discussed in this trial were top worked in the 2010 season. The trees produced their third crop in the 2016 season. Due to the high pressure of cross pollination in this variety block, most of the selections produced seeds. Valencias tend to keep their acids levels better compared to the navel selections. This trial site consists of a wide range of Valencia selections from early-, mid- and late maturing varieties.

Opsomming

Die Valencias wat bespreek word in hierdie proef was in die 2010 seisoen getopwerk. Die bome het hulle derde drag in die 2016 seisoen geproduseer. As gevolg van die hoë druk van kruisbestuiwing in hierdie proef perseel het die meeste seleksies saad ontwikkel. Die Valencias behou hulle suur vlakke beter as die Nawel seleksies vir hierdie produksie area. Die perseel is baie volledig wat Valencia seleksies aanbetref, want dit bevat die vroeë-, mid- en laat rypwordende Valencia seleksies

Objective

- To find suitable Valencia selections with superior characteristics for the semi-desert production area (Kakamas).

Materials and methods

Field evaluations and laboratory analyses were conducted on Bennie 2, Du Roi 2, Henrietta, Jassie, Kobus du Toit Late, Lavalle 2, Gusocora (G5), Louisa, McClean, McClean SL, Midknight 1, Moosrivier late 1, Moosrivier late 2, Ruby Red, Skilderkrans, Valencia late, Weipe.

Table 4.6.11.1. 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
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

*Interim internal fruit quality standards.

Table 4.6.11.2. List of Valencia selections evaluated at Mosplaas (Kakamas) during 2016.

Selection	Rootstock	Topwork
Bennie 2	X639	2010
Du Roi 2	X639	2010
Henrietta	X639	2010
Jassie	X639	2010
Kobus du Toit	X639	2010
Gusocora	X639	2010
Lavalle 2	X639	2010
Louisa	X639	2010
McClean	X639	2010
McClean SL	X639	2010
Midknight 1	X639	2010
Moosrivier late 1	X639	2010
Moosrivier late 2	X639	2010
Ruby Red	X639	2010
Skilderkrans	X639	2010
Valencia late	X639	2010
Weipe	X639	2010

Results and discussion

Bennie 2
The fruit size count peaked at count 72 to 64, medium fruit size and a good Valencia export size. Bennie 2 had a soft fibre strength compared to the other Valencia selections. Bennie 2 have a good acid percentage for the fruit; in a semi- desert production area the acids tend to drop quickly. Average seed count peaked at 2.3 seeds per fruit. There was no delay in external colour development (T1 to T3) before peak maturity. Bennie 2 developed a juice content of 55% towards peak maturity.

Gusocora
The external colour development of Gusocora ranged from T1 ti T2 and complied with the colour standards for export. Gusocora had a very good internal quality with average Brix of 10 and acids of 1.12% towards peak maturity. Even when Gusocora were over mature it still had a good acid level. Gusocora were completely seedless and will be regarded as a seedless selection for future plantings. The juice content of Gusocora were around 55% and the fruit size peaked at count 72 to 64.

Henrietta
The peelability of Henrietta were quite easy and the fruit shape was round. The rind texture was smooth with a medium rind oil on the fruit. The average seed count peaked at 4.6 seeds per fruit. Fruit size count range from (count 88 – 64). Henrietta had no problem with external colour development (T1) before peak maturity. Henrietta produced a good juice content of 56%.

Lavalle 2
Lavalle 2 cropped a good yield with medium to large fruit size on the trees. The fruit size range from count 72 to 48, excellent for Valencia production and export. The higher acid levels indicate that this selection was late maturing and will remain high in this semi-desert production area. Lavalle 2 was last selection to reach peak maturity at this trial site. The selection was completely seedless in a strong cross pollinated trial site. The juice content was above 53% towards peak maturity and there were no problems with the external colour development (T1 to T4) when the Brix: acid ratios were at 7; Brix level was below 12.

Louisa
Louisa remained completely seedless at this trial site. The fruit size range from count 72 to 64 and the rind texture were medium to fairly smooth; fruit peeled easily. The juice content of Louisa was 53.4% at peak maturity with a good Brix: acid ratio (10.2). The external colour development peaked from T1 to T4 at peak maturity.

Mc Clean and McClean SL
Mc Clean produced a bigger fruit size (count 56 to 40) compared to McClean SL (count 64). The juice content of the 2 selections were similar; McClean with 52.1% close to peak maturity compared to McClean SL at 53.7%. McClean SL developed a slightly higher Brix and both selections favourable acids. McClean SL mature about 4 weeks earlier than McClean. Both selections remained completely seedless. McClean had a better external colour development (T1 to T6) compared to McClean SL (T3 to T5).

Midknight 1
The fruit development peaked from count 64 to count 56. Midknight 1 bore round fruit on the trees with a medium to coarse rind, fibre strength were fairly soft and the fruit peeled easy. The juice levels of Midknight 1 were on the lower side (48%); below the minimum export standard. Midknight 1 were completely seedless during all evaluations. The colour development at peak maturity was T2 on the colour plate range.

Ruby Red
Ruby bore medium sized fruit on the trees with an average between count 88 and count 72. The juice content of Ruby Red increased towards peak maturity up to 57.9%. Ruby Red had one of the highest juice percentage of all the Valencia selections evaluated at the trial site. At peak maturity Ruby's external colour development were between T1 and T2 on the colour plate range. The selection was seedless with the first evaluation and produced an average seed count of 1.3 seeds per fruit during the second evaluation.

Jassie
Jassie produced a medium to large fruit size on the trees and peaked from count 64 to count 48. The fruit developed a coarse pebbly rind due the the young tree age and will become smoother with time. Jassie were seedless with the second evaluation and during the first evaluation there were an average seed count of 2.2 seeds per fruit. Internal quality was excellent close to peak maturity with high juice levels above 55%, high Brix of 11.3 and fairly high acids of 1.19%. External colour peaked from T1 to T2 with the final evaluations. Maturity seems to be mid to end of July based on the results in Table 4.6.11.3.

Moosrivier Late 1 and 2
Moosrivier Late 1 ranged from count 88 to count 56, while Moosrivier Late 2 peaked at count 64. The juice percentages of the 2 selections (Moosrivier Late 1 - 52% and Moosrivier Late 2 - 45,4%) were to low for export. Moosrivier Late 1 had the better internal quality as well as external colour development compared to Moosrivier Late 2. Moosrivier Late 2 was the earlier maturing selection and completely seedless while Moosrivier Late 1 averaged 2.3 seeds per fruit.

Skilderkrans
Skilderkrans developed a medium to large fruit size (count 64-48) on the trees. The internal quality was good with a juice content of 54.1%, Brix at 11.5 and acid content of 1.24%. The external colour development was good enough for export (T1 to T3). The fruit peeled fairly easily, rind thickness was thin and the fruitshape was round with rind texture medium to rough. The selection was completely seedless at this trial site. Based on the internal quality results in Table 4.6.11.3, estimated maturity will be the middle to end of July.

Weipe
Weipe is an early maturing Valencia selection and was first to reach peak maturity at this trial site. Internal quality Brix: acid ratio (12.6) was average with low juice levels (45%), Brix of 9.8 and acid content of 0.78%. The fruit was completely seedless and developed a deep orange external colour (T2 to T3). Maturity is estimated to be early to middle of May based on the results in Table 4.6.2.4.

Valencia Late
The Valencia Late produced small to medium fruit size and range between count 105 and count 72. Acid levels were high close to peak maturity (1.37%) when the second evaluation was completed, indicating the late maturity qualities of the selection. The juice content was 51.1% and Brix was above 12.7. The external colour development was good with T1 and seed counts varied from seedless to 1.6 seeds per fruit. Maturity will be late in the season and according to Table 4.6.11.3, peak end July to middle of August.

Du Roi 2
The fruit size peaked at medium (count 88 to 72), optimum for Valencia production. There were 2.9 seeds per fruit in the Du Roi 2 Valencia and the rind texture was fairly smooth. The internal quality of Du Roi 2 was good with juice levels above 50%, Brix of 11.3 and fairly high acids (1.38%), with good external colour development (T1-2). Du Roi 2 is one of the late maturing Valencia options and maturity seems to be end of July according to Table 5.6.11.3.

Du Toit Late
Du Toit Late produced a medium fruit size (count 88 to 56) on the trees, with average 2.7 seeds per fruit. The juice content was not so good, with juice levels below 50% (47,7%). Brix content was up to 11.6 and acid levels were higher for the later maturing selection (1.2%). External colour peaked from T1 to T2 and maturity seems to be middle to end of July according to Table 4.6.11.3.

Conclusion

McClean SL were the only selection with a delayed external fruit colour development. Henrietta, Du Roi 2, Bennie 2 and Kobus du Toit Late were the selection on average with the highest number of seeds per fruit. Lavalle 2, Louisa, Gusocora, Skilderkrans, McClean, McClean SL, Midknight 1 and Weipe were the only selections that were completely seedless. The Valencia selections with a juice content above 55% towards peak maturity or at peak maturity were Bennie 2, Henrietta, Jassie, Gusocora and Ruby Red. The fruit size varied quite a lot between selections, but all of them were good enough for export. The following selections produced a Brix above 11 and acids content above 1%; Du Roi 2, Jassie, Kobus du Toit Late, Lavalle 2. Louisa, Moosrivier Late 2, Ruby Red, Skilderkrans and Valencia Late. At this trial site the season started with Weipe and ended with Lavalle 2.

Table 4.6.11.3. Internal fruit quality data for Valencia selections at Mosplaas (Kakamas) during the 2016 season.

Date	Cultivar	Count	Juice %	Brix°	Acid %	Ratio	Avg. Seed	Colour
2016-05-16	Bennie 2	64	53,0	9,6	1,35	7,1	2,3	T5
2016-06-07	Bennie 2	72	55,4	10,8	1,26	8,6	2,2	T1-3
2016-06-07	Du Roi 2	88	55,0	10,1	1,43	7,1	1,7	T4-5

2016-07-06	Du Roi 2	88-72	54,4	11,3	1,38	8,2	2,9	T1-2
2016-06-07	Gusocora	64	55,5	10,0	1,12	8,9	0,0	T3-4
2016-07-06	Gusocora	72-64	53,8	11,3	0,99	11,4	0,0	T1-2
2016-06-07	Henrietta	64	57,9	10,2	1,37	7,4	1,1	T3-4
2016-07-06	Henrietta	88-64	56,3	10,5	1,32	8,0	4,6	T1
2016-06-07	Jassie	64	42,4	10,1	1,23	8,2	2,2	T5
2016-07-06	Jassie	64-48	56,7	11,3	1,19	9,5	0,0	T1-2
2016-06-07	Kobus du Toit Late	64	52,4	9,9	1,33	7,5	2,7	T5
2016-07-06	Kobus du Toit Late	88-56	47,7	11,6	1,20	9,7	2,6	T1-2
2016-06-07	Lavalle 2	64	53,6	10,5	1,78	5,9	0,0	T5-6
2016-07-06	Lavalle 2	72-48	53,1	11,8	1,68	7,0	0,0	T1-4
2016-06-07	Louisa	72	40,7	9,8	1,15	8,5	0,0	T4-6
2016-07-06	Louisa	72-64	53,4	11,2	1,10	10,2	0,0	T1-4
2016-07-06	Mc Clean	56-40	52,1	9,7	1,02	9,5	0,0	T1-6
2016-06-07	Mc Clean SL	64	53,7	10,1	1,00	10,1	0,0	T3 -5
2016-07-06	Mc Clean SL	64	51,6	11,3	0,97	11,6	0,0	T1-4
2016-06-07	Midknight 1	64	47,9	9,8	0,90	10,9	0,0	T2
2016-07-06	Midknight 1	64-56	49,9	11,6	0,97	12,0	0,0	T1-2
2016-06-07	Moosrivier late 1	64	40,4	9,3	1,60	5,8	0,1	T2-4
2016-07-06	Moosrivier Late 1	88-56	52,0	11,2	1,28	8,8	2,3	T1-2
2016-07-06	Moosrivier Late 2	64	45,4	10,6	0,88	12,0	0,0	T1-6
2016-06-07	Ruby Red	88	43,3	10,1	1,19	8,5	1,3	T6
2016-07-06	Ruby Red	88-72	57,9	11,6	1,18	9,8	0,0	T1-2
2016-06-07	Skilderkrans	64	38,0	9,8	1,33	7,4	0,0	T4-6
2016-07-06	Skilderkrans	64-48	54,1	11,5	1,24	9,3	0,0	T1-3
2016-06-07	Valencia late	72	54,3	10,7	1,32	8,1	1,6	T2
2016-07-06	Valencia Late	105-88	51,1	12,7	1,37	9,3	0,0	T1
2016-05-16	Weipe	64	45,4	9,8	0,78	12,6	0,0	T2-3
2016-06-07	Weipe	64	34,9	10,5	0,72	14,5	0,0	T2

4.6.12 PROGRESS REPORT: Cultivar characteristics and climatic suitability of mandarin hybrids in a semi-desert production region (Kakamas)

Project 964D by W. Swiegers and J. Joubert (CRI)

Summary

These mandarin selections were topworked onto X639 rootstock in 2010. None of these mandarin selections is under nets and sunburn was a problem for some of the cultivars. Fruit fly can be a problem in this trial site. Due to the climate it is important for the selections to keep their acids relatively high, because it is important for shelf life as well as good flavor. There is high pressure of cross pollination in this trial block.

Opsomming

Hierdie mandaryn seleksies was getopwerk op X639 in die 2010 seisoen. Geen van hierdie seleksies is onder net nie en sonbrand is ŉ probleem by sekere van die variteite. Vrugtevlieg kan ŉ probleem wees by die proefperseel. A.g.v. die klimaat is dit belangrik vir die seleksie om sy suur relatief hoog te hou vir rak leeftyd en smaak. Daar is hoë druk van kruisbestuiwing in hierdie proef blok.

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 semi-desert regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on mandarin hybrid selections from the semi-desert (Kakamas) region of the Northern Cape. A range of new mandarin hybrids have been added to this area and should bear fruit in the 2015 season. The following selections were evaluated; Furr, Etna, Edit x Nova, IRM 1, IRM 2, Nadorcott ARC, Nadorcott SL, Samba, Shani SL, Tango, Valley Gold (B17), Winola and Yosemite Gold.

For mandarin hybrids, 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 over mature. 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.

Table 4.6.12.1. List of mandarin hybrid selections in the semi-desert region (Kakamas) during the 2016 season.

Selection	Rootstock	Topwork
Furr	X 639	2010
Etna	X 639	2010
Edit x Nova	X 639	2010
IRM 1	X 639	2010
IRM 2	X 639	2010
Nadorcott ARC	X 639	2010
Nadorcott SL	X 639	2010
Samba	X 639	2010
Shani SL	X 639	2010
Tango	X 639	2010
Valley Gold	X 639	2010
Winola	X 639	2010
Yosemite Gold	X 639	2010

Results and discussion

Furr
Furr is used as a control for the mid-maturing mandarin selections. The selection has a very good rind colour development (deep orange). The juice percentage peaked at 52.9% being just over mature. Furr peels easily and has a very good eating quality with good flavour. The selection had a good Brix (12.2°). Due to the high cross pollination pressure, Furr produced the most seed; 14.8 seeds per fruit on average (it is less than the 2015 season with over 22 seeds average per fruit). Fruit size peaked at count 1xx. Furr tend to keep its acids quite well with acid levels being 0.91% past peak maturity. Furr had a delayed external colour development (T3 to T4).

Etna
Etna is an early mandarin selection that matures end of April to middle of May. The fruit size peaked at count 1xx with juice percentages of 54.7%. The selection will produce seeds in the fruit under cross pollination conditions. At this trial site Etna produced 1.2 seeds per fruit on average. Etna had low Brix (9.1°) and acid (0.87%) levels towards peak maturity. The external colour decelopment were T6 and degreening will be neccesary for Etna.

Edit x Nova
The fruit size count for this selection were between 1 and 1xx. At peak maturity the internal quality was very good with the juice content above 58%, Brix of 13° and the acid content were 1.09%. The external colour development were good and reached a range from T1 to T2 at peak maturity. The average seed count for Edit x Nova peaked at 3 seeds per fruit and the one evaluations were seedless.

IRM 1
The IRM 1 selection is one of two irradiated Murcott selections from Australia. IRM 1 has a slightly less intense external rind colour than the IRM 2 selection; has more ribbing and the fruit is firm and peels easily. The IRM 1 selection had juice percentages above 52%; higher compared to IRM 2 with 49.5%. Brix was high at 13.2° and the acid remained at 1% even when the selection were over mature. The fruit size count for IRM 1 peaked at count 2 to1x. External colour was a bit delayed (T1-T2) at over maturity and the selection had 0.7 seeds per fruit on average. IRM 1 reach peak maturity after IRM 2.

IRM 2
IRM 2 are ahead of IRM 1 in reaching peak maturity. IRM 2 developed some ribbing on the fruit, but less than IRM 1 and the fruit was firm and peel easily and clean. Fruit size count for IRM 2 were count 1 to 1x. Juice content were below 50%. There was external colour delay in some of the fruit (T1 to T3). The average seed count for IRM 2 were 2.2 seeds per fruit. Internal quality was good with Brix above 12° and the acid content were just below 1% (good shelflive).

Nadorcott ARC & Nadorcott SL
Nadorcott ARC and Nadorcott SL are induced Nadorcott mututions to minimise the average seeds per fruit. Both selections have the same growth habit and charactaristics as the Nadorcott, and fruit size peaked at count 2 to count 1. The juice percentages were over 55%; Brix above 10°, Nadorcott ARC had a slightly higher Brix. With acids of 0.90% close to peak maturity, Nadorcott ARC had a slightly higher acid %. The seed count per fruit on average ranged from seedless to 0.8 seeds per fruit, which is very good in a combined variety block like this. The external colour of the fruit were delayed (T4-5) close to peak maturity.

Samba
Samba is an early maturing mandarin selection that was one of the best performing soft citrus selections in this semi-desert production area. The external rind colour was excellent for this hot area (deep orange). Samba's fruit size peaked at count 2 to count 1. Brix levels were above 10 with an acid percentage of 0.90%. The juice percentages were good and above 56%. The selection will produce seeds if cross pollinated and under these heavy cross pollination conditions, Samba produced on average 0.4 seeds per fruit, much less compared to 2015 (average 9 seeds per fruit). The external colour on the colour plate close to peak maturity were T3 to T5.

Shani SL
Shani SL produced a fruit size ranging from count 2 to count 1x. The juice content for Shani SL were 53.4% towards build up to peak maturity. Shani SL had a high Brix content of 13.7 and a high acid of 1.33%. The selection had no problem with external colour development, being at T1 well before peak maturity. The seed count peaked at 2 seeds per fruit average.

Tango
Tango had a good colour development for this semi-desert production area; the selection was at colour plate T1 at peak maturity. Juice levels were low with percentages at 49.5% for this trial site. Tango was seedless in 2016 in this trial block. The acid levels remained high during the season and the Brix level reached 12.6°. Fruit size was on the smaller side and peaked between count 2 and 1x. The rind texture was smooth and shiny and the fruit peeled easily.

Valley Gold B17
Valley Gold had fruit splitting and fruit drop during the production season. The fruit size peaked at count 1xxx. Juice percentage was at 51.1% at peak maturity, Brix peaked at 11.6 and acids were 0.98%. The average seed count were 0.7 seeds per fruit. The external colour development ranged from T1 to T3.

Winola
Winola is a late maturing mandarin selection that has very high acid percentages. The selection is prone to alternate bearing patterns as well as Alternaria. Winola developed a deep orange-red external colour before peak maturity (T1). The selection had good juice percentages, reaching up to 54.6%, Brix peaked at 12.4° and the acids were at 1.32%. Winola produced 0.1 seeds per fruit on average. The selection cropped large fruit on the trees and peaked at count 1xxx.

Yosemite Gold
The selection had a very good juice percentage of over 55% and the Brix peaking at 11.9°, acids remained at 1.01% at peak maturity. Yosemite Gold was at colour plate T1 at peak maturity. The fruit size of Yosemite

Gold was large to xtra large and peaked at count 1xx. Yosemite Gold were completely seedless at this trial site with high cross pollination pressure.

Conclusion

This semi-desert production area is a marginal growing area for Mandarin hybrids and only certain selections will be profitable under these growing conditions. Furr was the selection with the highest seed count at 14.8 seeds per fruit on average. Tango and Yosemite Gold were seedless. Edit x Nova had the highest juice percentage of 58.8% and also the highest Brix (13°). Etna were one of the selections most affected by these harsh growing conditions. Etna developed large fruit with very coarse rinds as well as severe sunburn problems. Shani SL, Winola, Yosemite Gold, Tango and Edit x Nova had the best external colour development. Selections with the biggest fruit size count were Valley Gold and Winola

Table 4.6.12.2. Internal fruit quality data for Mandarin hybrid selections from the semi-desert region (Kakamas) during the 2016 season.

Date	Cultivar	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2016-05-16	Furr	1xx	52,9	12,2	0,91	13,4	11,3	T3-4
2016-06-07	Furr	1xx	59,6	13,0	0,88	14,8	14,8	T1
2016-04-14	Edit x Nova	1	57,4	10,4	0,99	10,5	2,0	T6
2016-05-16	Edit x Nova	1x	58,8	13,0	1,09	11,9	0,0	T1-2
2016-06-07	Edit x Nova	1x	53,3	14,6	1,16	12,6	3,0	T1
2016-07-06	Edit x Nova	1-1xx	56,0	14,3	1,18	12,1	0,7	T1
2016-04-14	Etna	1xx	54,7	9,1	0,87	10,5	1,2	T6
2016-07-06	IRM 1	2-1x	52,0	13,2	1,00	13,2	0,7	T1-2
2016-06-07	IRM 2	1	49,5	12,4	0,95	13,1	2,2	T1-3
2016-07-06	IRM 2	1-1x	40,6	13,2	0,76	17,4	1,8	T1
2016-04-14	Nadorcott ARC	2	56,3	9,7	1,07	9,0	0,0	T7
2016-05-16	Nadorcott ARC	1	57,8	11,2	0,99	11,3	0,0	T4-5
2016-06-07	Nadorcott ARC	1	57,7	12,1	0,89	13,6	0,8	T1-2
2016-04-14	Nadorcott SL	2	54,5	9,1	1,16	7,9	0,1	T7
2016-05-16	Nadorctt SL	1	58,0	10,2	0,90	11,3	0,0	T5
2016-06-07	Nadorcott SL	1	48,2	12,0	0,87	13,8	0,4	T1-2
2016-04-14	Samba	2	56,4	10,4	0,90	11,5	0,2	T3-5
2016-05-16	Samba	1	57,6	11,6	0,87	13,3	0,4	T1
2016-05-16	Shani SL	1	60,1	12,4	1,64	7,6	0,7	T2-3
2016-06-07	Shani SL	2	50,4	14,1	1,98	7,1	2,0	T1
2016-07-06	Shani SL	1-1x	53,4	13,7	1,33	10,3	2,0	T1
2016-06-07	Tango	2	49,5	12,6	1,09	11,5	0,0	T1
2016-06-07	Valley Gold (B17)	1xxx	51,1	11,6	0,98	11,8	0,7	T1-3
2016-06-07	Winola	1xxx	54,6	12,4	1,32	9,4	0,1	T1
2016-06-07	Yosemite Gold	1xx	55,3	11,9	1,01	11,8	0,0	T1

5 CITRUS IMPROVEMENT SCHEME (CIS)

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5.1 Summary

The purpose of the CIS is to enhance the standard of the South African citrus industry by ensuring that only horticulturally superior plants, which are free of viruses, diseases and pests, are supplied to growers and certified. The Citrus Growers Association of southern Africa (CGA) is responsible for the CIS and delegated

its authority to CRI. In order to achieve this objective, close co-operation is required between CRI, the Agricultural Research Council's Institute for Tropical and Subtropical Crops (ARC-ITSC), DAFF's Directorate of Plant Health (DPH) and citrus nurseries represented by the South African Citrus Nurserymen's Association (SACNA). Additionally, Cultivar and Pathology sub-committees co-ordinate the respective CIS activities. The organisations and committees, as well as all participating role players in the CIS are represented on the CIS Advisory Committee (CISAC), which advises CRI on the CIS operations as specified in its Procedural Guide.

The 2016/17 season exceeded all past records with 6.72 million certified buds supplied to nurseries. This is 49.8% more buds than in 2014/15 and 35.8% more than in 2015/16. Supply was mostly driven by unprecedented demand for lemon and mandarin buds. The demand for mandarins have surpassed lemons and a record of 2.5 million mandarins buds were supplied in 2016/17, compared to the 1.6 million in 2015/16 and 1.8 million in 2014/15. Whilst supply of lemon budwood increased by 6% from 2015/16 only, it was still extraordinary 2.23 million buds supplied in 2016/17. A huge increase in Clementine bud demand was also observed, with 121.4% and 76.4% more buds supplied in 2016/17 (572 thousand) than in 2014/15 and 2015/16, respectively; this is significant considering the 10-year average in 2013/14 was 61 994. The demand for other citrus types increased marginally. The CFB currently holds 360 cultivar lines of which 67% is privately owned or managed. This creates an additional challenge to meet the budwood demand as 47% of total budwood supply is of private cultivars, which must be coordinated with the owner or agent. Despite these challenges, the CFB continues to improve in its central supply objectives by reducing the percentage of certified buds cut in nurseries from 40% in 2012/13 down to 30% rolling average for the past three seasons and 33.4% in 2016/17. CFB also supplies 79% of the industry's rootstock seed demand, and surplus seed of most cultivars are stored as contingency measure. However, lemon demand dramatically increased the demand for Eureka compatible rootstock cultivars and these were in short supply. Rough Lemon and Volckameriana seed had to be imported from USA to meet demand. The CFB's rootstock orchards were expanded by 51% and first crops will be harvested next season. With the high demand for citrus trees, a 50% increase in nursery capacity was observed, with 25 nurseries presently certified by the CIS. Concomitantly, rootstock seed demand was also unprecedented. The phytosanitary status of the CIS is ensured by virus-elimination and diagnostic services prior to CIS introduction and was again confirmed through routine re-indexing of mother trees as well as multiplication blocks. Citrus biosecurity in southern Africa is safeguarded through the CIS activities and the industry's support and use of CIS certified plant material. Initiatives to promulgate the CIS as a compulsory statutory scheme under the Plant Improvement Act are ongoing.

Opsomming

Die doel van die SVS is om die standaard van die Suid-Afrikaanse sitrusbedryf te verbeter deur te verseker dat slegs bome van die beste hortologiese gehalte, wat vry is van virusse, siektes en peste, aan produsente gelewer en gesertifiseer word. Die Sitruskwekersvereniging van suider Afrika (CGA) is verantwoordelik vir die SVS en delegeer sy gesag aan CRI. Ten einde hierdie doelwit te bereik, word noue samewerking tussen CRI, die Landbounavorsingsraad se Instituut vir Tropiese en Subtropiese Gewasse (LNR-ITSG), DAFF se Direktoraat van Plantgesondheid (DPH) en die sitruskwekerye verteenwoordig deur die Suid-Afrikaanse Sitrus Kwekers se Vereniging (SACNA) vereis. Daarbenewens koördineer die Kultivar en Patologie komitees die relevante SVS-aktiwiteite. Hierdie organisasies en komitees, asook al die deelnemende rolspelers in die SVS, word op die SVS Advieskomitee (CISAC) verteenwoordig en adviseer CRI oor die SVS-bedrywighede soos gespesifiseer in die operasionele riglyn.

Die 2016/17 seisoen het alle vorige rekords oorskry en 6.72 miljoen gesertifiseerde ogies is aan kwekerye voorsien. Dit is 49.8% meer ogies as in 2014/15 en 35.8% meer as in 2015/16. Die verskaffing is meestal gedryf deur ongekende aanvraag na suurlemoen en mandaryn ogies. Die aanvraag na mandaryn ogies het dié van suurlemoene verbygesteek en ŉ rekord van 2.5 miljoen mandaryn ogies is in 2016/17 voorsien, in vergelyking met 1.6 miljoen in 2015/16 en 1.8 miljoen in 2014/15. Suurlemoen aanvraag het slegs met 6% gestyg, maar was steeds ongekend met 2.23 miljoen ogies voorsien in 2016/17. ŉ Reuse toename in Clementine aanvraag is ook waargeneem, met ŉ 121.4% en 76.4% meer ogies verskaf as in 2014/15 en 2015/16, onderskeidelik; hierdie is betekenisvol inlig van die 10-jaar gemiddeld van 61 994 in 2013/14. Daar was ŉ marginale toename in die aanvraag na ander sitrus variteitstipes. Die CFB het tans 360 kultivar lyne waarvan 67% in privaat besit is en so bestuur word. Dit skep 'n bykomende uitdaging siende dat 47% van die totale okuleerhout verskaffing, dié van private kultivars is wat met die eienaar of agent gekoördineer moet word. Ten spyte van hierdie uitdagings, het die CFB daarin geslaag om steeds hul doelwit van sentrale verskaffing te verbeter. Die behoefte vir die kwekery om self te sny het van 40% in 2012/13 verminder tot ŉ rollende gemiddeld van 30% oor die afgelope 3 seisoene en 33.4% in die 2016/17 rekordjaar. CFB verskaf ook 79% van die bedryf se aanvraag vir onderstam saad, en die surplus saad van meeste kultivars word gestoor as deel van ŉ gebeurlikheidsplan. Die suurlemoen aanvraag het egter die aanvraag na Eureka-verenigbare onderstam kultivars drasties verhoog en tekorte is ervaar. Growweskilsuurlemoen en

Volckameriana saad is vanaf die VSA ingevoer om in die aanvraag te probeer voldoen. Die CFB se onderstamboorde is 51% vergroot en die eerste drag sal aanstaande seisoen geoes word. Met die hoë aanvraag na sitrus bome, is 'n verhoging van 50% in kwekery kapasiteit waargeneem, met 25 kwekerye wat tans gesertifiseer is deur die SVS. Dienooreenkomstig het die aanvraag na onderstam saad ook ongekende vlakke bereik. Die fitosanitêre status van die SVS word verseker deur virus-reiniging en diagnostiese dienste voor introduksie tot die SVS en word weer bevestig deur roetine her-indeksering van die moederbome en vermeerderingsblokke. Sitrus biosekuriteit in suider Afrika word beveilig deur die SVS-aktiwiteite en ondersteuning deur die bedryf, asook die verskaffing van SVS-gesertifiseerde plantmateriaal. Inisiatiewe om die SVS as 'n verpligte statutêre skema onder die Plantverbeteringswet te promulgeer, word voorgesit.

5.2 Budwood

This report summarises the seasonal supply of budwood from July 2016 to June 2017. A total of 6 721 484 buds were supplied by the Citrus Foundation Block (CFB) and authorised for cutting in certified nurseries. This is 35.8% more buds than in the same period of 2015/16 and 49.8% more buds than in the same period of 2014/15. During this period 6 400 buds were exported to neighbouring countries. Budwood demand generally increased in volume was mostly from Western Cape (33.2%), Limpopo (28.3%), followed by the Eastern Cape (22.8%), Mpumalanga (4.0%) and North West (1.5%). Mandarin (37.5%) was the most popular citrus type, followed by lemon (33.2%), Valencia (10.1%) and Clementine (8.5%); in 2015/16 this proportion was 32.2%, 42.4%, 10.1% and 6.6%, respectively (Tables 5.1.1 and 5.1.2). The top 30 varieties comprised 93.7% of total number of buds supplied. Eureka Lemon was the most popular cultivar for the third consecutive year in 2016/17, followed by Leanri and ARC Nadorcott LS (Table 5.1.3). The very high demand for these two mandarin cultivars could not be predicted. The Leanri demand has increased from 3 030 to 67 506 to 590 633; and ARC Nadorcott LS has increased from 29 160 to 267 422 to 511 582 for 2014/15, 2015/16 to 2016/17, respectively and the majority of these buds was BCIN supplied in 2016/17 (86% and 76.6%, respectively). In general, the need for authorised cutting in nurseries has decreased from 40.0% in 2012/13 to 33.4% in 2016/17 (Figure 5.1.1), which is poorer than 2015/16 (27.8%), largely due to the aforementioned mandarin, which together with 2PH Eureka Seedless lemon comprised 51% of the total number of BCIN buds.

Table 5.2.1. Buds supplied during the period July to June 2014/15-2016/17.

Area	2014/15	2015/16	2016/17
Local	**4 415 748**	**4 939 876**	**6 715 084**
Eastern Cape	944 832	1 141 973	1 531 698
Gauteng			96 400
KwaZulu Natal	38 500	37 530	101 300
Limpopo	1 304 141	1 192 526	1 899 713
Mpumalanga	237 560	316 100	267 210
North West Province	167 900	117 600	99 280
Northern Cape	142 530	352 500	488 866
Western Cape	1 580 285	1 781 647	2 230 617
International	**72 214**	**9 490**	**6 400**
Netherlands			400
Other African States	72 214	9 490	6 000
Total	**4 487 962**	**4 949 366**	**6 721 484**

Table 5.2.2. Buds supplied during the period July to June 2014/15-2016/17.

Area	2014/15	Distribution %	2015/16	Distribution %	2016/17	Distribution %	% Increase on 2015/16
Local	**4 415 748**	**98.4%**	**4 939 876**	**99.8%**	**6 715 084**	**99.9%**	
Eastern Cape	944 832	21.1%	1 141 973	23.1%	1 531 698	22.8%	34.1%
Gauteng		0.0%		0.0%	96 400	1.4%	100.0%
KwaZulu Natal	38 500	0.9%	37 530	0.8%	101 300	1.5%	169.9%
Limpopo	1 304 141	29.1%	1 192 526	24.1%	1 899 713	28.3%	59.3%
Mpumalanga	237 560	5.3%	316 100	6.4%	267 210	4.0%	-15.5%
North West	167 900	3.7%	117 600	2.4%	99 280	1.5%	-15.6%
Northern Cape	142 530	3.2%	352 500	7.1%	488 866	7.3%	38.7%
Western Cape	1 580 285	35.2%	1 781 647	36.0%	2 230 617	33.2%	25.2%
International	72 214	1.6%	9 490	0.19%	6 400	0.1%	

Netherlands		0.0%		0.0%	400	0.0%	100.0%
Other African States	72 214	1.6%	9 490	0.2%	6 000	0.1%	-36.8%
Total	**4 487 962**	**100.0%**	**4 949 366**	**100.0%**	**6 721 484**	**100.0%**	

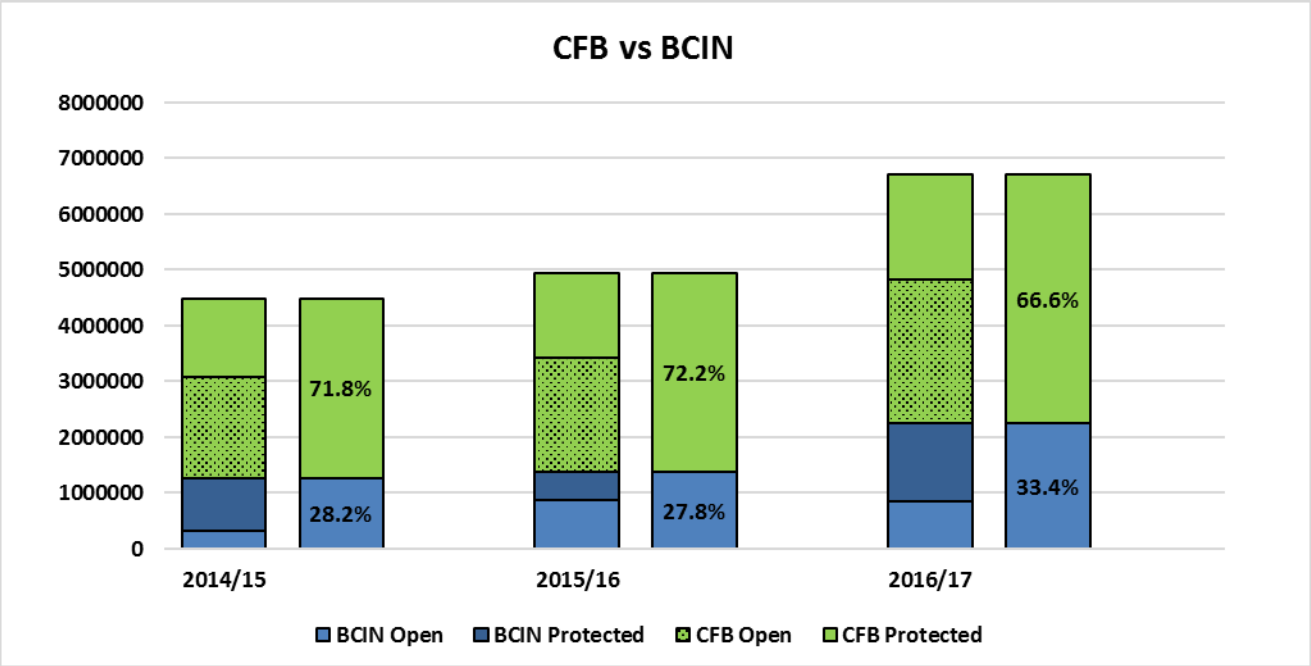

Figure 5.2.1. Budwood (% of total) of open and protected cultivars supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2014/15 to 2016/17.

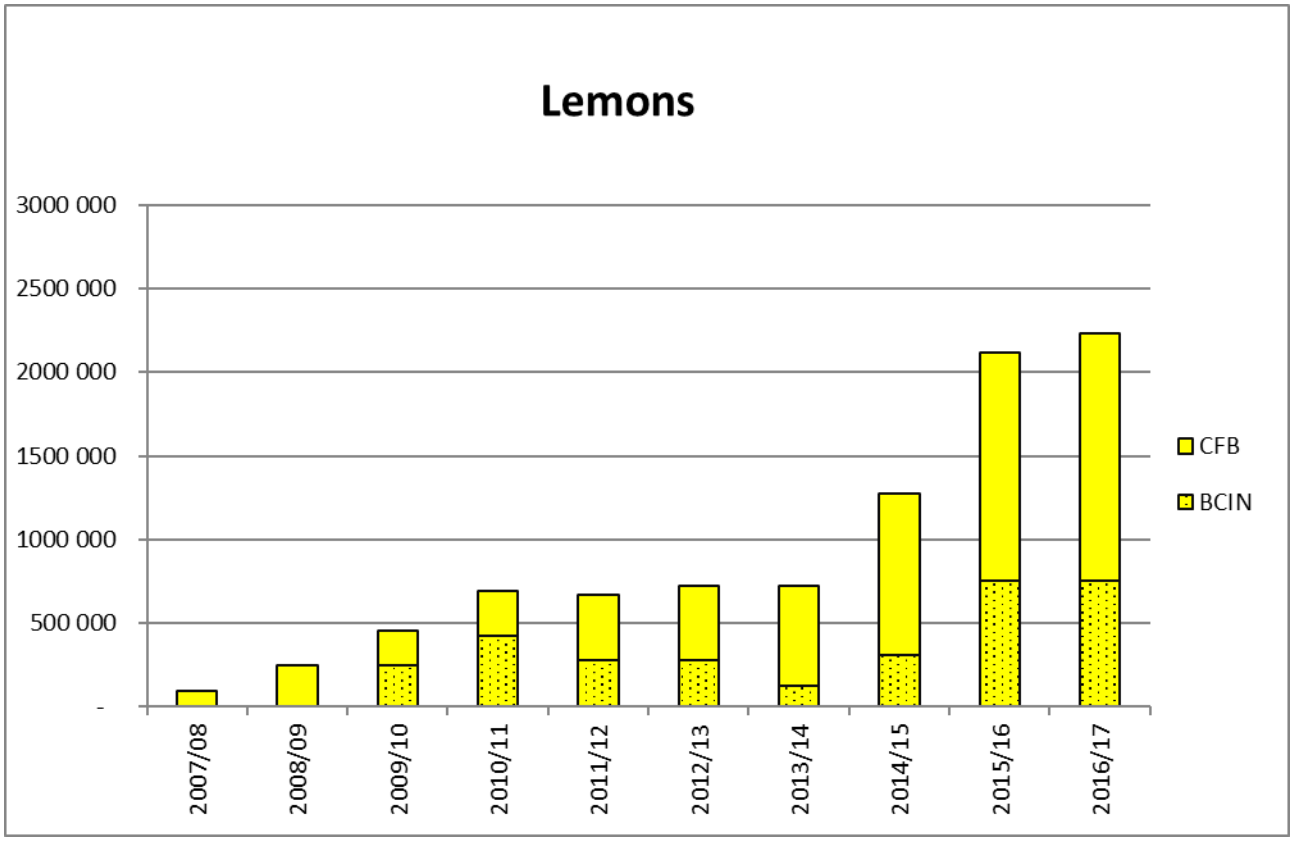

Figure 5.2.2. Lemon budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2007/08-2016/17.

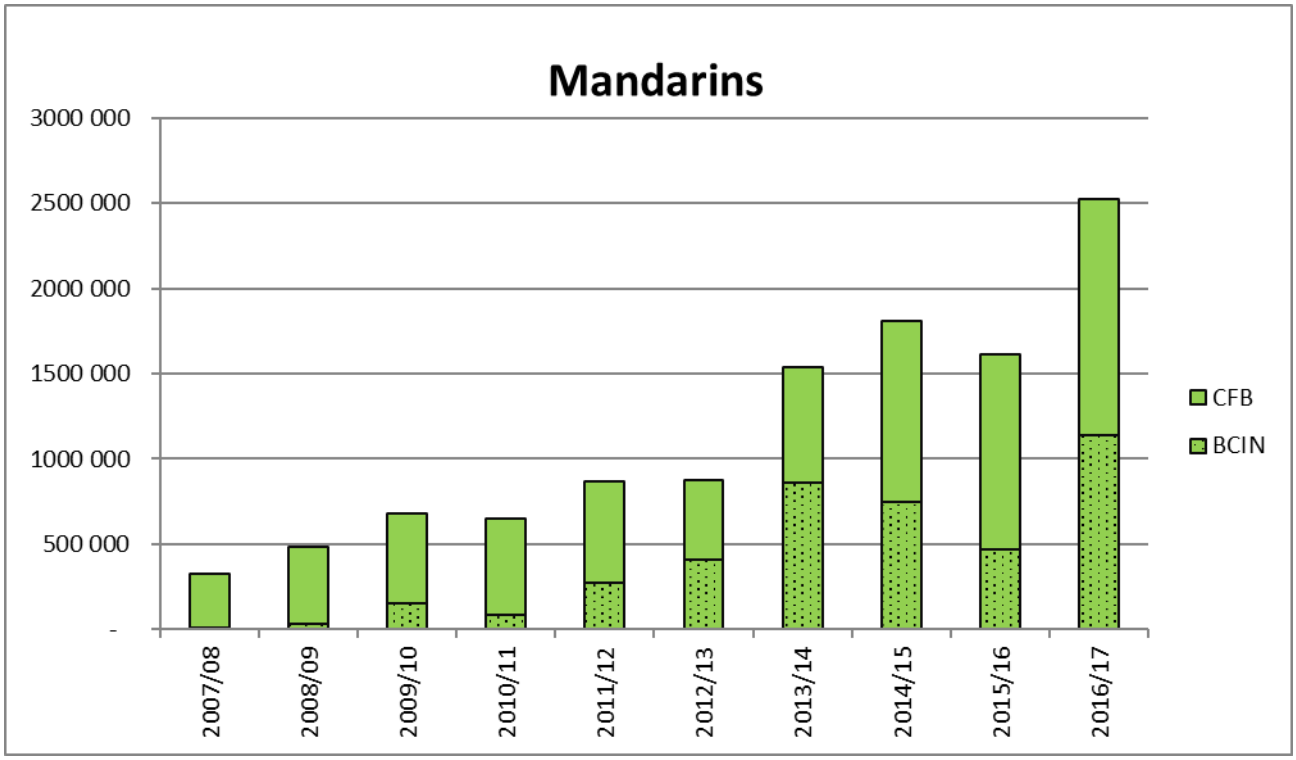

Figure 5.2.3. Mandarin hybrid budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2007/08-2016/17.

Table 5.2.2. Buds supplied per variety type per area (total number of buds per season) during the periods July to June from 2014/15 – 2016/17.

Variety Type	Year	EC	GP	KZN	LIM	MPU	NWP	NC	WC	Local	International	Total
Clementine	2014/15	37 759			29 350	330		7 000	183 981	258 420	4 450	**262 870**
	2015/16	56 601			28 920	1 010	150	26 200	211 566	324 447	1 100	**325 547**
	2016/17	87 133	5 250		34 420	580	2 450	44 400	397 920	572 153		**572 153**
Diverse	2014/15	90			8 300	2 450	2 800		4 445	18 085	14 200	**32 285**
	2015/16	710		1 500	7 480	4 490	7 000		6 723	27 903	240	**28 143**
	2016/17	710		1 000	1 030	3 900	3 000		15 755	25 395	200	**25 595**
Grapefruit	2014/15			1 500	21 050	14 600	3 500		700	41 350	700	**42 050**
	2015/16	660		3 000	31 770	49 650			2 092	87 172	500	**87 672**
	2016/17	15 025	15 500	5 500	65 943	39 815			2 645	144 428		**144 428**
Kumquat	2014/15				5 400	2 100	2 000		2 000	11 500		**11 500**
	2015/16					800			200	1 000		**1 000**
	2016/17	70				1 020			2 950	4 040		**4 040**
Lemon	2014/15	431 061		17 500	358 345	54 490	63 800	56 260	290 626	1 272 082	200	**1 272 282**
	2015/16	733 291		16 000	481 686	123 835	57 000	181 000	501 905	2 094 717	1 500	**2 096 217**
	2016/17	778 248	100	44 000	571 694	89 750	22 700	202 000	519 621	2 228 113	200	**2 228 313**
Lime	2014/15				2 000				4 030	6 030	450	**6 480**
	2015/16	1 550				2 500			4 600	8 650	100	**8 750**
	2016/17	20		1 500	250	2 250			2 700	6 720		**6 720**
Mandarin Hybrid	2014/15	330 685		5 000	406 586	66 285	20 500	47 450	931 797	1 808 303	600	**1 808 903**
	2015/16	233 686		2 500	248 980	65 380	17 300	109 100	914 224	1 591 170	750	**1 591 920**
	2016/17	384 259			903 871	85 710	30 580	213 466	902 562	2 520 448	1 000	**2 521 448**
Midseason	2014/15	20							4 958	4 978		**4 978**
	2015/16					15			3 368	3 383		**3 383**
	2016/17	50							9 692	9 742		**9 742**
Navel	2014/15	113 999		6 000	123 150	22 050	19 950	14 500	82 022	381 671	21 036	**402 707**
	2015/16	74 288		8 500	109 815	31 725	29 650	9 900	46 180	310 058	3 900	**313 958**
	2016/17	141 022	37 050	8 500	75 805	13 455	20 650	17 800	143 441	457 723	2 000	**459 723**
Pummelo	2014/15				2 400				20	2 420		**2 420**
	2015/16				4 400	15				4 415		**4 415**
Satsuma	2014/15	8 825		1 000	4 700	3 510	1 000	6 700	48 269	74 004		**74 004**
	2015/16	6 852		3 000	1 900	685	500	1 400	34 476	48 813	1 400	**50 213**
	2016/17	12 234			10 250	1 155	500	4 700	37 744	66 583	1 000	**67 583**

Valencia	2014/15	22 393		7 500	342 860	71 745	54 350	10 620	27 437	536 905	30 578	**567 483**
	2015/16	34 335		3 030	277 575	35 995	6 000	24 900	56 313	438 148		**438 148**
	2016/17	112 927	38 500	40 800	236 450	29 575	19 400	6 500	195 587	679 739	2 000	**681 739**

Table 5.2.3. Top 30 cultivars based on total number of buds supplied for seasons July to June from 2014/15 – 2016/17.

	2014/15			**2015/16**			**2016/17**		
	Cultivars	**BCIN**	**CFB**	**Cultivar**	**BCIN**	**CFB**	**Cultivar**	**BCIN**	**CFB**
1	Eureka LEM	192 173	553 967	Eureka LEM	659 875	787 971	Eureka LEM	498 227	936 185
2	Nadorcott 1 MAN	276 805	294 737	Tango MAN	131 000	214 930	Leanri MAN	509 643	82 990
3	Tango MAN	293 746	163 335	Lisbon LEM	55 325	234 366	ARC Nadorcott LS MAN	390 532	119 050
4	Midknight VAL		243 515	ARC Nadorcott LS MAN	210 755	41 852	2PH Eureka SL LEM	250 755	138 482
5	Lisbon LEM	27 100	188 122	Nadorcott 1 MAN	8 500	232 512	Midknight VAL	5 000	376 247
6	Nules CLE	90 961	99 460	Midknight VAL	10 000	197 662	Nules CLE	229 281	149 535
7	Witkrans 3 NAV	54 303	111 111	Nules CLE	93 422	108 503	Nadorcott 1 MAN	26 000	278 555
8	Or 4 MAN		161 335	Or 4 MAN	36 600	143 576	Or 4 MAN	89 610	191 596
9	2PH Eureka SL LEM	87 912	69 335	Valley Gold MAN		165 025	Nova MAN		266 245
10	Valley Gold MAN*	25 660	130 421	Nova MAN		150 151	Lisbon LEM		209 289
11	Nova MAN		139 354	Limoneira 8A LEM	8 200	141 475	Tango MAN	26 791	160 320
12	Late VAL		116 178	2PH Eureka SL LEM	14 500	125 410	Clemenluz CLE	20 244	127 320
13	RHM (Royal Honey) MAN	82 200	2 310	Witkrans 3 NAV	9 600	114 085	Cambria 3 NAV	9 200	133 291
14	Limoneira 8A LEM		84 024	Clemenluz CLE	21 600	75 138	Valley Gold MAN		109 484
15	Mor 26 MAN		78 005	Late VAL		75 210	Star Ruby GFT	1 100	105 058
16	Genoa LEM		62 743	Genoa LEM	3 900	62 448	Witkrans 3 NAV		101 336
17	Alpha VAL	7 200	48 065	Leanri MAN	41 100	24 391	Limoneira 8A LEM		99 219
18	Clemenluz CLE	23 469	29 850	Gold Nugget MAN	4 100	43 904	IR M2 MAN	58 610	21 309
19	Cambria 3 NAV		46 873	Delta VAL		40 081	Cara Cara NAV	45 502	28 680
20	IR M2 MAN	38 800	850	Cara Cara NAV	13 800	25 615	Genoa LEM	4 300	68 281
21	Bahianinha NAV		35 950	FE 1 (Jackson 1) GFT		35 135	Late VAL		67 365
22	Empress MAN	3 000	30 900	Mor 26 MAN		30 231	Queen MAN		64 240
23	Washington NAV		32215	Cambria 3 NAV		29 418	Bennie 2 VAL		55 650
24	Autumn Gold NAV	23 219	7 436	Star Ruby GFT		28 825	Turkey VAL		46 612
25	Delta VAL		30 323	Turkey VAL		28 415	Tambor MAN	33 116	5 130
26	ARC Nadorcott LS MAN**	20 800	8 360	Belabela SAT		26 360	Lavalle VAL		36 870
27	Belabela SAT		28 450	Palmer NAV		24 276	Belabela SAT	7 415	27 779
28	Gusocora (G5) VAL		28 385	Bennie 2 VAL		22 200	Esbal CLE	20 700	8 362
29	Bearss LIME		27 825	Queen MAN		20 362	Mor 26 MAN		27 248
30	Bennie 2 VAL		27 200	Washington NAV		20 013	Washington NAV		27 006
	Top 30	**1 247 348**	**2 880 634**	**Top 30**	**1 322 277**	**3 269 540**	**Top 30**	**2 226 026**	**4 068 734**

	> Top	**17 000**	**342 968**	**> Top**	**51 731**	**305 818**	**> Top**	**21 398**	**405 326**
	Total	**1 264 348**	**3 223 602**	**Total**	**1 374 008**	**3 575 358**	**Total**	**2 247 424**	**4 474 060**

* ARCCIT1614 (B17) (Valley Gold) MAN ** ARCCIT9 (ARC Nadorcott LS) MAN

5.3 Seed

During May to April 2017, 5593 litres of seed were supplied locally (Table 5.2.1) and 277 litres of seed were exported (Table 6.2.1). Carrizo Citrange remains the most popular rootstock (43.7%), followed by C35 Citrange (17.8%), Swingle Citrumelo (15.8%), Rough Lemon (8.6%) and X639 (4.1%). The supply did not meet the demand for Eureka compatible rootstocks (Table 6.2.1) and CRI imported Rough Lemon (RL) and Volckameriana (VA) on behalf of nurseries from USA. This seed was, however, defective and very poor germination was reported by nurseries (0% for VA and 19.3% for RL). In the order of 400 litres of seed were also imported directly by nurseries from USA (California) and Australia.

Table 5.3.1. Seed (litres) supplied by the CFB during the periods May to April 2014/15 – 2016/17.

Seed Supply	**2014/15**	**2015/16**	**2016/17**
Local	**4128**	**4856**	**5316**
Eastern Cape	649	1268	628
Gauteng		40	25
KwaZulu Natal	35	36	27
Limpopo	2160	1467	2055
Mpumalanga	75	473	343
North West Province	98	125	197
Northern Cape	157	267	362
Western Cape	954	1180	1680
International	**527**	**376**	**277**
Australia/NZ	15		
Botswana		2	2
Chile	316	111	201
Congo	12	4	
Egypt		9	
Mozambique		31	60
Portugal	140	213	
Reunion	10	10	
Swaziland			7
United Arab Emirates	6		
Zambia	26	-5	7
Zimbabwe	2	1	
Total	**4655**	**5232**	**5593**

Table 5.3.2. Seed (litres) supplied by the CFB during the periods May to April 2014/15 – 2016/17.

Rootstock Cultivar	**2014/15**	**2015/16**	**2016/17**
CFB	**4655.45**	**4045.77**	**5453.70**
79AB	3	0	6
79AC	1	0	10
BC		19	20
C35	670	575	993
CC	1980	1906	2446
CM	14		
FD	353	213	25
MXT	146	162	222
RL	461	334	483
SC	543	347	883
SXB		12	32
TC	2	13	
VA	58	125	93
X639	414	339	227
YC	10		15
Imported		**231**	**139**
SPIN *	Not determined	**955**	Not determined
Total	**4655**	**5232**	**5593**

*Seed produced in nurseries

5.4 Production

Budwood: CFB presently maintains 133 367 multiplication trees of approximately 361 cultivar lines with a potential annual budwood stock of >10 million buds. The STG facilities in Nelspruit, CRI has released 13 new and 8 existing cultivars to the CFB. The ARC also introduced 7 new cultivars and re-introduced 7 existing cultivars to the CFB. As the top 30 varieties comprise 93.7% of demand, multiplication tree stocks are being managed in order for CFB to be timeously able to supply demand of the sought-after varieties. During 2016/17 17 103 new multiplication trees were made in the rapid multiplication tunnels to address the shortage of high demand cultivars. Whilst CFB's multiplication system is addressing the budwood demand in the medium term (1-2 year lag phase), it was not sufficient to rapidly increase budwood stock to address short-term demand; for example, Nules Clementine, Leanri Mandarin and ARC Nadorcott LS Mandarin, for which the huge demand experienced in 2016/17 was not foreseen. CFB is investigating *in vitro* rapid multiplication systems as a potential solution. To address space constraints, 10 504 multiplication trees of low-demand cultivars were removed. All 4 multiplication blocks that tested positive in the re-indexing by direct PCR (see below) were also removed.

Two trees of 311 cultivars were planted out during October 2016 in newly established evaluation block at the CFB. These trees will be used for true-to-type evaluation. Newly released cultivars will be planted out during the following season.

Table 5.4.1. Cultivar introductions from 2014/15 – 2016/17.

Area	**2014/15**	**2015/16**	**2016/17**
ARC: New introductions	17	15	7
ARC: Existing lines with new CTV Strain			7
CRI: New introductions	18		13
CRI: Reintroductions from the Nucleus Block			8
CFB: Re-multiplication of existing cultivars	13	58	35
Total	**48**	**73**	**70**

Seed: As contingency and maintenance measure the CFB seed source was expanded from 2420 trees by planting another 1362 trees in 2014/15. These trees were planted at high density and will significantly contribute to the source in 2017/18 and 2018/19. Thereafter, every other tree will be removed or transplanted. Two nurseries have also requested to plant rootstock cultivars for seed supply. In 2016/17 CFB's seed stocks were completely sold out of popular rootstock cultivars. A reassessment will be made of CFB's seed orchards, particularly with regard the older orchards (replacement or heavy pruning options will be investigated). Another 318 rootstock trees of high demand and experimental cultivars were made and will be planted in the spring of 2017.

5.5 Tree Certification

There were 4,080,246 trees certified during April to March 2016-17. A high number of applications were received and were processed. Of the applications received, the trees not meeting the certification requirement were 28282, 64575 and 65523 for the last three consecutive years. This was mostly because of the Phytophthora status or tree age that exceeded 30 months after budding. The trees not meeting the certification requirements have increased slightly with 1% on the previous year. Nurseries are required to apply for certification for all trees supplied to industry, and in future the percentage of trees certified as a proportion of the total number of buds received will be used as a nursery certification criterion.

Table 5.5.1. Trees certified during the period April to March from 2014/15 – 2016/17.

Variety Type	Year	EC	GP	LIM	KZN	MP	NW	NC	WC	Exported	Total
Clementine	2014/15	3 780	310	670	2 200	20	2 334		24 711	3 647	37 672
	2015/16	19 144		7 215		2 060			21 079		49 498
	2016/17	27 459		30 898		10 470	1 700		111 526		182 053
Diverse	2014/15		300	523	1 500	2 533			500	300	5 656
	2015/16	1 355		3 800	200	1 441					6 796
	2016/17	1 000		7 800	1 140	1 750	4 600		2 536		18 826
Grapefruit	2014/15	626		4 874	1 112	9 014			500	100	16 226
	2015/16	2 300	12 735	17 930		17 270				6 460	56 695
	2016/17			23 939	10 000	7 484	2 300			1 000	44 723
Lemon	2014/15	154 667		126 672	16 520	34 403	12 407	600	60 695	14 940	420 904
	2015/16	312 660	8 000	127 102	7 500	106 660	9 530		47 009	7 935	626 396
	2016/17	501 011	2 465	381 298	25 970	207 138	11 620		342 264	7 300	1 479 066
Mandarin Hybrid	2014/15	204 757		109 015	5 150	153 123	59 460	22 247	158 071	21 064	732 887
	2015/16	172 946		126 454	1 020	285 210	13 680	3 320	263 909	13 640	880 179
	2016/17	290 737	1 000	352 355	610	168 881	23 370	4 150	667 244	1 784	1 510 131
Navel	2014/15	105 957		62 790		42 056	6 520	3 600	99 455	19 825	340 203
	2015/16	92 165		26 075	2 000	33 675	1 826	10 100	79 911	2 175	247 927
	2016/17	104 296		171 090		36 636	4 960	2 710	39 751	1 550	360 993
Satsuma	2014/15	20 240	4 010	5 703		1 458			18 957	717	51 085
	2015/16	7 429		3 154		2 350			13 530		26 463
	2016/17	3 173				1 400		10 000	22 071		36 644
Valencia	2014/15	18 213	330	236 375	520	84 030	19 305	6 410	33 500	37 295	435 978

	2015/16	65 659	12 735	168 950	300	68 645	1 450		21 430		339 169
	2016/17	55 243		286 428	12 364	44 829	12 300		33 276	3 370	447 810
Total		**2 164 817**	**41 885**	**2 281 110**	**88 106**	**1 322 536**	**187 362**	**63 137**	**2 061 925**	**143 102**	**8 353 980**

Table 5.5.2. Trees not meeting the certification criteria during the period April to March from 2014/15-2016/17

Tree Certification	**Year**	**EC**	**GP**	**KZN**	**LIM**	**MP**	**NW**	**NC**	**WC**	**EXP**	**Total**
Certified	2014/15	508 240	4 950	27 002	546 622	326 637	100 026	32 857	396 389	97 888	2 040 611
	2015/16	673 658	33 470	11 020	480 680	517 311	26 486	13 420	446 868	30 210	2 233 123
	2016/17	982 919	3 465	50 084	1 253 808	478 588	60 850	16 860	1 218 668	15 004	4 080 246
Not Certified	2014/15	13 216			5 390				9 676		28 282
	2015/16	38 753						4 729	21 093		64 575
	2016/17	8 880	563		42 310	3 000	2	499	10 219	50	65 523

5.6 Nursery Certification

Twenty-eight (28) nurseries were visited during the April-June 2016 audits. Twenty-six nurseries were certified, while 2 nurseries were provisionally certified. During the audit random media samples from *Phytophthora* sensitive rootstocks were collected. Of the 73 samples submitted to the diagnostic centre, 10 tested positive (13.7%).

Thirty (30) nurseries were visited during November-December 2016 audits. Twenty-six were certified, two were provisionally certified and two new nurseries were certified. Of the 83 samples submitted to the diagnostic centre, 14 tested positive (16.9%).

Twenty-nine (29) nurseries were visited during the May 2017 audits. Twenty-two nurseries retained their certification status, while four nurseries were provisionally certified and another three new nurseries were provisionally certified. One nursery has delayed their inspection, while another is relocating to new premises. Upon completion of the outstanding requirements, the provisionally certified nursery may be fully certified. 93 samples were submitted to the diagnostic centre, of which 26 tested positive (27.9%).

Table 5.6.1: CIS Certified Nurseries in May 2017

Nursery	Town / Province		Contact Person	Tel	Cell	Email
Apapanzi Kwekery	Kirkwood	EC	Nellis Meiring	042 230 1483	082 550 6210	nellis@srvalley.co.za
Atwell Citrus Nursery	Kirkwood	EC	Wayne Attwell	042 230 1560	072 463 7118	attwellcitrus@srvalley.co.za
Augsburg Kwekery	Clanwilliam	WC	Alta Laing	082 952 8127	079 527 0316	admin@augsburgnursery.co.za
BF Joubert Kwekery	Kirkwood	EC	Francois Joubert	042 230 0309	084 951 1922	bfjkweek@srvalley.co.za
Casmar Kwekery	Mooinooi	NW	Neville Wenhold	014 574 3152	082 881 4189	casmarnursery@absamail.co.za
Cederberg Tree Nursery	Citrusdal	WC	Patricia Willemse	022 921 3526	076 622 7007	info@cederbergtreenursery.co.za
Du Roi Kwekery	Letsitele	LP	Mariska Benn	015 345 1650	072 475 5568	mariska@duroi.co.za
Esselen Kwekery	Malelane	MPU	Leon Esselen	013 790 0160	083 325 0565	esselenk@mweb.co.za
Gamtoos Kwekery	Patensie	EC	Keuler Engela	042 283 0506	072 260 9813	keuler@rikusld.co.za
H J Joubert Kwekery	Montagu	WC	Herman Joubert	023 614 2237	082 578 5747	hopewell@breede.co.za
Henley Citrus (Letsitele)	Letsitele	LP	La-Ruscha Strydom	015 386 0211	063 292 7109	larushca@bigday.co.za
Henley Citrus (Welverdiend) **	Haenertsburg	LP	Callie Wagenaar		071 003 6080	callie@bigday.co.za
Letsitele Kwekery	Letsitele	LP	Barend Vorster	015 345 1600	083 259 5590	barend@mahela.co.za
Mabu Zest *	Bapsfontein	GP	Dr. Linda Meyer		082 374 7707	linda@mabucasing.co.za
Mistkraal Nursery	Kirkwood	EC	Tyna Ferreira	042 230 0614	082 789 5150	beans@srvalley.co.za
Moorland Seedlings **	Loerie	EC	Rian Moore	042 286 0605	082 2860 604	info@moorland.co.za
Groot Patrysvlei Kwekery	Clanwilliam	WC	Helgard Smit	027 482 2619	084 524 7417	nursery@capespanfarms.co.za
Ngwenya Kwekery	Malelane	MPU	Milanie v/d Merwe	013 790 3004	082 418 7693	milanie@riversidefarm.co.za
Oase Sitrus Kwekery *	Hartswater	NC	Gerrit Schlebusch	053 474 2080	082 907 1562	oasekwekery@lantic.net
Oranjerivier Sitrus Kwekery	Kakamas	NC	Blom Rossouw	054 441 0183	083 306 0622	osk@vodamail.co.za
Parma Kwekery **	Hoedspruit	LP	Albert Horn	087 806 5649	072 022 4356	parma@global.co.za
Paksaam Kwekery	Patensie	EC	Michael J. van Rensburg	042 283 0201	063 776 1347	paksaam@gamtoos.co.za
Rietvlei Kwekery	Tzaneen	LP	Lucas McLean	083 630 3236	083 630 3236	rietvlei@global.co.za
Sondagsrivier Hillside Kwekery	Kirkwood	EC	Willem Truter	042 230 0349	083 227 6655	willem@srvalley.co.za
Stargrow Kwekery	Citrusdal	WC	Hennie Prins	022 921 2232	084 563 4412	hennie@stargrow.co.za

Sundays' River Citrus Nursery *	Kirkwood	EC	Riaan Slabbert		072 184 8726	srcnursery@igen.co.za
Tulbagh Kwekery	Tulbagh	WC	Bredell Roux	023 230 0694	082 214 2520	admin@tulbaghnursery.co.za
Tweeling Kwekery *	Kirkwood	EC	Jan Potgieter	042 230 1408	082 560 2179	tweeling@srvalley.co.za
Waterfall Nursery	Adelaide	EC	Rudi van der Meulen	046 684 0738	082 695 3433	waterfall@intekom.co.za
Witkrans Kwekery	Boshoek	NW	Linda Grobler	014 573 3036	082 414 4739	Witkrans1@mweb.co.za

* Provisionally certified
** Provisionally certified – New Nursery

5.7 Statutory Improvement Scheme

The statutory CIS proposal was extensively discussed and debated in meetings with all participating citrus nurseries, a retail nursery, cultivar management companies and growers. A status document stating the benefits and detriments of a voluntary or compulsory statutory improvement scheme, including summarised feedback and inputs from all stakeholders, was discussed at a public workshop facilitated by the NAMC on 9 April 2014. The workshop was attended by 38 persons representing stakeholders, including growers, SACNA, nurserymen, cultivar managers, CGA, CRI and DAFF representatives. The workshop debated matters arising from the consultation process on which more clarity or consensus was required. The NAMC meeting concluded, as was reported in 2013/14, that a compulsory scheme offered the most advantages as well as protection from biosecurity risks for the citrus industry in South Africa, but that the needs of all role players including those not supportive of a compulsory scheme should be considered. Subsequently, meetings were also held with private cultivar managers and SACNA, of whom certain members opposed a compulsory scheme, as well as the ARC who did not attend the workshop. The issues raised by the ARC in its initial opposition of the proposal have been resolved on operational level, but official notification from ARC management is still pending. This issue is receiving ongoing attention. Another important outstanding item in the statutory CIS proposal is the Memorandum of Understanding that must be signed by the Minister of DAFF and the designated authority, CGA. This has been drafted and will be discussed with stakeholders.

5.8 Protective zone surrounding the Citrus Foundation Block

The legislation, declaring a radius of 5 km around the CFB as a citrus free area, was published in the Government Gazette on 21 January 2011. Orders to remove all citrus trees were issued by DAFF. Most residents have removed their citrus trees. DAFF has made several follow-up visits to owners refusing to remove trees. Two owners have agreed to either have their trees moved or destroyed; this has been done, and there is only one owner who still refuses to remove his citrus trees. DAFF is following up on this case.

5.9 Shoot tip grafting (STG), pre-immunisation and nucleus block management and diagnostic services

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Summary

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. Shoot tip grafting (STG) is used to eliminate graft transmissible pathogens from citrus material before release to the Citrus Foundation Block (CFB) and introduction into the Nucleus Block. Biological and molecular indexing is done on new introductions prior to release to the CFB as well as on accessions maintained at the CFB to establish whether graft transmissible disease agents are present that have been inadvertently introduced. Mother trees maintained at the CFB are indexed every two years on a rotational basis to confirm the presence of *Citrus tristeza virus* (CTV) as introduced by the CTV pre-immunisation programme and also to monitor for the presence of citrus viroids. General diagnostics and investigations into *ad hoc* problems or outbreaks, potentially relating to graft transmissible diseases are also conducted. The ongoing activities of these CIS functions are reported. Twelve new selections (combined totals for ARC and CRI) were received for STG and 11 were released to the CFB and added to the gene source. The gene source maintained at CRI currently comprises 365 accessions and these plants were indexed for viroids and CTV. Eight samples tested positive and will be re-submitted for STG. The biological evaluation of 224 mother trees of the CFB, to assess their CTV status, was concluded. No indications of severe stem pitting were found, but 67 trees of various soft citrus cultivars, did not contain CTV. A selection of soft citrus samples from the multiplication blocks were also screened for the presence of CTV. Fifty two percent of these samples did not contain CTV. These results indicate that the CTV cross-protection for soft citrus cultivars needs to be reconsidered. The screening of the

742 CFB multiplication blocks for viroids was completed and 1240 samples were processed. Sixteen viroid-positive multiplication blocks were detected and were removed.

Opsomming

Die sukses van die Sitrusverbeteringskema (SVS) berus op ŉ fitosanitêre program wat op ŉ diagnostiese opsporing van die teenwoordigheid van skadelike patogene gebaseer is. Die SVS behels die eliminering van die patogene en die onderhou en verspreiding van gesonde voortplantingsmateriaal. Groeipuntenting (GPE) word gebruik om sitrus materiaal te vrywaar van ent-oordraagbare patogene voor vrystelling aan die Grondvesblok (GVB) en toevoeging tot die genebron. Biologiese en molekulêre indeksering word gedoen op nuwe toevoegings, voordat die materiaal aan die GVB verskaf word, asook op moeder- en vermeerderingsbome wat by die GVB onderhou word, om te verseker dat daar nie besmettings per ongeluk plaasgevind nie. Die moederbome by die GVB word op ŉ rotasie basis elke tweede jaar geherindekseer om die teenwoordigheid van *Citrus tristeza virus* (CTV) soos deur die kruisbeskermings program toegevoeg, te evalueer en ook om moontlike besmetting van sitrus viroïede op te spoor. Algemene diagnostiese dienste en ondersoeke na probleme en uitbrake t.o.v. ent-oordraagbare siektes in die industrie word op ŉ ad hoc basis gedoen. Verslaggewing oor hierdie deurlopende funksies van die SVS word gedoen. Gedurende die jaar is 12 nuwe seleksies ingedien vir GPE (gekombineerde totale van LNR en CRI) en 11 vrygestel aan die GVB en genebron. Die genebron by CRI bevat tans 365 kultivars en hierdie plante is vir viroïede en CTV geïndekseer. Agt monsters het positief getoets vir viroïede of CTV en sal weer vir GPE ingedien word. Die biologiese evaluering van 224 moederbome van die CFB, om hul CTV status te evalueer, is afgesluit. Geen strawwe stamgleuf is op die indikatore nie gevind, maar 67 bome van verskillende sagte sitruskultivars het nie CTV bevat nie. Verskeie sagte sitrus monsters van die vermeerderingsblokke is ook getoets vir die teenwoordigheid van CTV. Twee en vyftig persent van hierdie monsters het nie CTV bevat nie. Hierdie resultate dui aan dat die CTV kruisbeskerming vir sagte sitruskultivars heroorweeg moet word. Sitrus viroïed indeksering van 742 GVB vermeerderingsblokke is voltooi en 1240 monsters is verwerk; sestien viroïed-positiewe vermeerderingsblokke is opgespoor en is verwyder.

Introduction

As with any commercial tree crop, citrus species are susceptible to various graft transmissible diseases (GTD) caused by viruses, viroids, bacteria, phytoplasmas and unidentified pathogens. 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. 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). 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 to mitigate the effects of severe *Citrus tristeza virus (*CTV) stains is a function of the CIS where specific 'pre-immunising' CTV sources are applied to all citrus cultivars, apart from lemons and limes, before supply to the Citrus Foundation Block (CFB) at Uitenhage.

Indexing, or establishing whether GTD disease agents are present in plant material, is primarily done by inoculating indicator host plants, sensitive to various graft transmissible pathogens. Molecular and serological detection techniques such as Reverse-Transcription Polymerase Chain Reaction (RT-PCR), PCR and ELISA are used to confirm biological indexing results.

Since CTV and its vector, *Toxoptera citricida*, is endemic in South Africa, virus-free material is pre-immunised with a suitable cross-protection source to mitigate the effects of severe CTV strains (Müller & Costa, 1987). Currently three CTV sources are used for cross-protection in the southern African CIS depending on the citrus type (von Broembsen & Lee, 1988; van Vuuren *et al.*, 1993a; van Vuuren *et al.*, 1993b; van Vuuren *et al.*, 2000). The STG and pre-immunisation procedures have been adapted to suite South African conditions (Fourie & van Vuuren, 1993).

Re-indexing of the mother trees at the CFB is done to ensure these trees remain free of graft transmissible

pathogens and that the CTV sources introduced, remain mild within these cultivars. Indexing for CTV and viroids are done biennially. Screening for other GTD such as *Citrus psorosis virus* (CPsV), *Apple stem grooving virus* (ASGV) and Citrus Impietratura disease (CID) are done every 10 years.

Objectives

A. Cultivar introduction (administration, establishment, STG, diagnostics, cross-protection and CFB and nucleus block submission)
B. Maintenance of the virus-free gene source
C. Biological and molecular re-indexing of mother trees and multiplication blocks at the CFB
D. Collaboration and duplicate indexing with ARC-ITSC laboratory
E. *Ad hoc* diagnostics for GTDs for growers and external institutions
F. *Ad hoc* investigations as required by CIS

Materials and methods

A. Cultivar introduction (administration, establishment, STG, diagnostics, cross-protection and submission to CFB and nucleus block)

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 or Rough lemon 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 in an incubator at a constant temperature of 28ºC in continuous darkness. When the seedlings have reached a height of 30 to 40 mm, they are stored at 4ºC in darkness.

Scion preparation: Method 1; buds of the source plant are budded on a standard rootstock in the glasshouse. After bud growth 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 or agar medium. The cultures are incubated at 32ºC 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 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 28ºC exposed to 16 h light/day.

STG plant increase. The shoot tip normally 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. RT-PCR is used to detect CVd, CTV, CPsV and ASGV. PCR is used to detect the bacterial pathogen causing citrus greening.

On average it takes 24 to 30 months to obtain a virus-free STG followed by 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.

B. Maintenance of the virus-free gene source

Virus-free STG plants are multiplied on virus-free rootstocks and maintained in an insect-free tunnel. Material derived from the gene source is multiplied and pre-immunised with suitable CTV cross-protection sources (van Vuuren and Collins, 1990), prior to release to the CFB at Uitenhage. 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. Photo records of fruit from each cultivar/selection are kept on the data-base to assist with the true-to-type evaluations.

C. Biological and molecular re-indexing of mother trees and multiplication blocks at the CFB

The population structure of *Citrus tristeza virus* (CTV) sources used for pre-immunisation can change by the segregation of strains or by re-combination events. These events may be induced by environmental conditions such as high temperatures or other factors such as host influences. All trees are therefore re-indexed every second year to establish the severity status of the CTV present.

Citrus Viroids (CVd) are mechanically transmitted by grafting and contaminated cutting tools, but are not vectored by insects. Re-indexing for CVds follows the CTV re-indexing schedule and is done every second year.

All CFB mother trees and seed source trees are inspected annually for symptoms of citrus greening disease by ITSC and CRI Virologists. PCR and/or biological indexing are conducted on plants showing suspicious symptoms.

Most other citrus viruses are transmitted by infected bud wood only, minimizing the infection potential at the CFB. Re-indexing is therefore done only every 10 years for ASGV and CPsV.

Screening of the multiplication blocks for the presence of viroids is done by direct RT-PCR of pooled samples of each cultivar. Each pooled sample consists of 20 leaves. A leaf is taken from every third tree and a sample is therefore representative of a block of 60 trees. Each cultivar is sampled separately and the number of sub-samples of a cultivar is proportional to the size of the block. Each sample is first tested with 2 group specific viroid primer sets, Apsca and non-Apsca. If a sample tests positive in either of these tests, viroid-specific tests are done to determine the specific viroid species present. If weak signals are obtained with the initial tests and are not confirmed by viroid specific tests, the specific accessions are resampled and retested.

D. CIS collaboration with ARC-ITSC

Shoot tip grafting for the CIS is done at both the CRI and ARC-ITSC laboratories. To confirm the pathogen-free status of new accessions prior to release to the CFB, duplicate molecular testing is performed.

E. Samples received for diagnostics from growers and external institutions

Field material received for diagnostics is generally budded on 3 indicator host plants. The plants are cut back to force new growth and maintained in glasshouses at various temperatures required for symptom expression depending on the disease being indexed. The indicators are monitored for symptoms for a minimum of 3 months post inoculation. Molecular or serological tests are performed as a confirmation of any biological result. Direct molecular tests are also done, depending on the diagnostic requirement.

F. *Ad hoc* investigations as required by CIS

Problematic disorders of unknown aetiology or sudden outbreaks are occasionally encountered and require investigation. Investigations may include biological and molecular indexing for the presence of graft transmissible diseases, surveys, trials or other analyses.

Results and discussion

Objective / Milestone	Achievement
Cultivar introduction (administration, establishment, STG, diagnostics, cross-protection and CFB and nucleus block submission)	Ongoing: - 30 accessions still in progress - 12 new selections received (6 CRI, 6 ARC) - 11 submissions released to the CFB
Maintain the virus-free gene source	Ongoing: 365 cultivars maintained CRI gene source indexed for CTV & CVd
Biological and molecular re-indexing of mother trees and multiplication blocks at the CFB	Mother trees were indexed for CTV and viroid indexing of CFB multiplication blocks completed
CIS collaboration with ARC-ITSC	CFB releases were tested at both facilities for various pathogens

Requests from growers and institutions to diagnose suspected material for GTD	Approx. 150 analyses conducted for various clients
Ad hoc investigations as required by CIS	Various tests conducted and assistance provided in the chimera investigation

A. Cultivar introduction for STG

Introductions for STG and subsequent releases to the CFB from 2012 to date are summarised in Table 5.9.1. Six new selections of five variety types were submitted for STG in the current year. At the end of this report period, 30 accessions are at various stages in the STG pipeline. A total of 172 STGs were done within this period, including failed grafts and 21 were successfully micro-grafted.

The ARC facility also received 6 accessions for STG, 4 from international sources and 2 local introductions.

To facilitate a faster turn-around with the STG process, new introductions are tested directly with PCR prior to STG to determine the original pathogen status and then again directly after STG as soon as sufficient material is available for testing. These additional steps allow quicker detection of pathogens not eliminated by the initial STG step. Re-STG can therefore commence quicker rather than waiting for completion of the biological indexing. This process does, however, not replace the final biological indexing and PCR to confirm the pathogen free status prior to final release of the accession. These additional tests are routinely done and the number of tests conducted is not reported.

Eight successful STGs were biologically indexed for CTV, ASGV and CVd. Seven of these STGs were negative and one remained positive for CVd. Fourteen STG's were also indexed for CPsV and Citrus Impietratura disease (CID) and were negative. Confirmation of the biological indexing was done by PCR by both the CRI and ARC-ITSC laboratories prior to final release and is indicated in Table 7.

Table 5.9.1. STG submissions in the pipeline for graft transmissible disease elimination and indexing.

Variety type[2]	STG introductions and releases 2012 to 2017[1]															
	2012			2013			2014			2015			2016/7			
	Bf	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	Balance
C	1	5	1	5	1	0	6	0	3	3	0	1	2	0	1	1
G	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
L	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	1
Mi	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1
Ma	1	0	1	0	4	0	4	0	0	4	1	1	4	2	5	1
N	29	10	5	34	2	4	32	0	13	19	2	2	19	1	4	16
V	6	2	2	6	0	2	4	1	0	5	1	0	6	2	1	7
Or	1	0	0	1	0	0	1	1	0	2	0	0	2	0	0	2
Rs	1	1	0	2	0	1	1	0	1	0	0	0	0	0	0	0
Total	**40**	**18**	**9**	**49**	**7**	**7**	**49**	**3**	**17**	**35**	**4**	**4**	**35**	**6**	**11**	**30**

[1] Bf = Brought forward from previous year; Balance = Balance for the current reporting year.
[2] Variety type: C = Clementine; G = Grapefruit; L = Lemon; Mi = Midseason; Ma = Mandarin; N = Navel; R = Reticulata; V = Valencia; Or = Ornamental; Rs = Rootstock.

B. Maintenance of the virus-free gene source

The CRI gene source currently comprises 365 accessions and the number of selections per variety type is listed in Table 5.9.2. Two trees of each accession are maintained and the duplicate tree is maintained in a separate greenhouse structure. These 730 plants were screened for the presence of CVds and CTV by direct RT-PCR of leaf samples. Three accessions were positive for CVd, 4 for CTV and one accession was positive for both CVd and CTV. The identity of the specific non-Apsca viroids were determined, but not yet of those of the Apsa viroids detected. The CTV strains present were also determined. Inconclusive results were obtained for 2 accessions and these will be retested. The eight positive accessions will be resubmitted for STG. Results are presented in Table 5.9.3.

Table 5.9.2. The number of accessions per variety type maintained in the CRI nucleus block.

Variety Type	No. of cultivars at CRI
Clementine	30
Diverse (Citron, Sour orange, etc.)	2
Ellendale	4
Grapefruit	23
Kumquat	1
Lemon	23
Lime	4
Mandarin hybrid	62
Midseason	34
Navel	84
Ornamnetal	4
Pummelo	8
Rootstock	23
Satsuma	8
Valencia	55
Total	**365**

Table 5.9.3. Results of the re-indexing of the CRI Nucleus Block for viroids and CTV

CIS accession no.	Cultivar	PCR Results			Follow-up procedure
		Non-Apsca viroids	Apsca viroids	CTV (strain)	
716	Yuma Citrange				Re-Test
1065	Owari		+ (CDVd[3])		STG
1213	Californian Lane Late	+ (HSVd[1], CBCVd[2])	+ (CBLVd[4])		STG
1252	Mias	+ (HSVd)			STG
1299	Cambria R (Royal Late)	+ (CBCVd)		+ (Undetermined)	STG
1420	Powel Summer			+ (RB1)	STG
1572	Sevillano			+ (HA16-5)	STG
1586	DPI 10-9 Valencia			+ (RB1, T68)	STG
1598	Bay Gold			+ (Undetermined)	STG
1600	West Indian Lime				Re-Test
1709	Krajewski Early Navel		+ (CDVd)		STG

[1] *Hop stunt viroid*
[2] *Citrus bark cracking viroid*
[3] *Citrus dwarfing viroid*
[4] *Citrus belt leaf viroid*

C. Biological and molecular re-indexing of mother trees and multiplication blocks at the CFB

The CTV status of 224 mother trees was determined by biological indexing as part of the CIS re-indexing program. The severity of the CTV sources were evaluated based on growth and the presence of mild or severe stem pitting on the indicator host, 'Mexican' lime, six months after inoculation. The absence of CTV, indicated by the absence of symptoms, was confirmed by means of ELISA (Table 5.9.4).

The screening of the 742 CFB multiplication blocks for the presence of CVds by direct RT-PCR of composite leaf samples was completed. Greenhouse structures 1, 2, 3, 4 and 5 were tested and 1240 samples were processed. Sixteen positive accessions were detected and these are indicated in Table 5.9.6.

A selection of mandarin, Clementine and Satsuma samples from greenhouse 4c and 5 were also tested for the presence of CTV by RT-PCR, using the same extractions prepared for the viroid screening of the CFB multiplication blocks. This was done to assess the transmission of CTV cross-protection sources to various soft citrus multiplication trees. The results are presented in Table 5. Various soft citrus cultivars tested negative for CTV after re-indexing of both mother trees and multiplication trees at the CFB. The CTV stains found in both GFMS12 and LMS6 sources do not propagate or translocate well in some soft citrus cultivars and the virus is subsequently lost. CTV virus-host reactions differ substantially and the cultivars therefore differ in their suitability as hosts for different CTV strains. These results, together with those of the CTV analysis of the mother trees, indicate that the CTV cross-protection for soft citrus cultivars needs to be reconsidered.

Table 5.9.4. Results of CTV indexing of 224 mother trees maintained at the CFB.

Cultivar	No. of trees	No. of trees with severe SP*	No. of trees with mild CTV	No. of trees negative for CTV
Furr (Clem x Murcott)	4	0	4	0
Nova 2	4	0	4	0
Yosemite Gold	4	0	0	4
Tahoe Gold	4	0	0	4
Shasta Gold	4	0	0	4
African Sunset (B24)	4	0	0	4
Phoenix (2PH low seed)	4	0	0	4
Morr 26 (2)	4	0	4	0
Nadorcott 1	4	0	0	4
Valle Gold (B17)	4	0	0	4
Or 4 (2)	4	0	0	4
Nadorcott Seedless	4	0	0	4
Empress	4	0	0	4
Gold Nugget	4	0	0	4
Mandalate	4	0	0	4
Tango	4	0	0	4
Tambor (Ortanique) 1 (2)	4	0	4	0
Tanorlate (2)	4	0	4	0
Michal 89/64	4	0	4	0
Orit (Edit x Nova)	4	0	4	0
Michal 6/47	4	0	4	0
Royal Honey	4	0	4	0
Nova ARC (2)	4	0	1	3
Nectar	4	0	4	0
IR M1 (QDP#237)	4	0	4	0
Meravit 119	4	0	4	0
Shani Seedless	4	0	4	0
Total Mandarins	**108**	**0**	**53**	**55**
Carninka Late	4	0	0	4
Fischer	4	0	4	0
Cambria 3	4	0	4	0
Chislett Summer	4	0	4	0
Bahianinha	4	0	4	0
Lane Late California	4	0	4	0
Clarke	4	0	4	0
Autumn Gold	4	0	4	0
Chislett M7	4	0	4	0
Palmer	4	0	4	0
Fukumoto	4	0	4	0
Fukumoto2	4	0	4	0
Newhall	4	0	4	0
Kirkwood Red	4	0	4	0
Powell Sumer	4	0	4	0

Lina	4	0	4	0
Glenora Late	4	0	4	0
Washington	4	0	4	0
Witkrans 3	4	0	4	0
Gloudie (Nuwelande)	4	0	4	0
Suitangi	4	0	4	0
HE Late	4	0	4	0
Habata	4	0	4	0
Cara Cara	4	0	4	0
Robyn 2	4	0	4	0
Addo Early (EH)	4	0	4	0
KS	4	0	4	0
Total Navels	**108**	**0**	**104**	**4**
West Indian Key Lime	4	0	0	4
Total Limes	**4**	**0**	**0**	**4**
Etrog	4	**0**	**0**	**4**
Total Rootstocks	**4**	**0**	**0**	**4**
Grand Total	**224**	**0**	**157**	**67**

Table 5.9.5. Results of CTV screening of soft citrus samples of multiplication trees at the CFB.

Sample position and code		**Cultivar**	**Variety type**	**CTV pre-immunisation source**	**CTV PCR**
GH4c	R15 CLU - C/40/MS12	Andes 1 - Clemenluz	Clementine	GFMS12	+ (4/4)[1]
	R15 MHCK5 (2) - R/65	Tanorlate 2	Mandarin hybrid	GFMS12	+ (5/5)
	R16 MHL - R/130	Leanri	Mandarin hybrid	GFMS12	+ (1/4)
	R16 MHRH - R/128	RHN (Royal Honey)	Mandarin hybrid	GFMS12	+ (5/5)
	R18 MHQ - R/131	Queen	Mandarin hybrid	GFMS12	+ (9/9)
	R19 CNU - C/01/106/P01-6	Nules	Clementine	LMS6	+ (2/7)
GH5	R2 MHSG - R/98	Shasta Gold	Mandarin hybrid	LMS6	- (0/1)
	R2 MHB24 - R/63	B24 African Sunset	Mandarin hybrid	LMS6	- (0/1)
	R2 MHLS - R/104	Phoenix (2PH Low seed Murcott)	Mandarin hybrid	LMS6	- (0/1)
	R2 MHAF - R/44/343/02	Nardorcott 1	Mandarin hybrid	LMS6	+ (1/1)
	R2 MHB17(I-12) - R/64	B17 Valley Gold	Mandarin hybrid	GFMS12	- (0/1)
	R2 MH04 (2) - R/56	Or 4	Mandarin hybrid	GFMS12	- (0/1)
	R2 MHAFSL - R/106	ARC Nardorcott LS	Mandarin hybrid	GFMS12	- (0/1)
	R2 MHEM - R/26/297/01	Empress Mandarine	Mandarin hybrid	LMS6	- (0/1)
	R2 MHGN - R/97	Gold Nugget	Mandarin hybrid	LMS6	- (0/1)
	R2 MHM - R/90/02	Manadalate	Mandarin hybrid	LMS6	+ (1/1)
	R2 MHTA - R/111	Tango	Mandarin hybrid	GFMS12	- (0/1)
	R2 MHOT - R/41/331/18	Tambor (Ortanique Tangor)	Mandarin hybrid	LMS6	+ (1/1)
	R2 MHM26 - R/71/01/1	Mor 26	Mandarin hybrid	LMS6	+ (1/1)
	R3 SMW - U/03/108/01	Miho Wase	Satsuma	LMS6	- (0/1)
	R3 SKU - (P/02?) U/02/109/02	Kuno	Satsuma	LMS6	- (0/1)
	R3 SS - U/15	Sonet	Satsuma	LMS6	- (0/1)
	R3 CNU - C/01/106/01	Nules	Clementine	LMS6	+ (1/1)
	R3 CES2 - C/17/352/02	Esbal 2	Clementine	LMS6	- (0/1)
	R7 MHB17(I-12) - R/64	B17 Valley Gold	Mandarin hybrid	GFMS12	+ (6/8)
	R8 MHB17(I-12) - R/64 P01	B17 Valley Gold	Mandarin hybrid	GFMS12	+ (2/2)
	R8 MHB17(I-12) - R/64 P02	B17 Valley Gold	Mandarin hybrid	GFMS12	- (0/7)

	R9 MHTA - R/111	Tango	Mandarin hybrid	GFMS12	- (0/9)
	R10 MHTA - R/111 P01	Tango	Mandarin hybrid	GFMS12	+ (1/6)

[1] '+' and '-' denote a positive and negative reaction, respectively; the number of positive sub-samples are indicated in brackets.

Table 5.9.6. Positive detection of CVds in the CFB multiplication blocks in greenhouse structures 1, 2, 3, 4 and 5. The cultivar, greenhouse and position in the structure are presented together with the CVd detected.

Cultivar (Structure, Row, Code)	**Citrus viroid detected**
Star Ruby Grapefruit (GH1, R5, P/03/135/09)	*Hop stunt viroid* (HSVd), *Citrus bark cracking viroid* (CBCVd)
Limoneira 8A Lemon (GH1, R9, S/08/P03)	CBCVd
Lavelle 2 Valencia (GH1, R8, V/58)	HSVd[1]
Cambria-R2 Navel (GH1, R32, N/53)	CBCVd
Tardif de Mars (LL) Clementine (GH1, R37, R/71/01/1)	HSVd[2]
Bahianinha Navel (GH2, R7, N/02/066/10)	HSVd (Sequence confirmed - CC-A4)
Lina Navel (GH2, R8, N/06/090/02)	HSVd (Sequence confirmed - CC-A4)
Or4 Mandarin Hybrid (GH2, R16, R/56/411/02)	HSVd[1]
Kedem Mandarin Hybrid (GH2, R20 R/72/01/1)	HSVd[1]
Late Valencia (GH2, R23, V/03/057/12)	HSVd[1]
Lisbon Yen Ben Lemon (GH2, R25, S/13/379/16)	HSVd[1]
Bearss Lime (GH2, R31, L/01/009/05)	HSVd[1]
Newhall Navel (GH3, R13, N/10/195)	*Citrus dwarfing viroid* (CDVd)[1]
Miho Wase Satsuma (GH3, R15, U/03/108/P03)	CBCVd
Ueno Satsuma (GH3, R21, U/12)	HSVd[2]
Limoneira 8A Lemon (GH4, R30, S/08/322/P03)	CBCVd

[1] confirmed with sequence data
[2] non-cachexia variant, confirmed by PCR

D. CIS collaboration with ARC-ITSC

Shoot tip grafting for the CIS is done at both the CRI and ARC-ITSC laboratories. To confirm the pathogen-free status of new accessions prior to release to the CFB, duplicate molecular testing is done on these accessions and the number of accessions tested for specific pathogens are presented in Table 5.9.7.

Table 5.9.7. Number of samples subjected to duplicate testing for various pathogens prior to final release to CFB.

Pathogen	**ARC-ITSC accessions**	**CRI accessions**
CTV [3]	4	27
CVd	5	8
ASGV	4	8
CPsV	6	14
'*Ca*' L. africanus	6	10

[3] Includes testing to confirm CTV pre-immunization

E. Samples received for diagnostics from growers and external institutions

Twig dieback and unthrifty plants of a CRI mandarin trial block that showed typical symptoms for Cachexia disease was investigated. Samples (117) were taken from symptomatic trees as well as from adjacent orchard blocks to determine the extent of the infection. The Cachexia-causing variant of HSVd (IIc) was detected in the symptomatic mandarin trees, which confirmed the visual diagnosis. Numerous other viroid species were detected in both old and newer adjacent orchards, indicating contamination over time with various cultural practices. This analysis has enabled the grower to prevent spread of the viroid to a newly planted, sensitive mandarin orchard.

Another 37 samples were analyzed for various pathogens.

F. *Ad hoc* investigations as required by CIS

As part of the investigation into the occurrence of chimeras on Valencia fruit in various regions, CTV and viroid analyses were conducted on trees showing chimeras and trees where no chimeras were observed. Direct RT-PCR analyses for viroids and CTV strain determinations were done. Samples were also grafted to biological indicators and these were also tested. No correlation was found with either CTV or viroid presence and the presence of chimeras.

Conclusion

Efficient pathogen detection and elimination enables supply of healthy budwood to the industry and is the primary objective of this project. Successful elimination of GTDs from new selections were achieved. New selections were added to the gene source and released to the CFB. Mother trees were indexed for their CTV status, the CFB multiplication blocks screened for viroids and the CRI gene source was tested for viroids and CTV. Diagnostic services were provided and analysis of industry problems relating to graft transmissible diseases were addressed.

Technology transfer

Symposium presentations:
J.H.J. Breytenbach, S.P. van Vuuren, G. Cook and C. Steyn. 2016. Elimination of graft transmissible diseases of citrus in southern Africa through shoot tip grafting. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.

C. Steyn, J.H.J. Breytenbach, S. P. van Vuuren and G. Cook. 2016. Citrus viroids: back with a bang. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.

C. Steyn, J.H.J. Breytenbach, S. P. van Vuuren and G. Cook. 2017. Citrus viroids: A re-emerging problem. 50th SASPP, Champagne Sports Resort, Drakensberg.

Further objectives and work plan

Quarterly milestones for Apr-Jun, Jul-Sep, Oct-Dec 2017 and Jan-Mar 2018

A. Cultivar introduction (administration, establishment, STG, diagnostics, cross-protection and CFB and nucleus block submission)
B. Maintenance of the virus-free gene source
C. Biological and molecular re-indexing of mother trees and multiplication blocks at the CFB
D. Collaboration and duplicate indexing with ARC-ITSC laboratory
E. *Ad hoc* diagnostics for GTDs for growers and external institutions
F. *Ad hoc* investigations as required by CIS

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6 INTERNATIONAL VISITS

6.1 Attendance of the 20th conference of the international organization of citrus virologists (IOCV) IN Chongqing, China 10 - 15 April 2016

Glynnis Cook, CRI, Nelspruit.

Aim:

- Participate in the conference by attending presentations and viewing posters
- Present a paper [Field performance of various *Citrus tristeza virus* cross-protection sources trialed in grapefruit in different climatic regions]
- Chair a programme session
- Participate in a one-day field visit to observe local diseases

Delegates: The conference was less well attended than previous meetings and representatives from approximately 10 countries attended including China, USA, Brazil, Argentina, Uruguay, Australia, Turkey, Pakistan, South Africa and Greece.

Papers and posters: 36 papers and 31 posters (Table 1).

Table 1. Topics of papers and posters presented at the conference[1].

	HLB	CTV	CVd	CPsV	CLV	CYVCV	Phytoplasma	Other	Tech/Gen
Papers	13	3	2	2	4	1	1	6	4
Posters	11	9	4	1	1	2		1	2

[1] Topics: HLB = Huanglongbing (greening); CTV = *Citrus tristeza virus*; CVd = Citrus viroids; CPsV = *Citrus psorosis virus*; CLV = *Citrus leprosis virus*; Other (*Citrus chlorotic dwarf associated virus* and other diverse topics); Tech/Gen = techniques, country overviews and general topics.

Notes on papers and posters of interest:

Huanglongbing (HLB) / Asian greening

The majority of presentations were focused on HLB.

Prof W.O. Dawson presented a novel approach using a *Citrus tristeza virus* (CTV) expression vector to control HLB by expression of foreign genes or silencing of genes by RNA interference (RNAi). The use of CTV as a vector to target both the pathogen and the vector is a uniquely specific approach due to the phloem limitation of both CTV and the pathogen as well as the phloem feeding nature of the vector. Currently certain CTV-antimicrobial peptide constructs are being commercialized. RNAi is a gene silencing mechanism which is triggered by the presence of double-stranded RNA which is processed by enzymes to smaller dsRNA entities of 21 to 24 bp called small interfering RNA (siRNA). The RNAi approach showed much promise in the psyllid caged trials presented, with a number of vector gene targets preventing psyllid progeny development. (*Using the CTV vector to attempt to control HLB, W.O. Dawson*).

A bio marker used to determine HLB severity that is useful for screening for resistance/tolerance in citrus was identified. This appears to be a combination of host-pathogen interaction and an adaption of bacterial populations. HLB symptoms were divided into grades. Briefly, blotchy mottle is normally associated with less tree die-back and indicates a lesser severity and was associated with phage presence. Yellow leaves indicate a more serious infection. Small leaves, without yellowing, is a milder symptom and the plant may even outgrow this symptom. (*Molecular mechanisms behind the HLB symptom variations and rapid selection for variant citrus plants with greater HLB resistance/tolerance, Y. Duan and M. Pitino*)

Comparative analyses of Liberibacter genomes help identify the putative virulence genes/factors shared among three HLB Liberibacters. Research is underway to confirm virulence genes. These results facilitate development of anti-virulence drugs that specifically target functional domains of the virulence genes and disarm pathogenicity. (*Development of genome-based therapeutics to control HLB, H. Lin*).

Citrus viroids

A new citrus viroid was identified from a non-symptomatic Lisbon lemon in New South Wales, Australia. It falls in the Apscaviroid group and tentatively named *Citrus viroid VII* and significantly differs from other known

viroids. (*A novel citrus viroid found in Australia, tentatively named citrus viroid VII, G.A.Chambers, N.J. Donovan, S.M. Jelinek and G. Vidalakis*). CIS viroid screening should be adapted to include this viroid.

Citrus viroid VI (CVd-VI) was identified in Chinese orchards (*Identification and molecular characterization of Citrus viroid VI isolates from China, M. Cao, et al*.)

These viroids are important to consider for future Post Quarantine Entry citrus accessions.

Citrus Leprosis Virus

A new virus was identified from sweet orange trees in Brazil showing typical leprosis symptoms. It is unlike previously described citrus leprosis viruses. (*Genome sequence of a new Dichoravirus associated to citrus leprosis nuclear type disease, C. Chabi-Jesus et al*.)

Citrus yellow vein clearing virus (CYVCV)
A virus disease of biosecurity importance for South Africa is *Citrus yellow vein clearing virus* (CYVCV). A planting of infected Eureka lemons was visited during the field visit (photos below) and the virus is clearly destructive to the host. The citrus host range is limited to lemons and sour orange and is especially severe on Eureka lemon. It is aphid transmitted although other vectors might be implicated. It was originally found in Pakistan, India, Turkey and China. Full genome sequencing for China isolates were obtained *(Deep sequencing and characterization of Citrus yellow vein clearing virus isolates from Chongqing and Yunnan Province, China, Y. Yu et al*.)

E-probes
The development of e-probes and graphical user interface developed at Stellenbosch University within CRI Project 1100 was very well received. Interest was shown for the use in citrus material transfer between gene repositories. (*Citrus virus detection in NGS data using e-probes, T.L. Jooste, M. Visser, G.Cook, J.T Burger and H.J. Maree*)

Field day

Delegates were taken to the Citrus Research Institute laboratories, a division of the Chinese Academy of Agricultural Sciences and a number of disease symptoms were displayed. Laboratories were well equipped and glasshouse and greenhouse structures well maintained. Of interest to see was the bud union abnormality on trifoliate orange obtained with *Apple stem grooving virus* (ASGV) (syn. *Citrus tatter leaf virus*) infected material. This symptom (Figure 1) is rarely observed in Southern African orchards due to supply of virus-free material through the Citrus Improvement Scheme. Symptoms of *Satsuma dwarf virus* (SDV) on Satsuma was also displayed showing stunting, leaf malformation and rosetting. Characteristically, the leaves produced in spring have the shape of an inverted boat or spoon (Figure 2).

Figure 1: Bud union abnormality on trifoliate orange due to ASGV infection

Figure 2: *Satsuma dwarf virus* on Satsuma showing stunting, leaf malformation and resetting.

The trip included a visit to a virus free nursery. Mother trees were well maintained in screened structures, however, nursery multiplications were not enclosed (Figure 3).

A. 

B.  C 

Figure 3: A typical Chinese virus-free nursery. Mother trees are maintained under screen (A), but multiplications are not enclosed (B & C).

A standard Chinese orchard was visited. All work is done manually and no mechanical spraying is applied. There is no weed control and mite control is done using a biocontrol agent *Neoseiulus barkeri*. (Figure 4).

A.  B. 

Figure 4. (A) standard Chinese orchard and (B) the packaging of the biocontrol agent *Neoseiulus barkeri*

A virus disease of biosecurity importance for South Africa is *Citrus yellow vein clearing virus* (CYVCV). A planting of infected lemons was visited. Foliar symptoms of yellow-vein clearing, defoliation of existing canopy leaves and malformation and stunting of new flush were observed (Figure 5). The citrus host range is limited to lemons and sour orange and is especially severe on Eureka lemon.

Figure 5. Yellow vein clearing on lemons, defoliation and stunted, malformed new flush due to CYVCV.

Contacts

Dr Hano Maree (University of Stellenbosch) and I spent time with Dr William Dawson to discuss collaboration regarding the CTV vector approach to control HLB for the South African context. He suggested that the relevant researcher spend time at the Lake Alfred laboratories to learn from their experiences. The current clones are available for use by other laboratories, but certain patents are being submitted regarding aspects of this approach. He was unsure how wide the patents will apply. Brazilian researchers are currently following this approach and are building an infectious clone in collaboration with Dr Dawson's group.

Arrangements were made to acquire the viroid group specific tests (Apsca and non-Apsca) from Dr Georgios Vidalakis, University of California, Riverside, USA. These will be implemented for quicker viroid screening of CIS material.

21st IOCV Conference 2019.

The next IOCV meeting will be held in California in 2019 and it will be proposed that it be combined the International Research conference on Huanglongbing.

Bibliography

A hard copy as well as an electronic version of the Abstracts is available on request.

Acknowledgement

I would like to thank Citrus Research International for the opportunity and financial support to attend the conference.

6.2 **Report on the International Citrus Congress** at Foz do Iguaçu, Brazil, on 18-23 September 2016

Attended by: Hannes Bester, Elma Carstens, Paul Cronje, Tim Grout, Vaughan Hattingh, MC Pretorius, Jakkie Stander, Teunis Vahrmeijer, Jan van Niekerk

Opening Ceremony

O1: The role of ISC in the world citrus industry – *Dirceu Mattos (IAC – Brazil)*

O2: The Brazilian Citrus Industry – *Eduardo Femino Carlos (IAPAR – Brazil)*
Rangpur lime rootstock was introduced as it was resistant to Citrus tristeza virus and replaced sour orange. Then sudden death arrived and they found Swingle citrumelo was resistant to that so the industry changed over to that, but it was not as drought resistant so irrigation then became important. Rangpur lime could survive for 9 months without rain. [TG]

Keynote Lectures

K1: Research for Innovation Strategies in São Paul, Brazil – *Carlos Henrique de Brito Cruz (Fapesp – Brazil)*
Sao Paulo State has 42 million people and economy larger than Argentina. Spends 1.7% of GDP on R&D. State generates 32% of country's GDP. They have 3 state universities and 3 federal universities. 60% of research expenditure is from industry and 40% split between state and national. In the rest of the country the government spends more on research than industry. FAPESP is Sao Paulo research foundation. The government must send 1% of their revenue to FAPESP every month. This provides stability. 25 000 proposals were reviewed last year and 14 000 were approved. Small Business Enterprises can receive research funding. These are companies with less than 250 employees. Some of these involve companies working on citrus problems. FAPESP in 2015 spent R$1.188 billion on research, of which the majority is for health services. Sao Paulo state produces more scientific papers than any other country in Latin America. Funding organisations provide adequate support for the researchers to reduce the administrative load through grant management offices in the research institutions. In 2007 there were only 3 of these offices and now 200. [TG]

K3: Sustainable Pest Control for Citrus Production in Brazil – *Jose R.P. Parra (ESALQ/USP – Brazil)*
Jose has written a book on pests and beneficial insects of citrus. Most growers want to focus on use of pesticides. *Brevipalpus* mites are problematic due to the leprosis virus and leafhoppers for transmitting Xylella (CVC). Aphids transmit CTV and cause sudden death. Also now *D. citri* and HLB. With all the sprays for vectors there is little chance for natural enemies. Selectivity tables are available to show which chemicals are softer on natural enemies and rotation of chemicals is recommended for resistance management, but growers do not use these.

First example: Citrus fruit borer *Gymnandrosoma aurantianum* similar to FCM causes losses of $50 m per annum. Can attack up to 350 fruit per tree and causes fruit drop. Female lays 200 eggs but only one per fruit. Action threshold was 5% of fruit for start of spraying but this was not very effective. Survives better in fruit with higher pH and growth is quicker at higher pH and higher temperatures. There are 8 generations in the north of the state and only 7 generations in the south where it is cooler. High RH results in a longer life span and eggs are not laid at low RH. At 50% RH, only 4 eggs per female but at higher RH get >10x this. Mating in the top of the trees; seen with fluorescent markers. Pheromones were developed. Traps placed near the top of the tree caught many more moths than in the middle of the tree. Traps and pheromone replaced every 30 days. Need to identify the males correctly. Action threshold of 6 or more males trapped per week. 1 trap used per 10 ha because moths are attracted from 175 m away. Using about 38 000 traps per year and monitoring in 10 to 14% of citrus area $1.32 billion saved with cost benefit ratio of 1 to 112 due to 50% less pesticides.

Second example is CLM. Brazilian parasitoids were not enough to control the insect. Imported *Ageniaspis citricola* from Florida which had previously come from Asia. Polyembryonic so get 3 to 10 pupae per leaf. Used small black plastic tubs. Parasitoid released all over the state. Both classical and augmentative approach to biocontrol in order to get fast results. Decline in populations within 6 yr. Use pheromone in citrus nurseries.

Before HLB, natural parasitisation levels of CLM were 44%, then after sprays for HLB started the parasitism increased to 51%. This is surprising.

Third example is *D. citri* and HLB. *D. citri* reported in Brazil in 1942 (Costa Lima, 1942) but HLB only reported in 2004. Some Rutaceae species only result in egg laying and no complete development. *Murraya paniculata* and *M. koenigii* are important hosts. Development based on honeydew quantity is better on *M. koenigii* than *M p.* but they rear it on *M.p.* Plant eradication for HLB since 2004 has resulted in the loss of more than 40 million citrus trees in SP state. Impossible to use predators because of the large number of sprays. *Tamarixia radiata* is ectoparasitoid and *Diaphorencyrtus aligarhensis* an endoparasitoid. *T. radiata* is considered more effective. Life cycle shortest at 30°C but best rearing temp is 25°C. Use a greenhouse for rearing *D. citri* on *M.p.* and *T. radiata* is reared in the lab. Releasing 400 parasitoids per ha. Got good results where no sprays being used such as 10% increase in parasitism causing a 90% reduction in population of *D. citri.* Percentage parasitism is declining due to all of the sprays. In 2005 it was 20% and in 2007 down to 5%. Now concentrating more on releasing parasitoids in abandoned groves, organic groves, backyard trees or where orange jasmine is abundant. This represents about 12 000 ha in SP state. Parasitoids can disperse more than 1.6 km into commercial areas. Numbers of trapped D. citri on orchard borders has dropped after releases started. Now have 6 insectaries producing *T. radiata*. Jrpparra@usp.br. More expensive to grow *M. k.* so that is why they use *M. p.* for rearing. [TG]

K4: RNAi-based Strategies Against Insect Vectors - *Bryce Falk (Univ. of California – USA)*
RNA interference first reported in 1928 although not known how it happened. Upper leaves of an infected plant uninfected due to an active RNA response on lower infected leaves. Used commercially against plant infecting viruses. Papaya in Hawaii immune to ringspot virus as a good example and fruit are sold and consumed. In transgenic plants and in insects there is the same mechanism. Potential in all eukaryotes including fungi. We can induce RNAi responses. Nat. Biotechnol. 2007 25: 1231. Nobel prize given for this technique in 2006. They have been looking at glassy winged sharpshooter, mealybug and *D. citri*. Three different pathways occur. They use sRNA pathway where small RNA fragments are replicated. Can induce specific and non-specific effects in cells of glassy winged sharpshooter. Interfering RNAs do not always provide a predicted phenotype. But this was only in adults so then they did more work on nymphs that prevented moulting to adult. Then moved to work on *D. citri* and Tomato psyllid. Can use artificial feeding with sucrose through membranes and add interfering RNAs in the sucrose. Food dye used to show feeding. Can also label RNA and watch where it goes in the insect. Different RNAs induce different levels of mortality. Looked at RNA expression in different parts of the insect. Some expressed more in different parts of the insect. Plants can transmit RNAi effects systemically but in insects there is not this type of movement. Therefore, it is important to know which are the best targets. Insert sequence of RNA in plant virus as a carrier. In Florida they are using CTV for this. Infect the plant with the virus and the plant has an RNAi response resulting in small interfering RNAs that the psyllid can take up. Nymphs are better targets than adults. Have found that they can kill citrus mealybug via TMV on tobacco resulting in death of crawlers, but does not kill adults. Need to try to make the RNAi effects more specific. Now trying microRNAs which are more specific. Allow you to make a specific mRNA that will only work in one molecule. Are making the microRNAs we want but lots of others that we do not want. Still have not solved the problem of spreading the RNAi around the body. Insects lack the polymerase that plants have that allows for the spread. But what about an Insect-infecting virus? Only one report of a virus in *D. citri*. Have now looked at populations from all over the world and looked at the RNA using high throughput RNA sequencing. They found lots of possible viruses in *D. citri* which have not been recorded on Genebank. Some viruses are only in certain populations of the insect. E.g. Californian population is different to the Hawaiian one. Can make DNA copies of most of the genome and manipulate them. Have a *D. citri* virus that is related to parvoviruses called *D. citri* densovirus that offers good potential. Insects respond to these viruses in a specific way. They found in Californian wild populations and in Texas the *D. citri* associated C virus is a simple RNA virus that has given the greatest success so far. DcFLV has a huge RNA genome which related to yellow fever. This one only infects *D. citri.* Efforts now focused on wildtype and/or recombinant viruses for potential biological control efforts for *D. citri.* Virus induced gene-silencing VIGS are being researched in tobacco. [TG] A large amount of research has gone into identifying viruses within *D. citri*. However, little has been done on using or investigating this avenue in the African greening pathosystem and for the control of that vector. This could be an interesting avenue to pursue to also manage this disease. [JvN]

K5: Citrus Genomics: The Path from the Past to the Superhighway of Future Genetic Improvement – *Fred Gmitter (Univ. of Florida – USA)*
He believes that there was an original sweet orange because nobody has managed to reproduce it by hybridisation. Still don't know which genes are responsible for CTV. 2003 meeting in Spain to sequence citrus genome started collaboration in this area. 2011 developed first publically available citrus genome in the form of Clementine. Admixtures: where some genes from different original species of citrus. Pac-Bio sequencing

allows for larger sequences than Sanger techniques although latter are more accurate. Can genotype individual pollen grains – Mike Roos. Genome Wide Association Studies. Can identify regions of genes involved with thorniness. GWAS being used to link genome parts to phenotypes such as bitter, seed, BRIX, etc. Huge interest in HLB. Have a hybrid of mandarin and pomelo that looks and tastes like an orange and is very tolerant to HLB. Genomics assisted breeding. CRISPR being used as well. Can remove and add to genome. Can insert native citrus RNA but currently using agrobacteria that must be eliminated. Florida growers were the first to put money on the table for the genome sequencing in 2011. Now can just change the DNA sequence without adding anything foreign but it still must be accepted as it is modified. [TG] The final availability of a complete citrus genome can open up exciting avenues to study pathogen/host interactions that would help in developing more successful disease management strategies. [JvN]

K6: Transgenic Strategies to Control Citrus Huanglongbing – *Leandra Peña (Fundecitrus/IBMCP-CSI/UPV – Spain)*

Liberibacter americanus almost displaced by Las. Lam also sensitive to heat. Probably why Las displacing it. In 2004 Las was found in Papua New Guinea and SP state in Brazil; and 2005 in Florida. Symptoms of Las. Peduncular end of fruit stained orange if cut off the top end under calyx. Colour inversion more common with Laf. 2015/6 in Florida 81m boxes and next year 60m, but previously in 2004 ≈ 235m boxes. In Brazil stabilised at ≈ 17% symptomatic trees. 10 commandments of control for success (took photo of slide). Area-wide control important. Five major diseases of citrus in SP: CBS, Las, canker, CVC and leprosis. These may depend on VOCs as CBS, Las and canker very closely related to CBS. May be able to modify production of VOCs. Linalool can repel aphids *Myzus persicae*. β-caryophyllene reduced infection by *Pseudomonas syringae*. This in *Arabidopsis* plants that genetically modified. In citrus, looking at d-limonene which most important monoterpene in citrus. If reduce expression of d-limonene, less attraction to Medfly. Rodriguey *et al.* 2011. Guava effect – interplants Prof. Chau. Effect discovered by grower where he could produce mandarins when planted with guava but not with bananas, etc. Difficult to find *D. citri* when guava trees touching citrus. β-caryophyllene very high in guava but only found in citrus leaves when they are damaged. Used 4-arm olfactometer with *D. citri* mated ferals. Only count if insects made a choice. Test for 10 different days with 12-20 insects per day. Air filtered in charcoal then humidified before going over citrus plants in a bag. Got significant repelling from guava (trans-caryophyllene = β-caryophyllene) 0,1 – 1,0 μg/μℓ β-caryophyllene was repelling. Air flow 0, 4 Lpm. Worked with *Arabidopsis thaliana* transgenic plants with or without β-caryophyllene. High β-caryophyllene in plant gave repellent effect. Pera orange plus β-caryophyllene also caused repellency. Working with 250 citrus lines. Monoterpene levels in leaves change through the year. Now getting similar repellency from citrus construct leaves as guava. No source of genetic resistance in citrus germplasm. Edge effect: 80% of insects in edges of groves so planning to use borders with plants with RNAi. Kills all nymphs within 5 d. Field experiments in 2017 with 20 ha of modified plants. Intercropping of guava in Florida did not work – Tim Gottwald. Have not got fruit yet to test for β-caryophyllene levels in fruit that may be attractive to fruit fly (my comment). [TG]

K7: Potential Impacts of Climate Change on Citrus Water Requirements Across Some of the Major Citrus Producing Areas in the World – *Ali Fares (Prairie View A&M Univ. – USA)*

The objective of the study was to investigate the potential impact of future climate change scenarios on citrus water requirements and water cycle components, (e.g. effective rainfall, evapotranspiration (ET), canopy interception and excess water drainage below the rootzone) in some of the major citrus producing regions across the world such as: Cape Town – South Africa, Mersin – Turkey, Riverland – Australia, Nabeul – Tunisia, Riverside – California, Fort Pierce and Lake Alfred – Florida, Brownsville – Texas and Sao Paulo – Brazil. Optimum irrigation water requirements of citrus crops were predicted with the model, Irrigation Management System, for the current and two future periods 2046-2065 and 2081-2100 under different temperature, carbon dioxide (CO_2) and rainfall scenarios. Evapotranspiration was calculated using a modified Pennman-Monteith equation to account for the impact of CO_2 concentration on crop ET. Results obtained from this study indicated that irrigation water requirements vary between the different locations. The highest reduction (34%) in irrigation water requirements was found for Cape Town and the decreases in irrigation water requirements at higher CO_2 concentrations are associated with a decrease in ET. Evapotranspiration is predicted to decrease by 20-30% during the period 2046-2965 and by 24-37% during 2081-2100. However, under the same CO_2 concentrations an increase in temperature will result in an increase in ET and irrigation water requirements. [TV]

With no greenhouse gases, average temperatures would be -19°C not 14°C. UN Intergovernmental panel on Climate Change (IPCC). As CO_2 increases, ET decreases and the irrigation requirement IRR will decline. Effective rainfall ER will decrease with time. CO_2 increase reduces ET and overrides increasing temperatures that would increase ET on its own. [TG]

K8: Advances on Citrus Nutrition: 30-year of Research in Tropical Conditions – *José A. Quaggio (IAC – Brazil)*
The citrus industry in Brazil produces approximately 18Mt fruit of which 12 Mt is used for juice production and the rest is sold on the fresh fruit market. Brazilian production represents 85% of world juice volumes. Approximately 200 m citrus trees in Brazil. In 1980s 95% of rootstocks were Rangpur lime. With HLB, increased tree density to 550 trees/ha or even 700/ha. At higher density, had to reduce initial N applications. Now looking at nutrition requirements and diseases. CVC being controlled by psyllid sprays. Stronger cell walls and vessel diameter increases with calcium. Structure of parenchyma cells stronger with more calcium. Soil fertility and plant nutritional standards for tropical soils were developed from long term field trials. Their results showed that soil analyses correlate with leaf analyses when soil samples are not taken in the drip line and that different rootstocks have different nutrition demands, e.g. Swingle Citrumelo has a high demand for boron. Boron is taken up 3-times more efficiently through the roots than when applied as a foliar application. Magnesium is important in tropical soils. Lemon's demand for N is lower than oranges. Swingle has much bigger response to N than RL and Cleopatra. A linear relationship between calcium (Ca) and nitrogen was found, with NO_3:NH_4 ratio playing an important role in Ca uptake and fruit yield. Ca^{++} and NO_3^- ions balance charges rather than using NH_4^+ which is also positive. Recently they developed sap analysis. Cut off twigs, take off leaves, chop up stems and soak in ether and place in a freezer at -16°C. The peak of N use is in bloom and bud initiation in April. Ammonium nitrate gives a pH of 3.5 in soil but calcium nitrate a pH of 6.6 which is perfect for root growth. Too much NH_4^+ reduces Ca absorption. 6x NO_3^- to NH_4^+ can give 75 tons/ha but 2x NO_3^- to NH_4^+ has half the yield. Five times more roots are produced with Calcium nitrate than with ammonium nitrate. Over 10 years, this resulted in 30 tons/ha more. [TV + TG]

Scientific Sessions

S1: Economics, Trade and Marketing Policies

S1- 103: Worldwide markets and welfare impacts from introducing GM oranges to manage HLB – *Singerman A & Lence SH*
Third talk also a fill in from Coca Cola on orange juice. Input costs are up considerably in Florida. From $400 per acre to $2000 due to HLB. Pushing price of juice up so less consumption. Also more concern about consuming too much sugar. The production costs in Florida have increased three fold due to HLB. The rate of HLB infection in Brazil is ±18%. Prices of GM oranges must never exceed conventional. Consumers in US better off with GM product. Brazilian prices will decrease if GM is consumed in the US. [TG]

S3: Pre- and Postharvest Biology and Technology

S3-37: Alleviation of chilling injury caused by cold quarantine treatment in sweet orange fruit – *Singh Z, et al.*
Cold quarantine treatment of 1°C for 22 days causes chilling injury in sweet oranges. The effects of hot water treatment alone or in combination with thiabendazole (TBZ), salicylic acid, methyl jasmonate dip and fumigation of nitric oxide and ethylene were investigated. Hot water treatment of 50°C for 5 minutes in a dip alone or in combination with TBZ reduces chilling injury caused by cold quarantine treatment without adversely affecting fruit quality. [TG] [HB]

S3-102: Involvement of carotenoids in the response of mandarin hybrids to postharvest cold storage – *Rey, F et al.*
External rind colour has been identified as a feature influencing the tolerance of the fruit rind to Chilling Injury (CI), although the basis of such an effect is not well known. Selecting three phenotypes (light, dark-yellow and orange) mandarin cultivar as storing the fruit for 8 weeks at 2°C this observation was tested. The results indicate the highest incidence of CI developed in the dark-yellow compared to the orange and light-yellow fruit. The increase in CI was followed by an increase in ethylene synthesise indicating that it was a response to CI damage. The data suggest the carotenoid complement and content may influence the susceptibility of mandarin to CI. This research could be used in identifying susceptibility to chilling injury in selections during breeding. [PC]

S3-140: Towards the understanding of juice sac development – *Sadka A, et al.*
Yemonite citron does not have juice sacs. [TG]

S3-212: Postharvest management of citron (*Citrus medica* L.) Peel colour – *Klein JD, et al.*
Named *C. medica* because it originates from Medea (Persia). Costs $1.5 per fruit to produce but can get up to $100 when sold. Most acquire chilling injury at 11°C and go orange. $CuCl_2$ used as substitute for ethylene.

10 ppm made fruit a little yellower. Cu can accelerate colour change. Citron peel extracts can have insulin-like effects. $CuCl_2$ degreens without losing the calyx. Colour change can take a week at 20°C. [TG]

S3-223: Citrus stem end rot: an old disease without new solutions – *Cerioni L, Bennasar PB, Lazarte D, Sepulveds M Rapisarda VA & Ramallo J*
Stem-end-rot (SER) is an important postharvest disease that can be controlled with benomyl. Due to the increase of restriction regulations in the overseas markets, alternative chemicals had to be investigated. Postharvest fungicide effects on SER *Phomopsis citri* controlled by all fungicides. *Lasiodiplodia* more difficult to control. Lower pH and lower temperature gives better control. TBZ is the best option to reduce SER. [TG][HB]

S3-233: Okitsu satsuma postharvest rind breakdown: relationship with environmental conditions, fruit degreening and waxing – *Zocalo, P et al.*
Satsuma mandarin make up 26% of Uruguay production and have serious problems with rind breakdown. In a project evaluating pre and postharvest condition resulting in this disorder it was found that oil glands and cuticle was not affected, however, higher maximum temperature and lower RH and less rain during the 2 weeks before harvest resulted in an increase in rind breakdown. Degreening did not lead to higher incidence and wax application (polyethylene and shellac 10% solids) significantly reduced rind breakdown. The results suggest dehydration on the tree leads to higher incidence of rind breakdown. [PC]

S3-240: Preliminary analysis of the impact of quarantine cold treatment on different sizes and colour of lemons – *Sala H et al.*
Exporting lemons from Tucuman to Japan at 3°C for 24 days for fruit fly leads to chilling damage. By selecting fruit of two colours i.e. green and silver as well as size i.e. 162 and 125 the impact of these two aspects was studied. The results indicate that neither colour nor fruit size affected the development of chilling injury in this study. [PC]

S4: Mites, Pests and their Control

S4-65: Citrus insect pests and their nonchemical control in China – *Zhang HY, et al.*
800 citrus pests recorded in China. Three different trunk borers. Removal of infected trees with HLB. Ploughing to kill FF pupae. Removal of summer shoots to get more synchronised autumn flush. Covering crops can improve microclimate. Warmer temps in winter and cooler in summer. Fruit bagging for piercing moth and fruit fly. *Amblyseius cucumeris, A. barkeri* and *A. eharai* used in biocontrol. *A. cucumeris* used for rust mite and red mite, and *Eotetranychus*. *Amblyseius eharai* feeds on thrips and whitefly. *Aschersonia placenta* fungus effective against white flies in humid orchards. A lot of oil is used in China. Frequency trembler grid lamps especially used for Lepidoptera. Black light lamps also attract Lepidoptera and beetles. Not as good as trembler, but cheaper. Sugar-acetic acid and ethanol mix for fruit fly in bottles. Using CRISPR Cas9 for SIT without radiation – research. [TG]

S4-70: SIVANTO™ PRIME – A unique tool for pest management in citrus – *Bell JW, et al.*
Flupyradifurone butenolide 200SL registered in USA 2015; Brazil will be late 2017. IRAC 4D. PHI 45d as soil treatment. Excellent safety for bees. Can spray in bloom. Strange feeding cessation. Xylem mobile and translaminar knock-down and residual control. Imidacloprid also has good feeding inhibition 35% of the time, just walks around and only 44% stylet penetration with 5.6% phloem penetration. When tried with SIVANTO, 86% of time walking around and only 14% stylet penetration. No phloem feeding. [TG]

S4-276: Young shoots affecting the efficacy of insecticide spray applications against *Diaphorina citri* – *Carli LF de, et al.*
Marcel Mirands recently introduced management strategies outside the grove. Psyllid management, releasing *T. radiata.* CLas transmission more efficient on flush. Time to reach phloem 5-15 min, but in hard leaves ± 30 min. Imidacloprid 4 g a.i./hl spray. On day of treatment, all treatments were effective. After 7 days, worse on young but not significantly. After 14 days, there were differences between young and mature shoots. Best on immature leaves. Rain seven days after treatment reduced efficacy. Thiamethoxam and Dimethoate better than Imidacloprid and Bifenthrin on younger shoots. [TG]

S4-341: Spatial and temporal distribution of viruliferous *Brevipalpus* spp. mites and leprosis in citrus orchards - *Andrade DJ, et al.*
Leprosis costs $80 m/annum. No trans-ovarial transmission. Two types of virus: CiLV-C and CiLV-N. Environmental conditions are important. Fruit picked into paper bags. 5 mites/PCR test. Will results change your sampling plans/technique? All mites with leprosis were from plants with symptoms. [TG]

S4-393: Studies on the pheromone chemistry of the citrus borer, *Diploschema rotundicolle* – *Amorós ME, et al.*
Teflon coated traps. Light traps were best but only females were caught. Generic compounds caught lots of other cerambycids whereas light was more specific. [TG]

Poster: Efficacy of flupyradifurone (sivanto 200 sl) in drench application for managing *Diaphorina citri* (Hemiptera: Liviidae) in citrus – Sulzbach, F. et al.
Flupyradifurone gave promising control of *D. citri* at 0.6 g per m^2.

S6: HLB Pathosystem (Plant Vectors and Bacteria)

S6-22 Metabolic profiles of citrus rootstocks with different response to Huanglongbing – Albrecht U
Differences have been observed in rootstock responses to HLB infection. These were compared using GC-TOF MS metabolomics. This comparison showed that the tolerance is not associated with accumulation of protective metabolites but rather the presence of these metabolites in high concentrations in more tolerant rootstocks. The research question is if this type of analyses can be used to compare rootstocks or selections within one rootstock with regard to their reaction to *Phytophthora.* [JvN]

S6-24 Diagnosis, characterization and management of citrus greening disease in India: Current scenario and future research needs – Ghosh DK
Survey and genomic studies of different CLas individuals indicated that the population in India is quite diverse. Question to ponder is whether the genetically different individuals are different with regard to their ability to infect and causes disease? Are some more aggressive than others? [JvN]

S6-28 Distribution of *Candidatus Liberibacter asiaticus* above and below ground in Texas citrus – Louzada ES
Symptomatic leaves expressed higher levels of CLas and with distance from the trunk the expression of CLas in leaves also declined. In roots CLas titre was higher in horizontal roots versus vertical roots but again declined with increased distance from the trunk. [JvN]

S6-31 Comparisons of miRNA profiles and miRNA target gene expressions in response to Huanglongbing in citrus roots – Yun, Z
Micro RNA's (miRNA) play and integral part in host/pathogen interactions. The poster reported on solexa sequencing that was done to identify changes in miRNA profiles in roots of control and HLB infected trees. The expression of certain of the identified miRNA's showed that their associated target genes are implicated in stress responses, plant growth and development, transcription and metabolism. Understanding this interaction could lead to effective control strategies. What has been done to understand this interaction in the case of African greening host/pathogen interaction? [JvN]

S6-67 Insect vectors of Huanglongbing in China – Cen, YJ
Studies in China have revealed that apart from the Asian citrus psyllid (ACP) the pomelo psyllid, *Cacopsylla citrisuga* is also very effective in transmitting HLB. How effective will ACP be in transmitting not only HLB but African greening? Will its presence affect the disease dynamics of African greening, making our management practices less effective? [JvN]

S6-68 Drug repurposing: A new antimicrobial against *Ca. Liberibacter asiaticus* – Gardner CL
CarD is an essential RNA polymerase binding protein involved in pathogenesis, antibiotic resistance and stress response in *Mycobacterium.* In CLas the CarD homolog was identified and using molecular screening small molecules were tested for inhibition of this homolog. Some of these molecules were found to eliminate CLas infection in highly symptomatic citrus seedlings. Could this technology be used in other ways managing other pathogens as well? [JvN]

S6-78 Primary spread has a more important role than secondary spread in Huanglongbing managed citrus orchards – Arruda JH
Disease progression studies showed that the primary infection of HLB in managed areas is at the edges of orchards and that it is often originating from non-commercial or neglected neighbouring orchards. [JvN]

***S6-110 Diaphorina citri* ovipositon host preference is determined by volatile organic compound profiles –** Amorós, ME

Volatile organic compounds (VOCs) play an important role in attracting ACP to its host and also play a role in ovipositon preference. In a comparative study comparing 6 citrus species showed that there is marked differences between the species with regards to the preference in ovipositon of the ACP. There could possibly be potential in using some of the less preferred hosts as buffer trees around commercial plantings. [JvN]

S6-161 Water stress and plant nutrition influence *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) take-off – Tomaseto, AF
Studies showed that ACP was less prone to take-off from plants that were optimally irrigated opposed to take-off from water stressed plants. Take-off was also higher in non-fertilized trees compared to fertilized trees. This indicates why abandoned citrus groves are such good sources of ACP – take-off from these trees is very high while the commercial orchards are attractive hosts. [JvN]

S6-192 Asian citrus psyllid and Huanglongbing in California, USA – Kumagai, L
Spread of HLB in California was very rapid and it underlines the importance of speedy action once ACP and HLB is detected in a country. [JvN] ACP trapping and treatments as well as HLB testing continues in collaboration with the industry, state and federal agencies. [HB]

S6-215 Behaviour of fruit drop and productivity of HLB-infected Valencia and Pineapple orange trees on 6 different rootstocks in Jamaica – Torres, JB
In 100% HLB infected orchards it was shown that the combination of Carrizo and Valencia or Pineapple orange were one of the worst performers with regard to productivity. On the other hand, combinations containing Swingle or Cleopatra as rootstock were significantly better. [JvN]

S6-256 Program for monitoring *Diaphorina citri* in Tucumán, Argentina: Joint work by public and private entities – Martínez, D
The poster underlined the importance of monitoring not only for HLB symptomatic trees but also for the presence of ACP and that with early detection quick action is possible. Partnerships between public and private entities are also very important in successful management plans. [JvN]

S6-286 Recovery of vigour and production of orange trees with Huanglongbing through pruning, nutrition and plant growth regulators – *Medina CL*
Application of nutrients and plant growth regulators directly after drastic pruning to recover trees with HLB were tested. Plants with symptoms in about 60% of the canopy were treated with various foliar fertilizers and PGRs after pruning and evaluated over two years. Nutrition and the use of PGR's significantly improved rehabilitation of trees with HLB. [HB]

S6-342: Enhanced acquisition rates of *CL*as by the Asian citrus psyllid in the presence of vegetative growth in citrus - *Alabi OJ, Setamou M, Kunta M, Jifon JL & Graça JV da*
*C*Las transmission is enhanced in flush. Hall *et al.* 2016 JEE. Young shoots influence the dynamics of the vector and disease. Must focus control on each flush. [TG]

S6-422: Variations in physico-chemical characteristics of flush shoots and phloem sap during ontogenesis and their influence on Asian citrus psyllid populations – *Setamou M, Simpson RC, Akabi JO, Nelson DS & Jifon LI*
HLB. More psyllids accumulate on young shoots which are easier to feed on. Lemon mature leaves are softer than grapefruit leaves. It takes the insect 3 – 15 min to reach the phloem in soft shoots but 30 min in hard leaves. Young shoots have higher N and less Ca than old. With principle component analysis we can separate old from new shoots. Young shoot has 3 – 15x more free amino acids. Range of amino acids also differs between old and young shoots. Ca and B levels are negatively correlated with psyllids. If you are not careful with plant nutrition, you could stimulate psyllid growth. Young shoots attract psyllids with colour and volatiles. Then the softness facilitates feeding and Las uptake better. Nutrient sprays without psyllid control programme could make things worse. [TG]

S7: Citrus Genetics and Breeding

S7-89: Citrus rootstockbreeding programme in Turkey – *Yesiloglu T, et al.*
Sour orange is still the main rootstock for 90% of citrus in Turkey. High pH soils are a problem in Turkey. Iron chlorosis screening studies. Resulted in 9 hybrids that are being tested against Phytophtora. [TG]

S8: Biotechnology

S8-71: Control of stomatal aperture – a new way to save water in citrus plants – *Granot D, et al.*
SPV – smart plant valves. Guard cells swell to open stomata. Osmolytes said to make guard cells open. But sugars seen to close stomata. Hexokinase closes stomata. Hexokinase in guard cells is a sugar sensor. Sugars get carried with water which transpired and when gets to a certain concentration, stomata close. Guard cell hexokinase concentration increases temperature. [TG]

S8-101: Characterization of new lycopene-accumulation mutants of sweet orange – *Cuellar F., et al.*
Lycopene accumulation in citrus is due to a mutation resulting in the down regulation of the lycopene cyclase gene group. By using various cultivars such as Kirkwood red and Ruby Valencia more insight in this mechanism was gained. The biochemical and molecular data indicate only partial blockage in the carotenoid biosynthetic pathway, probably at the carbohydrate cyclization step. In addition, it was first shown that the accumulation of lycopene can also occur in the phloem and more so in older branch structures. The results reveal possible functions in specific tissue of the carotenoid pathways. The research is leading to an improved understanding of the mechanism underlying the rind colouration process. [PC]

S8-27 Overexpression of a cyclic nucleotide gated ion channel gene in citrus induces broad spectrum disease resistance – Louzada, ES
The overexpression of a citrus cyclic nucleotide gated ion channel (CNGC) gene in various citrus cultivars and rootstocks showed that it did not have a negative effect on the phenotypic characteristics of the cultivars or rootstocks. However, in the transgenic plants resistance to citrus canker infection of leaves and root infections by *Phytophthora nicotianae* were observed while the wild types were successfully infected by both pathogens. Can the expression of this gene be used to compare or explain the level of disease resistance between cultivars and rootstocks?

S8-46 Transgenic sweet orange rootstocks overexpressing a osmotin-like PR-5 protein show resistance against *Phytophthora* foot rot after seven years of growth in the field – Peris, JE
Many plant defensive antimicrobial proteins have been identified. Proteins in the PR-5 group are known to have *in vitro* activity against fungi and oomycetes. By overexpressing some of these PR-5 coding sequences from tomato in Pineapple sweet orange seedlings led to the seedlings displaying resistance to *P. citrophthora* infection in potted plants. In field trials the one transgenic line, 9.1a have remained healthy for a period of 7 years under severe soilborne pressure by *P. nicotianae.*

S8-165 Engineering citrus to resist Huanglongbing – Dutt, M
Citrus cultivars engineered to contain the SAR inducing genes NPR1 and SABP2 have shown in field trials that they remain tolerant to HLB infection 4 years after planting. Do these genes also play a role in resistance to other pathogens?

S8-295 Pepper BS2 gene is functional in citrus and increases canker resistance – Sendín, LN
The Bs2 gene in peppers confers resistance to *Xanthomonas campestris* pv. *Vesicatoria* (Xcv) pathogenic strains that contain the avrBs2 avirulence gene. This avirulence gene is highly similar to the avrBs2 avirulence gene in *X. citri* subsp. *citri* (Xcc). Stable expression of the Bs2 gene in transgenic orange trees showed these trees to show reduced symptom expression when infected with the above-mentioned citrus canker bacterium. [JvN]

S9: Other Bacterial Diseases

S9-12: Soil drench and foliar spray application of Tiviant for citrus canker control – Graham *et al.*
The application of Tiviant, a Bayer product, as a soil drench or foliar spray significantly reduced the incidence of citrus canker on fruit and was comparable to copper hydroxide sprays. Its effect as a soil drench is interesting and its potential for the control of other fruit and foliar diseases can be of interest. [JvN]

S9-96: Exogenous NAD activates SAR and induces disease resistance in citrus – *Alferez et al.*
The application as foliar or soil of nicotine adenine dinucleotide (NAD) were shown to reduce canker lesions on bacterial inoculated seedling leaves, up to 2 months after treatment. This is through the activation of plant defence mechanisms of the plant. This application can also be tested for other plant/pathogen interactions in citrus. [JvN]

S9-221 Serenade®, a bio-fungicide with further promising properties, against bacterial diseases and for crop establishment
Serenade® was developed as a foliar fungicide. It is derived from the fermentation products of *Bacillus subtilis* QST713. It showed good activity against various bacterial diseases of citrus. Recently it was trialed through

soil applications and was shown to have beneficial effects on plant growth and yield. This could be a new use for this fungicide on citrus. [JvN]

S10: Citrus Germplasm, Phylogenetics and Genomics

S10-88: Citrus origin and domestication: an evolutionary paradigm for the genus citrus – *Wu GA, et al.*
Poncirus is an outsider. Chloroplast trait is closer to the geographical origins. Oldest citrus fossil has similarities to *C. maxima*. Think there was a radiation that led *to C. reticulata*, *C. maxima* and *C. medica* from summer monsoon. All main citrus types east of India in China. Mandarins moved east to Japan. Limes moved south to Australia. 7,5m years ago had the initial radiation period. Second radiation was in the Pliocene (2 mya). West to East movement was responsible for the origin of many plants in Australia. Most flora and fauna on islands came from the China mainland. Butterflies followed the same route. Centre of origin for citrus is between Assam, N. Myanmar and Yunnan. [TG]

S10-323: The phylogenetic origin of limes and lemons – Curk F*, et al.*
Four original taxa *C. micrantha, C. reticulata, C. maxima* and *C. medica*. Mexican lime from *C. medica* and *C. micrantha*. 1975 theory. Looked at 93 lines of lemons. Eureka lemon from *C. micrantha, C. reticulata* and *C. medica*. Same origin for Marakech lime, Mexican lime *C. aurantifolia, C. micrantha* and *C. medica*. *C. latifolia* from all 4 original citrus spp. Bergamot from sour orange and lemon. See: Curk, *et al.* 2016. The phylogenetic origin of limes and lemons. [TG]

S11: Fungal and Oomycete Diseases

S11-6: Citrus black spot: sexual reproduction provides new insights into an old problem – *Tran NT, Miles AK, Dietzgen RG & Drenth A*
Have managed to get CBS mating in the lab and the production of ascospores. This will be published soon. [TG]

S11-136: Evaluation of *Macleaya cordata* extract to control *Geotrichum citri-aurantii* causing citrus sour rot
Extract from *Macleaya cordata* was shown to inhibit fungal mycelial growth and spore production by the sour rot pathogen in vitro. In simulated commercial trials the application of this extract at 1000 ppm to Ponkan reduced the incidence of sour rot by 58.33%. Plant extracts could be a valuable avenue to follow to find alternatives to fungicides. [JvN]

S11-283: Efficacy of azoxystrobin-fludioxonil for stem end rot on lemon
This fungicide is known for its efficacy against Botryosphaeriacea pathogens that causes rots on various fruits. It is not often used on citrus but can have potential, especially in combination with prochloraz where a strong synergism was shown on mangos in controlling postharvest rots. [JvN]

S11-346: The use of *Bacillus subtilis* QST 713 (Serenade) for citrus postbloom fruit drop disease control
This product showed good potential in combination with other fungicides to control postbloom fruit drop caused by *Colletotrichum acutatum.* As mentioned previously it also has potential in a soil application and with its foliar fungicide properties should be tested in our current disease management programmes. [JvN]

S12: Cultural Practices

S12-425: Effect of different systems of pruning on the productivity and quality of Clemenules – *Cornejo J*
The effect of different pruning systems to improve productivity, fruit size and quality were evaluated on Clemenules clementines in Chile. No differences in internal fruit quality and fruit size were found between the four systems tested. The “east door” pruning system presented the highest productivity, percentage fruit for export, best coloration of fruit and reduced the number of workers during pruning and harvest. [HB]

S13: Citrus Tree and Fruit Physiology

S13-29: A possible mechanism for the low citrate accumulation in an orange mutant - *LIU YZ; Shi CY; Guo LX; Ning DY; Liu X; Jin LF; Peng SA*

In order to elucidate the mechanism of low citrate accumulation in citrus a model plant Hong Anliu – a mutant from the Anliu was used. The results indicate the mutant plant has a reduced ability to transcribe citrate biosynthesis and utilization of related genes. In addition, the proton pumps in the mitochondria activity were increased. This led to the lower citrate accumulation in the cell. Furthermore, by feeding the data into a model the reduction in proton pump activity was identified as the key factor to lead to the low citrate accumulation and the comprehensive metabolite alteration in this mutant. This study could potentially be used in screening of cultivars as well as manipulating the citric acid metabolism to improve fruit quality. [PC]

S13-86: Efficacy of pre-harvest sprays to reduce rind pitting on 'benny' valencia - *Cronje PJR.; Ehlers J.L; Alférez F; Theron KI*
Preharvest application of 2,4-D will reduce pitting of Benny Valencia oranges if applied at 50% petal fall. GA_3 had no impact. Will work more on 2,4-D in Stage 1 and 2,5,6-TPA. In addition, applying s-ABA and TBZ two weeks prior to harvest also reduced the pitting incidence but we need to figure out the mode of action of TBZ. The result indicates the possible use of plant growth regulators during different fruit development stages in order to decrease the sensitivity of fruit to postharvest pitting, but TBZ use in the orchard could lead to green mould resistance. [PC & TG]

S13-152: Effect of rootstock-scion interaction on metabolomic composition of phloem sap in citrus reticulata – *Srivastava.R., Raveh E, et al.*
The effect of grafting sour orange, Volkameriana lemon, SB-812 rootstocks grafted with Merav, Orra, Shani and Michal showed that the interaction of genetic material influences the balance in primary metabolites i.e. sucrose, fructose as well as malic acid. The composition of phloem sap is therefore not only due to the scion but also the rootstock. The research could increase the information on why certain rootstock-scion combinations result in higher quality fruit. [PC]

Salt levels can be high in fruit due to high water salinity. Sodium levels in leaves have doubled over the last 20 years; chloride levels have also doubled over this period. Levels in other crops are also higher than USDA norms. All crops also have 100% more sodium than EU norms. In 2008 desalination started so sewage water also had less sodium and in orchards where recycled water was used. Desalination also removes magnesium so then had a shortage of magnesium in citrus. [TG]

S13-156: Relationship between floral intensity and floral gene expression in 'washington' navel orange in response to low-temperature, water-deficit, and ga3 treatments – *Tang Lisa; Lovatt CJ (UCR)*
Flowering in citrus is independent of daylength. Low temperature and water stress induce flowering. For 'Washington' navel orange, 8 weeks of low temperature (15/10°C, day/night) plus 3 weeks of warm temperature (24/19°C, day/night); and /or 8 weeks of water deficit (-2.86 MPa) followed by 3 weeks of re-irrigation can successfully induce flowering and expression of key flowering genes. Tang and Lovatt showed that foliar GA_3 (50 mg L-1) applied weekly during the 8 week-long low-temperature or water-deficit treatments significantly reduced flowering and expression of all the key flowering genes, to levels equal to warm-temperature exposed and/or well-irrigated trees, except for the FT gene, i.e. GA_3 downregulates genes for flowering. [JS & TG]

S13-176: Response of phenological stages to climatic extremes affecting citrus yield and quality – *Khurshid T; & Khurshid, JE*
Khurshid and Khurshid presented data on the effects of climate change on the production of 'navel' sweet oranges in Australia, with specific reference to the impact on phenological events. During the last ten years, temperatures increased significantly, and important phenological phases such as time of flowering, time of colour break, and fruit maturity were slowed down, and/or shifted earlier. The biggest driver of these was higher temperatures and the subsequent effects on the accumulation of heat units. 32 000 ha of citrus in Australia and navels produce 35-45 t/ha. 75% of citrus is in Sunraysia, Riverland & Riverina in S. Australia. They have a phenology calendar. Optimal range 13-15°C with 23-28°C max. Temperatures above 36°C during bloom causes flower drop. Pattern of earlier flowering each year. Fruit set is optimal at 25-30°C and above 35°C results in fruit drop. In very hot years, the fruit expands faster. Warm winters are poor for blood oranges. Predicting more days >40°C in the future with sunburn from 40+. No netting research is being done in Australia. [JS & TG]

S13-269: An investigation of the role of carbohydrates in alternate bearing of mandarins –*Stander OPJ; Barry GH; Cronjé PJR*
To better understand alternate bearing under South African conditions, Stander *et al.* measured seasonal leaf and root carbohydrate concentrations and tree phenological responses of 'on' and 'off' 'nadorcott' mandarin trees at monthly intervals, as well as responses to fruit removal and girdling treatments. In the presence of

fruit, summer vegetative flush and return bloom was inhibited, despite leaf carbohydrates being elevated by girdling. The presence of fruit affected the subsequent vegetative flush and return bloom, however, inhibition thereof is unrelated to leaf carbohydrates. Small mandarins have higher acidity so picking is delayed. In an off-year, sugars are higher in roots. Off-trees main component is sugars and On-trees, carbohydrates. Root growth is the main source of cytokinin production. Two times more sugar than starch in the roots could be used as an indicator for an On-year the following season. [JS & TG]

S15: Virus and Virus-like Diseases

S15-25: Developing molecular diagnostics for rapid and sensitive detection of important virus/virus-like pathogens infecting citrus and implementing citrus budwood certification program in India – *Gosh DK & Ladaniya MS*
Diagnostics in India. ICAR = Citrus Research Institute in Nangpur. Have Citrus Mosaic Badnur Virus. Have CIS in India and covered nurseries. [TG]

S15-210: Characterization of *Mandaviruses* and development of a multiplex rt-pcr for graft transmissible pathogens in citrus – *Baranwal VK, Meena RP & Prabha K*
The genus *Mandavirus* was named for Indian citrus ringspot virus but probably several viruses are involved. Citrus Yellow Vein Clearing Virus was placed in the same genus above. Symptoms only visible in winter. Multiplex works but needs simpler technique. Often occurs with HLB. Not known to have an arthropod vector. [TG]

S15-267: Deepsequencing analysis of RNAs from sweet oranges showing citrus sudden death symptoms – *Matsumura EE, et al.*
Citrus Sudden Death – still do not know what is causing disease but suspect variant of Ctv or a new virus. CSD is mainly on sweet orange on Rangpur lime. CSD virus titre 5x stronger in symptomatic plants so probably responsible. [TG]

S15-372: Disclosing three citrus leprosis-associated viruses of the new genus *Dichorhavirus*, family Rhabdoviridae – *Chabi-Jesus C, et al.*
Dichorhavirus – nuclear type of virus and *Cilevirus* is a cytoplasmic type of leprosis virus. Nuclear type associated with *B. californicus* and *B. obovatus* has been eliminated from U.S.A. *B. phoenicis* sensu *stricto* transmits the new nuclear virus. Can cause localised lesions on *Arabidopsis thaliana*. [TG]

S16: Irrigation and Nutrition

Irrigation overview
Most of the presentations on irrigation focused on the aspects of water quality and the supplemental use of irrigation to increase yield in regions with a high rainfall. Water quality, especially salinity, is a major concern in regions with a semi-arid climate (South Australia, Mediterranean regions, California and Texas). Water salinity has a negative impact on plant growth and water movement in the plant. Shoot and root dry mass, and leaf water and osmotic potentials are more reduced under uniform salinity than under non-uniform salinity. The addition of calcium sulphate to saline water reduced the salt damage to sour orange seedlings. In order to improve water quality, regulations need to be implemented to minimise grove operations that have a negative impact on water quality and the environment. A better understanding of the influence of climate variables on water resources, especially rainfall frequency, is needed and water management and irrigation efficiency need to be improved. Some results presented from Argentina indicated that the water productivity of Valencia oranges ranges between 146 and 291 L kg^{-1}.

Although the annual rainfall in Brazil and Florida is more than 1 000 mm per annum, water availability for irrigation is a challenge. Rainfall is supplemented with irrigation during periods of low rainfall that occur frequently during flowering and fruit set. During the early 1990s nearly all citrus production was rain fed, while currently approximately a third of the citrus producers supplement the rainfall with irrigation. Surface water that needs to be filtered, to prevent the blockage of irrigation systems, is mainly used for irrigation. Yield increased by 31% from the 1990s and fruit size and the life span of the orchards also increased.

Results to validate direct transpiration measurements and different sap flow techniques, were presented. The use of moderate drought stress to achieve adequate flower bud induction in regions with not enough cool temperature hours was also discussed and results presented. [TV]

Nutrition overview

Results on foliar applied copper (Cu), zinc (Zn) and boron (B) indicated that the highest Cu uptake was achieved with copper sulphate, although Cu oxicarbonato (micro particles of Cu) was also absorbed. More B was absorbed from foliar applied boric acid than foliar applied zinc borate, while more Zn was absorbed from zinc borate than foliar applied zinc sulphate. In orchards with Cu toxicity, due to the excess use of cupric fungicides, the root application of both nitrogen (N) and (Ca) can reduce the oxidative stress injuries caused by the excess Cu. It was found that high levels of N enhance the antioxidant enzymes activity and Ca reduces the absorption of Cu by the roots.

In fertigation experiments it was found that $CaNO_3$ application increased the fertiliser productivity in temperate soils. Fruit yield and N-use efficiency were greater for trees (Valencia oranges) that received calcium nitrate. Maximum Ca uptake coincides with maximum N uptake and a linear relationship was found between NO_3:NH_4 ratios and Ca absorption (Ca concentration in leaves) and yield. Lemon trees are more vigorous than most other citrus species and have the highest nitrogen-use efficiency (NUE). The highest fruit yields for lemon trees were obtained when the concentration of N was 18 g N kg^{-1} DM (dry matter) compared to 27 g N kg^{-1} DM in orange tree leaves. Lemon trees have better photosynthetic NUE despite lower N concentrations per unit of leaves. This indicates that lemon trees invested more N and biomass production in the leaves, whereas orange trees invest more in root-biomass production. For both species, with low N supply, the trees increased investment of biomass and nitrogen accumulation in roots. [TV]

S16-13: Nitrogen source and nitrification inhibitors affect soil nutrient status and 'star ruby' grapefruit performance – *Erner Y, Bar-Tal A, Tagari E, Levkowich I and Bar-Yosef B*
Israeli citrus producers struggle with high pH soils. Erner et al. determined the effects of nitrogen (N) source and nitrification inhibitors (NI) on soil solution ionic composition and nutrient availability, and grapefruit yield and quality, with specific reference to fruit acidity. Fertilization with ammonium sulfate resulted in soil acidification during the irrigation season from pH 7.4 to 6.1 and increased P, Mn and Ca concentrations in the soil solution relative to the ammonium nitrate treatment. Consequently, under ammonium sulfate treatment, P and Mn uptake, chlorophyll content in leaves and fruits and TSS in fruits, were higher. Application of NI had a negligible effect on fruit yield and quality but enhanced the capacity of ammonium sulfate to raise N uptake above that achieved with ammonium nitrate. It also reduced the N leaching from the soil. [JS]

S16-59: Calcium nutrition of orange and its impact on growth, nutrient uptake and leaf cell wall – *Kwast A, et al.*
In potted trials the importance of Ca was studied in Navelate grafted on *Citrus macrocarpa* rootstock. $Ca(N0_3)2$ were applied in the soil at 160 mg/L. The treatment resulted in increases in rate of photosynthesis and root volume. In addition, higher cell wall material was reported in the leaves, which could indicate a structure more resistant to insect penetration. The essential aspect in relation to Ca nutrition was confirmed such as better growth, optimal nutrient uptake and enhanced pest/pathogen resistance, in addition to higher yield. [PC]

S16-193: Fruit yield and quality were all improved by combining applying calcium, magnesium and boron fertilizers to citrus paradisi cv. changshanhuyou – *Tan Qiling, et al.*
Due to soil acidification in the Zhejinang province and un-availability of Ca, Mg and B deficiency of these elements results in leaf etiolation and yellowing. By application of these mineral nutrients in combination trials the yield was increased. In addition, SPAD values show a significant positive correlation with Mg applications. The treatments reduce the TA and increased the ratio (TSS:TA) significantly in *Citrus chanshanensis*. It was recommended to their growers to fertilize with Ca, Mg and B in order to recover the yellowing of the trees and improve yield and quality. [PC]

S16-250: Nutritional and horticultural management minimize copper toxicity in citrus trees – *Hippler FWR; Boaretto RM; DOVIS VL; QUAGGIO JA; ZAMBROSI FCB; AZEVEDO RA; MATTOS-JR D*
Copper (Cu) toxicity has been occurring in Brazilian citrus orchards due the excess applications of cupric fungicides to control diseases such as citrus canker. Hippler et al showed that the increase of either nitrogen (N) or calcium (Ca) supply reduced stress caused by excess Cu. N enhanced the activity of antioxidant enzymes, whereas Ca reduced absorption of Cu by roots. Activity of antioxidant enzymes in leaves were higher in trees grafted on Rangpur lime compared to Swingle. Interestingly, the rootstocks accumulated more of the applied Cu than any other plant part (up to 80%). [JS]

S16-450: Nature of global warming and potential impact on citrus production in the Americas – *Albrigo LG*
From now until 2050 meteorologists expect an increase of 1.5°C in temperature and one metre sea rise due to global warming. Citrus is adapted to a wide range of climates. Warmer climates would accelerate fruit

development, decrease fruit quality and increase tree growth and disease and pest pressure. Wetter or dryer climates could increase hurricanes or lead to severe drought pressure. [HB]

S17: Scion and Rootstock Varieties

S17-113: Rootstock trials for 'tango' mandarin – *Roose ML; Kupper RS; Federici CT*
Tango mandarin is one of California's most popularly-produced mandarin cultivars, and also recently gaining a lot of interest in South Africa. Roose et al. reported on results from three rootstock trials for Tango conducted in California: at 1) Porterville, an area with a clay loam soil and soil pH of 7.7 to 8.3; 2) Arvin (a warmer site), and 3) Orosi (the most northern site). The two latter sites have a lower soil pH and more sandy loam soils. Throughout all the evaluations, Tango performed best on C35. At Porterville the highest yields were recorded on C35, Carpenter, Bitters and an unreleased Sunki x Flying Dragon hybrid. The largest trees were on C35. Most trees had good health ratings, except trees on trifoliate orange which showed symptoms of severe iron chlorosis and grew poorly. Results at the other sites were quite different and the largest trees were produced on Santa Barbara Red lime, Schaub rough lemon, Furr (Arvin), C35 (Orosi) and Volk. [JS]

Workshops

W1: Talking GMOs: Reaching Out to the Public

Communication and public perceptions about GMOs around the world – *Margaret Karembu (ISAAA Africenter – Kenya)*
Perception about GMOs is that you hear more from people against GMOs than those for. Selective hearing and feelings override facts. Perception influenced by: trust, benefit, control, fairness. Effective communication on GMOs must be planned. Netmapping – helps identify key opinion leaders. Always have opponents, proponents and undecided majority. Empathising with stakeholders is important. Public is more interested in the "so what?" Need to engage the mass-media. Need a social media team. Address myths and misconceptions promptly. Anticipate. Prepare. Practice. [TG]

W2: Organic Citrus Production

Long term experience on organic citriculture in Italy – *Giancarlo Rocuzzo*
Three organic fertilizers were compared to a mineral fertilizer. Organic fertilizers showed an increase in soil C, nutrient use efficiency of P, K & micronutrients and some fruit quality parameters. A key issue of organic agroecosystems is to increase the soil organic matter content over time. The combined action of cover cropping and conservative soil management techniques can act to increase the economic and environmental sustainability of organic citriculture. [HB]

W3: Mechanical Harvest

Mechanical harvesting of citrus: challenges and opportunities – *Reza Ehsani*
Different factors have contributed to the slow adoption of mechanical harvesting machines and their lack of success. Some of these factors include the high cost and massive size of these machines, as well as concerns of growers on the adverse effect on tree health, structural damage and yield reduction. The challenges of dealing with exotic diseases and the continuous growing costs of citrus production provides an opportunity for growers to consider alternative orchard designs and production systems that work better with mechanical harvesting. [HB]

W4: Joining Hands for Innovation and Sustainability for Healthy Citrus

Selectivity on beneficials using the new active ingredient flupyradifurone – *Pedro Takao Yamamoto (ESALQ/USP – Brazil)*
Sivanto selectivity. *Iphiseiodes zuluagai* is an important predator of *Brevipalpus*. Sivanto selective to *I. zuluagai* with only ± 28% mortality from spray. Mortality to residual contact with *Tamarixia:* Sivanto residues harmless. Direct spray on lacewing is harmless and with *Trichogramma pretiosum.* On most natural ememies Sivanto considered harmless. Sivanto in Brazil will be 200SL and can be used on soil, stem or foliar for psyllids, aphids and whiteflies. Trunk application: 90 dat similar control to Confidor for *D. citri*. Drench 3-5 ml/m of canopy height. Foliar spray 25-40 ml/hl. Recommended treatments during bloom. There is a restriction on maximum amount of a.i./ha. [TG]

W6: Researches and Strategies for Management of HLB – Research Perspectives

Research strategies for management of HLB in Florida – *Harold Browning (CRDF – USA)*
Formerly >900 000 acres of citrus in Florida and humidity and climate means constant control is required. Declined from 2005 to 2011. In 2011 growers stopped removing trees. 20% of acreage is not managed now. Increased costs. CRDF developed as grower-based research finding organisation. Priorities and gaps analysis then funding. Between 2006-2009 the whole state became infected with HLB. Root weevils and Phythopthora become worse if the roots are compromised. Since 2008, ±$15m per year has been spent on research. 2015/6 budget $19,2m. Money does not solve the problem. Coordination with CRB and Federal funds important. Focus now on bactericides and thermal therapy. Still spending $3m on canker – from industry. 53 regions in Florida for coordinated treatments. Citrus Health Management Areas. Numbers of *D.citri* increasing now as people cut back on vector control. Starting field trials with RNAi. 10-12 field applications per year are required for vector control and 60m trees are infected. Thermal therapy has been commercialised but not clear how long the impact lasts. 1 tree at a time. Bactericides are to slow decline and allow young trees to recover. Know we cannot cure trees. Streptomycin & Oxytetracyline. CRDF overseeing 60 field trials. Streptomycin 3x/year and Oxytetracycline up to 8x/year. Many field trials for HLB resistance selection. Unmanaged trees are a serious problem now. NuPsyllid project where want a psyllid that cannot transmit HLB and breeds with wild ones. *Murraya* sp. where only eggs are laid could work as a good trap crop. *Murraya* flushes when citrus is not, but when citrus is flushing, it is not very attractive. In Florida, still having court cases about removing backyard citrus trees. [TG]

Research and strategies for management of HLB in Brazil – *Joseph Bové (in memoriam) (INRA – France) and Renato Beozzo Bassanezi (Fundecitrus – Brazil)*
No resistant varieties. No feasible control methods. 3-pronged approach: Healthy trees, Reduction of inoculum and Control of vector. Advantage was that previously had canker and CVC so the nurseries were already insect-proof. Had experience inspecting orchards and controlling vectors. Early detection strategies being developed. Strategies to eliminate diseased trees. monitoring of *D. citri* – yellow traps – where to put them, etc. Control: residual control and selectivity is important. Large square blocks less affected by neighbour than long narrow blocks. Strategies to maximise control on orchard boundaries. Trap crop with systemic treatment on edges possible. Wanting more effective traps. Repellent semiochemicals. In last 10 years have spent $36m on research. Try to do control on area-wide basis. Have now improved the 3-pronged system. In SP state, 83% of trees have no symptoms but certain regions are highly infested. [TG]

W7: Management of Modern Citriculture: Rootstock Selection

The citrus rootstock breeding program developed by IVIA – Maria Angeles Forner Giner (IVIA - Spain) [PC]

W9: HLB and Roots/Rootstock Interaction

HLB and roots/rootstock interactions – *Jim Graham (Univ Florida – USA) & Eduardo A. Girardi (Embrapa/Fundecitrus – Brazil)*
Las movement in rootstocks. On potted plants. Las moved 2cm /day down to roots. 28 d after inoculation, had moved >10 cm. Las did not move laterally through the xylem and had to go to the roots to spread. [TG]

W9-62: HLB and citrus rootstocks: what the greenhouse and field assays tell us? – *Stuchi ES, Coletta-Filho HD, Santos M & Mourão Filho FAA*
Embrapa. Flying Dragon and Sunki mandarin with less symptoms. [TG]

W9-237: Characteristics that define an HLB tolerant rootstock – *Bowman KD, McCollum G & Albrecht U*
Field tolerance in rootstocks US 896 and US 942 are tolerant. Few tolerant trees died. Tolerant rootstocks have superior canopy health. Tolerant rootstocks may also have lower Las titre in tissues. Phythopthora does not make things worse. Seedy varieties are more symptomatic than seedless – disputed by others. There are rootstocks where Las probably does not replicate. seedy and seedless Murcotts just as susceptible. Some rootstocks maintain low titres of Las and seem to transfer tolerance to scion. Don't understand what happens at bud union. Tolerant interstocks show improvement so inarching may too. [TG]

W9-268: Citrandarin – *Ca.* Liberibacter asiaticus interactions – *Boava LP, et al.*
Consider *Poncirus trifoliata* to be tolerant to Las. [TG]

W9-259: Yield of HLB-infected trees treated with different nutritional programs in São Paulo, Brazil - *Bassanezi RB, et al.*

HLB infected trees and crop loss. Enhanced Nutritional Programmes (ENPs) 1st year reduction of 50% production. 2nd year further 30%. ENP had no effect. 13 different ENPs show no reduction in yield loss. [TG]

W9-446: Root infection and damage: rootstock and canopy responses –*Johnson EG, Graham JH & Grosser JW*
Found some lateral movement within 4 cm below inoculation site. Heat treatment induces strong leaf flush. 30-50% root loss occurs before canopy symptoms are visible. Increased root growth in HLB tree. HLB roots die after 4 months instead of 9-12 months. More carbohydrates therefore sent to rootstocks. [TG]

W9-448: Constant enhanced nutrient supply to roots increases HLB tolerance – *Grosser JW*
Problem in roots much more severe than above ground. Treating soil improves production. Swingle rootstock had no HLB in roots. Managed to increase yield in 100% infected orchards. [TG]

Parallel Citrus Irrigation Symposium – included in S16. [TV]

Presentations by CRI Researchers

S3-20: Lycopene and chilling tolerance during postharvest cold storage of grapefruits – *Lado J, Cronje PJR, Rodrigo MJ & Zacarías L.*

S3-177: Sanitisation of fungicide drench solutions and effects on green mould and sour rot control in citrus – *Erasmus A, Christie C, Lennox CL, Savage C & Fourie PH.*

S4-182: The relative importance of citrus pests in Southern and Eastern Africa - *Grout TG.*

S4-366: Development and validation of a systems approach for phytosanitary pest risk mitigation – *Hattingh V, Moore SD & Kirkman W.*

S8-101: Characterization of new lycopene-accumulating mutants of sweet orange – *Cuellar F, Cronje PJR, Barry G, Rodrigo MJ & Zacarías L.*

S11-54: Mefenoxam sensitivity of *Phythophthora citrophthora* and *P. nicotianae* isolates in South African citrus nurseries – *Van Niekerk JM, Basson E, Olivier C & McLeod A.*

S11-181: Citrus black spot: what have we learnt in the past four years? – *Fourie PH, Kellerman M, Carstens E, Schutte GC, Hattingh V, Dewdney MM, Miles AK, Magarey RD & Gottwald TR.*

S11-195: Population structure of the citrus black spot pathogen *Phyllosticta citricarpa* from five continents – *Carstens E, Linde CC, Slabbert R, Miles A, Donovan N, Li H, Dewdney MM, Glienke C, Schutte GC, Fourie PH & McLeod A.*

S11-199: germination parameters of pycnidiospores of *Phyllosticta citricarpa* – *Kellerman m, Dewdney MM, McLeod A & Fourie PH.*

S11-311: epidemiology and control of *Phytophthora* trunk and branch canker – *Schutte GC, Kotze C & Pretorius MC.*

S13-86: Efficacy of pre-harvest sprays to reduce rind pitting on benny valencia – *Cronje PJR, Ehlers JL, Alerez F & Theron KI.*

S13-269: An investigation of the role of carbohydrates in alternate bearing of mandarins – *stander OPJ, Barry GH & Cronje PJR.*

S16-95: Influence of late nitrogen soil applications on mandarin fruit quality – *Cronje PJR, Joubert J, Marais H, Hoffman EW & Zacarías L.*

S16-203: Citrus water use in a mediterranean climate - *Vahrmeijer JT, Taylor NJ, Banda M, Sam M & Everson CS.*

S16-206: Validating sap flux density measurement methods in potted *Citrus sinensis* - *Banda M, Taylor NJ, Everson CS & Vahrmeijer JT.*

Visit to Fosfoquim by Tim Grout, Santiago, Chile before the International Citrus Congress

This company (www.fosfoquim.cl) has been in existence for more than 30 years. In 2001 they developed the pure phosphine fumigation system. Cytec was previously the company selling their products but it was recently taken over by Solvaid who own the trademarks. Fosfoquim develops the technology for application. Their system, being free from ammonia, allowed them to treat fruits without burn and they mostly use phosphine for mealybug. One advantage of phosphine fumigation is that it can be done at low temperatures without breaking the cold chain, e.g. for mealybug on apples, 24 h at 0°C. Citrus is usually fumigated at 5 to 10°C for 24 h. Fumigation must be within 15 d of export for regulators to approve and prevent reinfestation. Citrus with copper residues can be problematic so must be washed. Fruit under nets could therefore be a problem but there is not much citrus in Chile under nets. With phosphine there is not much dosage response and the period of fumigation is more important. They use 48 h for codling moth in apples but this does not provide Probit 9 level of mortality but 95 to 98% and therefore needs to be followed by a cold treatment too. They have been treating apples in Argentina since March 2016.

Their units incorporate a blower that circulates gas through the cold room. It is not necessary to have high pressure like MeBr so Isowall walls for cold rooms are suitable. A CA room can also be used but degreening rooms may not seal well enough. Phosphine changes to phosphates in UV light. It is important to monitor temperatures and gas levels for authorities and this is part of their service. A slightly longer treatment may be required for internal orange pests. Medfly is more tolerant than *Drosophila suzukii* and there are differences between species of fruit flies. A system approach has been developed for Fullers rose beetle by Spencer Walse (USDA) that involves washing, waxing and phosphine. Their biggest commercial challenge appears to be *Brevipalpus chiliensis* where adults and eggs are very tolerant. Walse says all *Brevipalpus* spp seem to be very tolerant. Fosfoquim is doing research on the control of woolly apple aphid and some armoured scales. They are also developing a technique to show a colour change in a solution if a squashed scale insect is alive. This idea was published in the 1970s but is not being used. This could be very useful for bioassays involving sessile insects or eggs.

6.3 Technical visit to Brazil citrus nurseries and growers during September 2016

Jan van Niekerk, MC Pretorius and Paul Fourie

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Introduction
Brazil is the 2nd largest producer of citrus in the world with the citrus industry mainly situated in the state of São Paulo with production representing 85% of Brazilian production. Virtually all the frozen orange juice concentrate in Brazil is produced in this state. Despite the large production, several important citrus pests and diseases are present in Brazil.

Most important diseases include citrus canker, caused by the bacterium *Xanthomonas axonopodis* pv. c*itri.* This disease is spread by infected plant material over larger distances while short distance dispersal is by means of wind-driven rain. The presence of the citrus leaf miner, *Phyllocnistis citrella*, has furthermore exacerbated the problem due to its feeding on leaves helping the bacterium to enter leaf tissue and cause disease. Management is based on clean nursery material, control of leaf miner, regular inspection of orchards and eradication of infected trees.

Another severe disease is Citrus variegated chlorosis (CVC) caused by *Xylella fastidiosa*, a bacterium which blocks the xylem vessels of the plant. It can be transmitted to other orange groves by contaminated young trees and the insect vector, glassy-winged sharpshooters. The management strategy is again based on several measures such as the use of young trees from insect-secure nurseries (nurseries in fixed structures, covered by plastic and laterally protected by insect screens), pruning of branches that show initial symptoms of CVC, eradication of plants with advanced symptoms, and chemical control of sharpshooters.

Citrus leprosis virus, transmitted by mites in the genus *Brevipalpus*, is another important disease. Vector control, pruning or eradication of diseased trees and the use of virus and vector free planting material forms the cornerstone of management.

Citrus tristeza and citrus black spot are also important diseases, and are well known in South Africa.

The most important disease, however, is Asian Citrus greening or Huanglongbing (HLB), which is caused by the phloem-limited bacteria *Candidatus* 'Liberibacter asiaticus'. This pathogen can be spread via infected planting material over long distances and within and between infected orchards by the Asian citrus psyllid *Diaphorina citri*. This disease can devastate orchards and has already caused a >60% reduction in citrus yields in Florida, USA since it broke out in 2005. **Management in Brazil is based on a three-pronged method of (1) use of disease free planting material from insect-secure nurseries, (2) vector monitoring and control along with (3) regular inspection of orchards and eradication of diseased trees in orchards.**

HLB is especially worrying to the South African citrus industry as the pathogen and vector is known to occur in the Mascarene Islands (Mauritius and Reunion), HLB is present in Ethiopia, and the vector was recently detected in central-east Africa. Given the flow of people and goods between these countries and South Africa, this disease is seen by the South African citrus industry as an imminent threat.

Given the success and experience of the Brazilian industry in management of HLB and other serious citrus diseases, a visit to important nurseries and big growers was deemed important.

Visit aim

The first aim of this visit was to investigate the preventative management strategies employed by the two biggest citrus nursery operations in São Paulo state, which are aimed at producing nursery trees that are free from the abovementioned pathogens and vectors. The second aim was to visit citrus farms to investigate the pest and disease management practices employed at farm level. The knowledge gained with this visit will help the South African industry to improve its biosecurity preparedness and measures.

Nursery visits

Citrograf nursery, Ipeuna and Rio Claro
15 September 2016
Hosts: Cesar Graf and Carlos van Parys de Wit

The nurseries are located at least 15 km from any other citrus production. The total production of Citrograf is 100% in insect-secure structures (Figure 1) and spread across four different nursery sites. This is due to the threat of citrus canker: regulations state that if any canker lesions are found in the nursery, all trees in the nursery must be destroyed and the nursery is placed under quarantine.

Upon entering the main nursery gate, all vehicles are sprayed thoroughly with a QAC solution (Figure 2). Vehicles are not allowed into the nursery area and staff and visitors are required to leave vehicles and walk through a copper foot bath and wash hands in a QAC solution (Figure 3). Trucks doing deliveries or coming to collect trees are not allowed inside the main nursery grounds. These trucks are also washed and the drivers need to go through the same procedures as described above.

Before entering any nursery structure, all employees and visitors are required to shower and put on clothes and shoes provided by Citrograf (Figure 4 and 5). All personal belongings such as cellular phones, pens, notebooks and watches are required to be left in the change rooms where they are locked away. Employees and visitors are furthermore not allowed to bring any citrus into the nursery and employees can also not have any citrus trees in their home gardens. The employee homes are inspected monthly by management to ensure that no citrus is grown in their home gardens.

All the structures containing trees or seedlings have double entrances and the whole structure is covered by a layer of insect-proof netting as well as plastic sheeting (Figure 6). Rootstock seed is stored in Xtend® bags in cold rooms (Figure 7). The special bags retain seed quality better than normal plastic bags and allow for longer term storage. Seeds are planted directly into seedling trays with 2-3 seeds per compartment. The seedling mixture contains sphagnum peat and rice husk and seedlings are watered by hand (Figure 8). Yellow sticky insect traps are placed inside the seedling greenhouses, particularly in entry rooms, to monitor for insect/psyllid presence. This is an internal monitoring measure by the nursery and not compulsory (Figure 9).

The rootstocks that are most commonly used are Swingle, Rangpur Lime and Volkameriana. No Rough Lemon is produced due to its susceptibility to blight. Seedlings are transplanted into 4 - 6 L bags that contain 100% coir. Before use, coir is washed to reduce the EC to < 1.0 mS/cm (Figure 10). Planted rootstock seedlings and budded trees are irrigated by hand once a week in the winter and twice weekly in summer (Figure 11). On

average one worker can irrigate 30 to 35 thousand trees per day. Irrigation water comes from a bore hole. At the Rio Claro nursery excess salts are removed from the water using reverse osmosis.

After budding at a height of 12 – 15 cm, rootstocks are bent (Figure 12). Bending of the rootstock leads to better flushing and growth of the scion bud. Once the bud has grown sufficiently, the rootstock shoot is cut off.

The nursery has its own budwood multiplication trees and 4 mother trees per cultivar. In total they have 14 cultivars available. All tools used in the nursery production are sterilized using a QAC solution. Before trees leave the nursery they are treated with an imidacloprid drench to give initial protection against psyllid in the orchard. Trees from this nursery are produced within 12 – 14 months but the warmer Bahia state nursery can produce trees in 6 – 8 months. In the Ipuena nursery 400 000 – 500 000 trees are produced annually by a staff of 26 people. Key to the high productivity of the workers is regular, thorough training of workers and a good system of backup checks that are in place.

Figure 1. View of covered greenhouses at the visited Citrograf nursery.

Figure 2. Spray booms and wheel baths at the nursery entrance where vehicles entering the nursery is sprayed with a QAC solution.

Figure 3. Handwashing and foot baths located at nursery entrance that visitors should pass through.

Figure 4. Entrance to showers where all visitors must shower and change into provided clothing.

Figure 5. Cesar Graf (front) and Carlos van Parys de Wit, Paul Fourie, MC Pretorius, Jan van Niekerk and Hennie Prins (back, left to right) dressed in the clothing provided after entering the nursery and going through sanitation procedures that includes washing hands and showering.

Figure 6. Double entrance into green houses that is covered by insect-secure net (sides) and plastic sheeting (roofs).

Figure 7. Xtend® bag containing treated seed. These are stored in cold rooms.


Figure 8. Seedling tubes from seedling trays into which seeds are planted directly.

Figure 9. Yellow sticky trap inside the green house entry room for psyllid monitoring. Handwashing and foot bath is also visible.

Figure 10. Coir mixture used in the 4 – 6 L bags.

Figure 11. Watering of seedlings done by hand using a showerhead applicator that provides a stream with coarse droplets.

Figure 12. Rootstocks bent at 12 – 15 cm height where it is then budded.

Vasconcellos Citros, Limeira
Hosts: Luiz Vasconcellos and Carlos van Parys de Wit (Figure 13)

Figure 13. Pictured above from left to right Hennie Prins (Stargrow), MC Pretorius, Luiz Vasconcellos, Jan van Niekerk and Carlos van Parys de Wit.

This nursery was started in 2002 and the arrival of CVC in Brazil forced the nursery to have all trees in insect-secure screen houses. The nursery is located 15 km from any other citrus production, but its seedling production is situated in a citrus area. According to Brazilian legislation the nurseries should be at least 3 km from any other citrus production. The nursery produces 240 000 trees per annum.

They produce all rootstock seed themselves in a seed source that is 100 km away from the main nursery. The rootstock seed source trees are tested every year for important diseases. Seed are treated with captan and air dried before being stored at 6 – 8 °C for 4 – 12 months, depending on the rootstock cultivar. Seedlings are also grown at this site until they are 15 – 25 cm tall before being transported to the main nursery. When transported the seedlings are bare-rooted. Prior to planting, seeds are dehusked by hydrating before applying a caustic soda and chlorine treatment. After dehusking, seeds are planted directly into 600-mL torpedo tubes, two seeds per tube. The torpedo tubes contain a mixture of composted pine bark, rice husk and organic turf. Prior to transport to the main nursery, the seedlings are graded and all media removed from the roots. Murcott is also used as a rootstock cultivar, and purportedly has some tolerance to HLB and good tolerance to *Phytophthora*.

At the main nursery the seedlings are transplanted into 7-L planting bags. Before transplanting root pruning is done to prevent bent roots in the big planting bags. These bags are drip irrigated and regular tests done for *Phytophthora* and nematodes. However, many seedlings showing severe *Phytophthora*–like symptoms could be seen. It was also evident that over irrigation is taking place, as seen from the water puddles and moss growth on the greenhouse floor. Similar to Citrograf nursery the plants in this nursery were on benches raised at least 0.4 m from the ground level (Figure 14).

Similar to Citrograf and Citrosol nurseries, this nursery has its own budwood multiplication trees. These trees are in a greenhouse covered by insect screen on the sides and insect screen as well as plastic sheeting on the roofs. According to the owner this leads to suboptimal levels of light penetrating the structure. The budwood mother trees are kept for 4 years and budwood for new mother trees are received from the government mother blocks. Currently 9 cultivars are in stock. The multiplication trees are sprayed every 15 days with an oil, Envidor and abamectin mixture. A new multiplication greenhouse has been established. In here the rootstock seedlings have been planted directly into the ground and will be grown until big enough for grafting with the cultivar. This

practice was questioned as its sustainability is doubtful if root disease management on these trees is not done properly.

This nursery was less high-tech than the Citrograf and Citrosol nurseries with less stringent phytosanitary measures. Unfortunately, this was also evident from the quality of trees and seedlings in the nursery, and a seedling with HLB-like symptoms was observed inside the nursery.

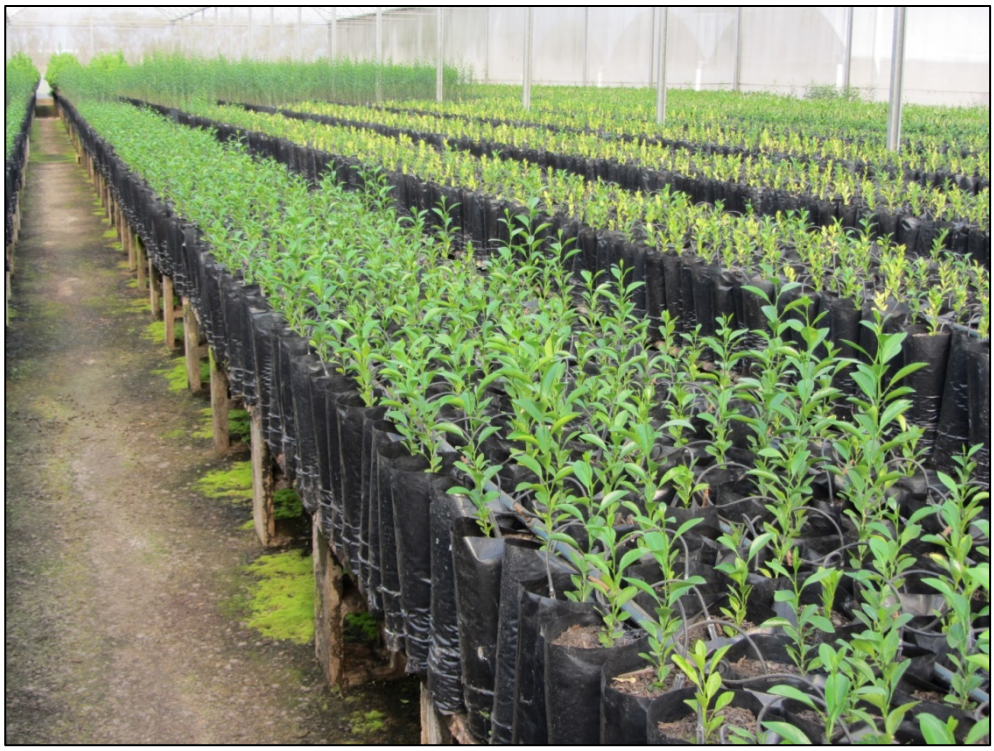


Figure 14. Seedlings in 7 L bags, 0.4 m above soil level. Some yellow seedlings are visible.

Citrosol, Mendonca
17 September 2016
Host: Juliano Ayres

The nursery spans 8 ha and is contained within 20 screen covered greenhouses. Of these 17 are used for the production of citrus trees with 130 personnel. Currently they annually produce 1.3 million trees but have capacity for 1.9 million. The entry procedures into the nursery are the same as at Citrograf nursery: vehicle sanitation, showering and changing of clothes before entering the nursery (Figure 15). The nursery is inspected by government officials every 2 months and if any breech in the insect proof netting is observed, it must be repaired.

Apart from the greenhouses being closed with insect-secure screen, they also have blue plastic roofs that have a 2°C cooling effect. They furthermore have 35% aluminium netting in the ceilings that can be drawn closed. This reduces the inside temperature by a further 5°C (Figure 16).

The floors are cement with the plants raised 40 cm from the floor (Figure 17). This reduces the risk of *Phytophthora* infections and if positive plants are found they are destroyed. In a newer expansion, the nursery will use benches that are raised to 60 cm to allow better worker productivity.

Budwood and seeds are stored in 100 µm plastic bags. The seeds are planted in a fine-grain coir while the big bags are filled with a coarser coir. Irrigation water pH is reduced to 5.5 through the use of phosphoric acid. Watering is done by hand. Uniquely, a clean water irrigation is first done and followed by a fertigation round; this saves a lot of fertilizer as bags are flushed with clean water. Plants on the benches are spaced into 4-5 rows in broader plastic bags that are better for plant development as light penetration is better. Watering and other management practices are then also easier. It was also found that a stronger tree is grown in bags with a bigger diameter.

Rootstocks are bent, similar to other nurseries, but prior to budding. A popular cultivar, Pera Rio is not compatible with a commonly used rootstock, Swingle citrumelo, and a Valencia interstock is used (Figure 18). Trees are also produced in plastic pots, which are placed on elevated tables and can be re-used. Trees are removed from pots and supplied to growers in biodegradable socks (Figure 19 and 20).

Budwood multiplication is done in a standard size pot and only one budwood shoot per tree is produced. From each tree 10 buds can be harvested 3 – 4 times. According to Juliano Ayres, Citrandarine 1710 is a very good rootstock for their conditions. It is *Phytophthora* tolerant with good yield and fruit quality.

Figure 15. Pictured in front of Citrosol offices are Hennie Prins (Stargrow) on far left, Juliano Ayres third from left, Paul Fourie, Jan van Niekerk and MC Pretorius. White clothes and caps provided by Citrosol as special visitor and employee attire.

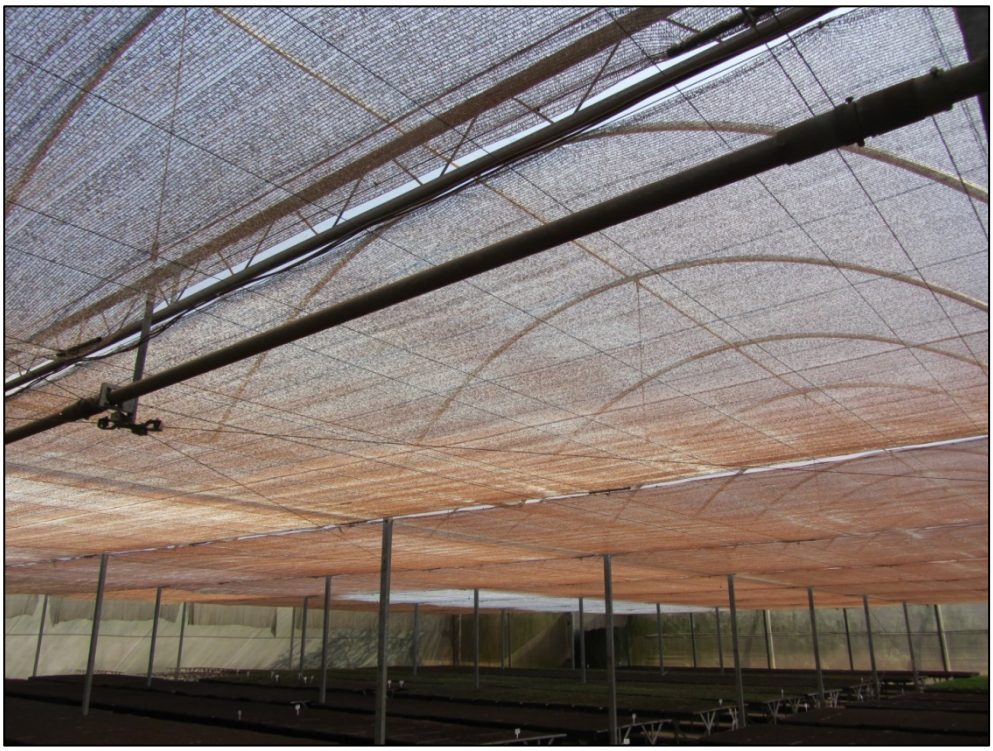


Figure 16. Aluminium netting drawn closed inside a greenhouse to further reduce the temperature.

Figure 17. Cement floors and raised benches with citrus trees as seen inside the greenhouse.

Figure 18. Bent Swingle citrumelo rootstock with grafted Valencia interstock and Pera Rio cultivar.

Figure 19. Trees in re-usable plastic pots.

Figure 20. On right, crate with trees in re-usable plastic pots and on left, crate with pots removed and tree roots inside biodegradable socks.

Notes and recommendations following the nursery visits:

1. Disease-free citrus tree production in nurseries in insect-secure structures that are remote from citrus production areas is one of the cornerstones of the Brazilian citrus industry's success against dreaded diseases.
    a. A cost-benefit analysis is needed to motivate and justify the structural and procedural changes that need to be implemented by the South African citrus nurseries.
    b. All nurseries experienced higher tree production rates in greenhouses compared with open-ground propagation.

2. Statutory regulations prescribe minimum guidelines and nurseries are regularly inspected by government inspectors to ensure compliance.
3. Relatively few cultivar options are available, compared to the South African situation.
4. Economy of scale management has been optimized: for example, separate water and fertilizer irrigation; a greenhouse unit will be planted in full and simultaneously so that similar size and age plants can be managed more efficiently.
5. Regular *Phytophthora* tests are conducted and trees destroyed in rows that test positive; treatment is not an option as susceptible rootstocks are commonly used.
6. Extremely strict vehicle and human traffic protocols are in place to safeguard the biosecurity of nurseries.
    a. The highly stringent measures regarding sanitation of workers and equipment followed in the Brazilian nurseries are points that can be emphasized more in the South African nurseries. These are relatively cheap measures that will improve disease management in the nurseries.
7. Bending of rootstocks prior to or after budding resulted in a much stronger and longer flush from the budded bud; trees are often sold after this single strong flush has hardened off.
8. Light penetration is essential and stronger trees were grown in broader bags or when fewer trees were placed on benches.
9. Raising of pots to higher levels (0.6 m) significantly improved productivity.
10. Nurseries must develop insect monitoring systems (yellow sticky traps) to determine risk and efficacy of control programmes.
11. Murcott and Citrandarine1710 could be interesting rootstock options for South Africa.

Farm and orchard visits

Citrosuco, Matão
16 September 2016
Hosts: Juliano Ayres, Citrosuco staff

Citrosuco is one of the biggest juice producers in Brazil and the farm visited is one of their production units where they produce oranges for juicing. The orchards on the farm is planted at a higher than traditional density of 700 trees/ha; the higher density is a result of the anticipated infected tree eradication programme. Citrus canker and HLB control starts again at the farm gate where vehicles are sanitized, including inside foot mats (Figure 21). Passengers' shoes and hands are also sanitized.

In the orchards canker control is done by planting windbreaks to limit spread, as well as 6 – 8 copper fungicide sprays per year. Leaf miner control is also done extensively.

HLB management is based on the three-pronged approach, which includes (1) planting of disease-free trees, (2) scouting and eradication of infected trees, (3) psyllid management, which includes monitoring with yellow sticky traps and chemical control. For the first two years after planting, scouting for infected trees is done on foot. From then on it is done from a raised platform that is drawn behind a tractor at 4 km/h (Figure 22). At this speed 5000 trees can be inspected per day. If an infected tree is seen, the tree is marked with a red marker ribbon. The row in which the tree is located is also marked. Within 30 days after this detection, a team comes that cuts down the infected trees and sprays the stump with glyphosate herbicide to prevent sprouting (Figure 25). The tree is then removed and burnt. The stump is not removed and a new re-set tree is immediately planted in its place. Inspection of orchards occurs 4 – 5 times per year and the scouts are trained twice a year. The survey and eradication strategy is followed in an orchard until such time that the number of infected trees in the orchard reaches 4%. At this stage the whole orchard is eradicated and replanted. Trees with suspected HLB symptoms are also marked during the scouting and tested, free of charge, by Fundecitrus to determine HLB status.

Psyllid control is done chemically along with monitoring using yellow sticky traps that are placed on poles in the orchard at a height similar to the top third of the tree canopy where psyllid is most prevalent (Figure 26). Fundecitrus also operate these traps independently from the growers. If the numbers show an increase, area-wide sprays are done. Aerial sprays are also employed to good effect at a volume of 3 L/ha. In orchards, chemical sprays start during flush initiation to protect the new flush. The spray volume used is 400 – 600 L/ha. This is done using D3 hollow cone nozzle. Driving speed is at 7 – 8 km/h and pressure of 17 – 20 bar. This spray only covers the outside of the tree and the lower volume does not compensate for any calibration problems.

With regards to CBS control the spray calibration above is slightly modified. Driving speed is reduced to 4 km/h, which increases the spray volume to 1200 L/ha. The spray programme consists of copper sprays at 50 g metallic copper/m^3 tree canopy that are alternated monthly with QoI fungicide applications. A total of 6 sprays are applied per season. Mite control sprays are done at 2000 L/ha, which is achieved by further reducing the driving speed to 2 km/h. To put the spray volumes in perspective, trees are 3 – 3.5 m high. Spray volumes are therefore much less than used in RSA.

Figure 21. Entrance to Citrosuco farm with wheel bath and spray nozzles pictured that is used to spray down vehicles entering the farm.

Figure 22. Raised platform with two scouts drawn by a tractor that is used to inspect orchards for HLB infected trees.

Figure 23. Citrus leaves with typical HLB symptoms.

Figure 24. Fruit with internal symptoms of HLB, note aborted seed and yellowing of vascular tissue.

Figure 25. HLB infected tree cut down and X cut into stump which were sprayed with glyphosate herbicide.

Figure 26. Pole with yellow sticky trap at the height of the top third of the tree that is planted in the orchard to monitor for psyllid activity.

Figure 27. Spray cart used for insect and disease control spraying at Citrosuco.

16 September 2016
Cambuhy Agricola Ltd, Matão

Hosts: Juliano Ayres, Cambuhy staff

Figure 28. Juliano Ayres pictured with Cambuhy manager at a new planting of citrus trees.

This estate spans 14000 ha of which 6400 ha are citrus orchards. The remaining land is planted to rubber, coffee, sugar cane and natural forest. In total 650 people, which include harvesters, are employed.
Their HLB management is also based on the three-pronged approach, as well as an area-wide system where they also co-operate with neighbours to manage the disease and vector. They have annual meetings with their neighbours to plan HLB management. On a monthly basis, Cambuhy sprays, at their cost, 200 ha orchards of their neighbours. They also spray backyard trees and eradicate abandoned orchards. For biocontrol of the psyllid, *Tamarixia* is also released in the neighbouring properties, especially where the offer of chemical control is rejected. Prior to psyllid management in neighbouring orchards, HLB incidence increased at an alarming rate. This practice, as well as the diligent three-pronged approach and area-wide management practices were attributed to the successful management of HLB. The HLB management programme costs 8% of total input costs, with 1% of this cost spent on the psyllid control at the neighbours. This intensive HLB management system is very successful with only 0.1% of trees on the estate being infected.

Orchards are scouted for HLB symptoms every 2 months, using the system described above, including strict eradication of diseased trees. Vector control sprays are done every 1-2 weeks; more regularly at the edges of the farm. Aerial sprays are also used when psyllid counts exceed thresholds. For timing of the latter, they use area-wide scouting information as provided by Fundecitrus. Up to an age of 2 years, trees are sprayed only with systemic insecticide. After planting young trees receive 3 to 4 imidacloprid applications on top of the systemic insecticide sprays. In order to keep up with this chemical management programme they use 40 spray machines that are operated continuously. Spray application volumes vary according to the target pest. Psyllid control is done at 800 L/ha, CBS at 800 – 1000 L/ha with mite control done at 3600 L/ha. Trees used for new plantings are also obtained from certified nurseries and are free from HLB. Planting densities are currently at a dense 6.5 x 2.2 m, to compensate for the HLB eradication programme. However, this is regarded as too dense and it will be changed to allow more space between rows.

CVC (Figure 29) was not that commonly observed, and is effectively controlled through insect vector control as well as planting of disease-free trees from insect-protected nurseries. Leprosis (Figure 30 and 31) also occurs on the estate. Leprosis is difficult to control, and mainly involves controlling mites and pruning of infected trees.

Figure 29. Leaves displaying typical CVC symptoms.

Figure 30. Fruit with leprosis symptoms.

Figure 31. Shoot displaying leprosis symptoms.

Notes and recommendations following the farm visits:

1. HLB management must incorporate all aspects of the three-pronged approach, which includes (1) planting of disease-free trees, (2) scouting and eradication of infected trees, and (3) psyllid management, which includes monitoring with yellow sticky traps and chemical control. To be effective, none of these can be done in half measures.
2. Neighbouring farms, neglected orchards, backyard trees and alternative hosts of ACP can greatly compromise the success of HLB management programme and control efforts must include these to be successful.
3. The management of HLB must be done on an area-wide scale to be effective.
4. Regular training of employees in the recognition of HLB and the management practices is important.
5. Preventative disease control measures include very strict human and vehicle sanitation protocols. Whilst these are specifically aimed at preventing the incursion of Citrus Canker, these measures also create a certain discipline highlighting the importance of all disease and pest control measures.

6. Whilst fruit is produced for the juice market with less stringent quality and phytosanitary thresholds, spray application at lower spray volumes and faster tractor speeds are much more time efficient and less costly compared to the high volumes employed in South Africa.
7. The research, technical and coordinative support from Fundecitrus is pivotal in the successful HLB management.
8. A trial site of Citrandarin 1710 was seen and it appears to be a good rootstock.

6.4 **International Congress of Entomology (ICE), Orlando, USA, 20 September - 2 October 2016.**
Martin Gilbert

Itinerary

20 Sept – SA336, Cape Town to Johannesburg; LH573 Johannesburg to Frankfurt

21 Sept – UA9065 Frankfurt to Orlando, Florida

1 Oct – UA221, Orlando to Washington; UA7228, Washington to Johannesburg

2 Oct – SA367, Johannesburg to Cape Town

Places visited

1. University of Florida, Citrus Research and Education Centre, Institute of Food and Agricultural Sciences, Lake Alfred, Florida.
2. International Congress of Entomology, Orlando, Florida.

1. Citrus Research and Education Centre, Lake Alfred

The object of visiting this facility (in company with Wayne Kirkman and Sean Thackeray) was to see at first hand the situation regarding Huanglongbing (HLB) (also known as Asian Greening) in Florida and to find out if research had yielded any promising results in combating this extremely serious bacterial disease. This disease has yet to enter South Africa although it occurs as close as northern Tanzania.

Our guide around the research station was Dr. Megan Dewdney, associate professor in plant pathology and an extension specialist. At the citrus plantings on the facility grounds, the presence of the Asian Citrus Psyllid (ACP) *D. citri*, and symptoms of Asian Greening were obvious (see photos). There were also many symptoms of citrus canker and citrus leaf-miner (which is known to aid in the spread of canker). Despite the fact that HLB is widespread throughout Florida, emphasis is still placed on controlling ACP as much as possible. This message has to be reinforced with local growers. Continued control of the psyllid vector by growers is vital as research has shown that trees decline and die quicker with increasing disease load. Therefore, any opportunity must be taken to control ACP when present and prevent increase in disease load in a tree.

Figure 1. Severe HLB symptoms on 3-year-old citrus

Figure 2. Asian Citrus Psyllid (ACP) and citrus leaf-miner

Figure 3. Severe leaf-miner symptoms.

Research into HLB in Florida has focussed both above and below ground. One aspect of above ground research is that of thermotherapy where trees are subjected to steam which has been shown to kill *Liberobacter asiaticus* and so to clean up the tree under controlled conditions. However, research is ongoing in order to try to develop a practical system of heat treatment. The exact time and temperature of treatment to give reliable results has not yet been confirmed. Further problems are associated with the inability to culture *L. asiaticus* and determining live vs. dead bacteria. The method though shows considerable promise. Other research work and aspects of ACP behaviour are detailed below and in the ICE section of this report.

It has been found that *Liberibacter asiaticus* infects citrus tree roots before symptoms are visible. 30-50% of fibrous roots are lost before visible foliar symptoms occur. 70-80% of roots are lost as the tree canopy thins. There is a need to preserve roots by minimising stress and optimise the ground pH to suit the rootstock concerned. Irrigation should not be excessive and soils should be well drained. Although root growth is initially stimulated by the presence of HLB these roots do not persist and any short-term gain is soon lost as the tree progressively loses condition. This temporary root growth occurs at the expense of fruit load. In order to grow citrus in the face of HLB infection, growers try to get their trees into production earlier (from 5 years down to 3 years).

We were able to meet Dr. Larry Duncan, professor of nematology, in order to discuss his work on entomopathogenic nematodes (EPNs) and entomomopathogenic fungi (EPFs). He is working on finding naturally-occurring EPNs and EPFs and has so far found 15 species of EPNs and 7 of EPFs in Florida groves. Another aspect of his work is to try to preserve naturally occurring EPNs by modifying the orchard planting in order to preserve sufficient humidity for their survival without causing problems to the citrus tree. Differing orchard practice regimes are being investigated e.g. daily irrigation and fertilization vs. conventional periodic irrigation and granular fertilization, and their effect on EPNs and EPFs. Altering the soil properties may also be of value.

2. International Congress of Entomology, Orlando, Florida

This congress took place at the Orlando Convention Centre. Approximately 10 000 delegates were present. There were many concurrent sessions so choices were made as to which presentations to attend. Notes were compiled at the most relevant talks and are presented below along with the name of the presenter.

Mamoudou Setamou, *Texas A&M University* - Effects of wind speed and direction on the host selection and field distribution of Asian citrus psyllid

Both ACP and HLB exhibit strong edge effects in citrus groves with higher ACP densities recorded on southeastern borders corresponding to dominant wind directions in Texas. Incidence is higher at the upwind side of an orchard. When it is windy the psyllids “hunker down” and orientate themselves into wind. When the

wind abates they fly upwind because they are pointing in that direction. The planting of windbreaks on the upwind side of orchards may therefore prevent or reduce further upwind spread. ACP densities were strongly related to trap height with >90% of all adults recovered from traps placed at <8 feet high. The highest numbers of ACP were caught on traps placed at 4 and 6 feet high.

Sandra Allan, *USDA - ARS, Gainesville, FL* - Examining color vision in the Asian citrus psyllid

ACP is strongly influenced by visual cues. A better understanding of the role in visual orientation of psyllids can provide insight into surveillance and management. Yellow traps are in standard use for monitoring of ACP but these are poor at catching psyllids at low densities. There was a peak in sensitivity to green / yellow with four photopigments being involved. In assays involving flight, attraction was strong to a UV light emitting diode and to ultraviolet transmitted light only with strong responses also seen to yellow-green. Exclusion of UV decreased attraction to both yellow and green targets with a larger decrease observed for yellow targets. Polarization did not appear to affect attraction of ACP to targets. Age affected attraction of ACP to yellow and green targets with greater attraction at 1 day of age compared to 4-7 day old psyllids.

Lukasz Stelinski, University of Florida, IFAS - Patterns and drivers of Asian Citrus Psyllid movement behavior

D. citri tends to move upwards and towards light. The species has been found to disperse up to 2 km and is capable of 3 hours of continuous flight. Immatures are more prone to disperse than mature individuals. Infected ACPs fly more readily than uninfected individuals and are preferentially attracted to already infected trees. Abandoned orchards are a particular problem in Florida in that they serve as a source of inoculum of HLB. A drop in barometric pressure suppresses flight activity and may aid survival during bad weather. Spraying of the borders of orchards is seen as an important aspect of preventing spread of ACP as are the planting of windbreaks. The UV phototaxis displayed by ACP will encourage movement to the tips of branches where there is new soft flush suitable for feeding and oviposition.

An interesting experiment had been conducted comparing different ACP management regimes. These were classified as: managed (rigorous ACP control), organic (no chemical fertilizers or sprays), abandoned (no inputs at all) and intermittent (some ACP control but not complete i.e. a cheaper programme). When the experiment was evaluated it was found, perhaps surprisingly, that the “intermittent” orchard had the highest incidence of HLB. The orchard where control was rigorous had the lowest incidence of ACP. The abandoned orchards had the second highest incidence of ACP and the organic orchards had a lower incidence than the abandoned orchards probably due to the lack of chemical fertilizer suppressing the production of flush. The “intermittent” orchards were thought to have the highest incidence of HLB because of the destruction of some degree of biological control without eliminating the psyllid. Also a fully managed commercial orchard would have chemical fertilizers applied and therefore abundant flush. So the message was clear, control of ACP has to be rigorous otherwise a farmer may be indirectly increasing the incidence of HLB unintentionally.

Xulin Chen*, Department of Entomology and Nematology, University of Florida, Immokalee, FL* - Functional response and implications for mass rearing of *Tamarixia radiata* (Hymenoptera: Eulophidae), parasitoid of the citrus greening disease vector *Diaphorina citri* (Hemiptera: Psyllidae)

This was an extremely interesting talk as we don’t often (if ever) consider biological control as a practical measure when dealing with a disease vector. However, it was not good news as percentage parasitism of ACP by *Tamarixia radiata* was found to decrease with increasing host patch size. Parasitism was highest with 10 hosts at 43% and least at 18.5% with 60 hosts. More parasitoid probing was observed on ACP-infested branches which were free of previous parasitism. Host marking by *T. radiata* reduces effective parasitism in an orchard by also causing nearby unmarked hosts to be rejected by a subsequent parasitoid individual visiting the host patch. How this obstacle to biocontrol can be overcome is not known.

[This probably accounts for why, in South Africa, parasitism of our *Trioza erytreae* is always poor.]

Jawwad A. Qureshi*, Department of Entomology and Nematology, University of Florida, Immokalee, FL* - Integrated use of conventional and organic insecticides and biological control for management of Asian citrus psyllid

Three separate organic programs, organic insecticides applied alone (program 1) or with horticultural mineral oil (HMO) (program 2) and insecticidal soap (program 3) were compared with one conventional program for impact on ACP and beneficial insects including release of parasitoid *Tamarixia radiata* in bearing citrus in southwest Florida.

During the dormant winter season, Pyganic (organic pyrethrum) alone or with 435 oil or M-pede applied in November, December and January and Danitol (fenpropathrin) applied in November and January all provided significant reduction in ACP with residual effects lasting longer for Danitol. Pyganic with 435 oil or M-pede

performed better than Pyganic alone. Organic programs 2 and 3 rotated organic insecticides with 435 oil or M-pede resulting in 50% reduction in use of organic insecticides while providing better ACP control than program 1 with organic insecticides only. However, ACP was reduced more by the conventional programme. *Tamarixia radiata*, ectoparasitoid of ACP nymphs was released in all programs but recovered more from ACP nymphs in the organic program than the conventional program. Monthly applications of Pyganic with 435 oil and M-pede during dormant season and rotation of organic insecticides with oil or soap during growing season appear to be reasonable options for ACP management in organic citrus.

The treatment threshold used is 0.1 ACP / yellow trap. *T. radiata* was badly affected by the conventional spray progamme. The untreated control showed 20-30% parasitism.

Philip Stansly*, Department of Entomology and Nematology, University of Florida, Immokalee, FL*

Strategies for bringing young citrus trees into production in the face of HLB

Florida citrus production has plummeted by almost 50% in the last 10 years, from 345 000 ha in 1998 to 203 000 ha in 2010. ACP was first identified in Florida in 1998 and HLB presence was confirmed in 2005. The soil applied systemics are the most effective with heavy dependence on neonicotenoids in protecting young citrus trees.

Despite this, many Florida growers still maintain yields in spite of Huanglongbing (HLB) by increasing inputs of insecticides and nutrients. Consequently, focus is shifting to health and profitability of the next generation of trees. Area wide Asian citrus psyllid (ACP, *Diaphorina citri*) management and new planting systems are needed to maintain tree health and bring new blocks into rapid production.

The research work focused on the use of metalized UV reflective polyethylene film as ground cover to repel ACP from newly planted citrus in Florida. This exploits the UV-positive phototaxis of ACP as noted in some of the research detailed above. Metalized mulch significantly reduced ACP populations and HLB incidence compared to whiteface mulch or bare ground. The tree condition in comparison to the control was very encouraging with the differences very marked. The mulched trees looked like completely normal trees. This could be a very positive way of combatting ACP in young plantings.

The mulch plus drip system increased soil moisture, reduced weed pressure, and increased tree growth rate. Chemogation proved more efficient than soil drenches for delivering systemic insecticide after root system establishment. Composted horticultural waste incorporated into the beds prior to planting increased cation exchange capacity, water holding capacity and microbial diversity, further accelerating growth. Thus, metalized mulch and chemogation can augment current ACP control measures for young trees based solely on systemic insecticides. The potential to more than compensate for additional costs by shortening time to crop profitability has inspired several large-scale trials in young citrus plantings threatened by HLB.

Alan L. Knight*, USDA - ARS, Wapato, WA* - Does acetic acid finally give us the focus for following female tortricids?

Development of sex pheromone lures for all the major tortricid pests of horticultural crops worldwide was a major accomplishment that significantly improved monitoring and management of this moth group. Pest managers developed predictive correlations based on the timing and magnitude of male catches to predict their seasonal population dynamics. However, the dispersal capacity and often broad host range of these species reduces the utility of only estimating male populations. Furthermore, sex pheromones have been widely adopted for many of these pest species and this technology negatively influences the ability to trap males.

Focus is finally shifting to catching females. The big advantage of catching females is that they can be monitored under (male) mating disruption regimes. There is a better correlation between trapped females (rather than males) and subsequent larval density in a host.

There are many tortricid pests in the world. They typically lay eggs 48-72 hours after eclosion. Multiple mating is not necessary for full egg complement. The question was asked “Do adults feed?” Certain moths are known to suck up fluids and may be attracted to fermenting sugar. There is not much information available regarding this topic. From an evolutionary viewpoint it would be advantageous for adult moths to be able to gain energy from other resources to aid dispersal. The best bisexual lure would contain a host cue and a food cue. Pear ester has been identified as a potent attractant for codling moth.

Eduardo Fuentes-Contreras*, Centro de Ecología Molecular y Aplicaciones Evolutivas en Agroecosistemas Núcleo Milenio, Universidad de Talca, Talca, Chile* - Boosting bisexual moth catches of oriental fruit moth in sex pheromone-treated orchards

In Chile, early monitoring of Oriental Fruit Moth (OFM) during the 1940s was carried out using terpinyl acetate (TAS) + sugar baits. Since the 1970s the OFM sex pheromone could be used for monitoring. In the 1980s mating disruption began to be used as a control material. However, monitoring of OFM under the MD regime became problematic due to only males being caught (similar to FCM).

Work has been undertaken recently to attempt to improve female catches of OFM in traps and can be summarized as below:

ATTRACTANT LURE(S)	OFM MALES CAUGHT	OFM FEMALES CAUGHT
OFM pheromone	20	0
TAS + sugar	0.6	7.8
OFM pheromone + TAS	19.8	12.8

The mixture of OFM pheromone + TAS is able to monitor both sexes of OFM efficiently.

[Could this have application to FCM monitoring?]

Pablo Liedo, Arthropod Ecology and Pest Management, El Colegio de la Frontera Sur, Tapachula, Mexico - Two in one: Use of sterile insects to transmit entomopathogenic fungi and induce sterility -

Beauveria bassiana was used to infect sterile insects including Medfly, tsetse fly, codling moth and pink bollowrm. The kill of the steriles has to be slow, 4-5 days is ideal. In the case of flies there should be enough time for the flies to join leks and spread the disease and/or mate. Mating accounted for an 80 % fungus transfer level. The sexual performance of treated sterile males was not affected during the first four days' post-treatment. Field trials confirmed transmission to 44% of wild *Anastrepha* flies captured in traps. There was no conidia transmission to non-target organisms. The very small amount of fungi conidia required and the possibility of affecting the wild population through sterility induction or mortality due to fungi infection make this approach worth further consideration and development.

En-Cheng Yang, Department of Entomology, National Taiwan University, Taipei, Taiwan - The development of monitoring systems for pests based on internet of things (IOTs) technology

In Taiwan, Oriental Fruit Fly (OFF) is a serious pest and is monitored by a system of 613 traps spread over the island. Visiting all the traps takes 10 days and a lot of time/manpower. Research is being conducted to develop infrared sensors in traps that will count the flies upon arrival in the trap. The results presented were very promising with a very good linear regression fit. Obviously other insects / species must be excluded from the trap but the use of a selective lure has yielded promising results.

Bodil Cass, Department of Entomology and Nematology, University of California, Davis, CA - An ecoinformatics approach to citrus pest management in California.

The citrus industry in California, USA, is facing major disruption from the invasion of the Asian citrus psyllid, which vectors the devastating citrus disease Huanglongbing. Shifts in pesticide use to suppress psyllid populations may cause substantial changes in arthropod pest dynamics. A new, comprehensive database includes up to 11 years of records of insect pest and natural enemy sampling, pesticide use, plant nutrition, yield, weather, and geographical descriptors, collected from 330 orange and mandarin groves in the San Joaquin Valley. Present analyses focus on factors affecting arthropod pests directly damaging to the harvested citrus fruit, including citrus thrips, California red scales, katydids and cutworms. Data collection is ongoing, as is the input from farmers and pest management consultants about the most pertinent directions and questions for the ecoinformatics analyses. Ecoinformatics affords enhanced statistical power and the ability to scrutinize many variables from the full range of growing conditions. Evaluating combined data sets with sampling methods, and spatial and temporal scales directly relevant to farmers, has the potential to provide tailored information in a timely manner, to increase productivity, and improve pest management practices, especially in rapidly changing environments.

Summary

More talks were attended than are reported upon. I have selected those with the most relevance to our situation especially bearing in mind the threat of Asian Citrus Psyllid (ACP) and Huanglongbing (Asian Greening) to our citrus industry. What was encouraging was to be able to see and hear how the Floridians are living with HLB. The use of UV reflective mulches was particularly interesting and promising in the effective suppression of ACP.

Acknowledgements

I would like to thank the Board of Directors and management of Citrus Research International for giving me the opportunity to visit Florida. It was a privilege to be able to visit Lake Alfred Research Station and attend the International Congress of Entomology 2016.

6.5 Citrus production practices in Queensland, South Australia and Victoria (Feb 2017)

Tim Grout

I was invited as a keynote speaker to the Citrus Technical Forum in Mildura, Victoria so used the opportunity to catch up on citrus production in other parts of northeast and south Australia. Production practices were similar in all areas visited except that South Australia has not yet got Queensland fruit fly *Bactrocera tryoni* and they are considered fruit fly free. I have therefore grouped the information according to the practices for easier reading. I visited the Munduberra citrus region in Queensland and various sites along the Murray river in South Australia, New South Wales and Victoria. The Munduberra climate is similar to Letsitele but the Murray river is more like Citrusdal or the lower Orange river.

General

Alan Jenkins said that citrus growers pay $3.20 per ton for research. This is matched by the government. There are 24 000 ha of citrus in Australia. Citrus growers in Queensland all use consultants who do their own monitoring. The trend is similar in the Murray river production areas.

Pest management

Etoxazole (Smite in South Africa) is used for mites. Spined citrus bugs are important but are sporadic. Few growers are requesting *Aphytis* releases for the control of red scale in citrus because of the widespread and effective use of Movento. The prevalence of loopers has increased in the recent past but they could not say whether this was associated with increased use of neonicotinoids. Loopers were controlled with Runner (methoxyfenozide). Chlorpyrifos 60 ml/hl plus 0.5% oil is often used for mealybug and katydid control and thiomethoxam (Actara) sprays are sometimes used for thrips. Their Movento treatments also control thrips but their dosage is 30-40 ml/hl whereas ours is 10 or 20 ml/hl. Gall wasp is a serious indigenous problem (Fig. 1) that has now spread from Queensland to all production areas in the country. There is only one generation per year but it attacks the new spring growth and results in woody branches that do not bear much fruit. There are big differences in the susceptibility of different cultivars to the gall wasp with most mandarins being quite tolerant and Eureka lemon being damaged less than Lisbon. Clothianidin is being used as a soil drench (via drippers) for gall wasp and is also controlling Fuller's rose beetle in the soil. Chemical stem sprays with long residual pyrethroids must be used to prevent Fuller's rose beetle access as part of the protocol for export to China, Thailand and S. Korea. Automatic apparatus on the front of a tractor (Fig. 2) is used for this purpose but can result in drift onto fruit and branches and can lead to red scale problems. Surround (kaolin) is often sprayed with DewWet adjuvant to improve coverage. It is used to prevent sunburn, young tree stress and bleaching of fruit colour. They use Movento sprays to prevent red scale repercussions from the kaolin.

Figure 1. Navel orange branches infested by gall wasps

Figure 2. Automatic apparatus for spraying chemical stem bands for Fullers rose beetle

Fruit fly management

Queensland fruit fly has now reached Melbourne and is high risk for stone fruit, feijoa, pome and zucchini. It is present in Mildura, VIC but has not reached Renmark in South Australia yet and the latter region is considered fruit fly free. Area-wide control of Queensland fruit fly involves the hanging of MAT devices 3 times a year. They apply fruit fly bait by quad bike using 15 L per ha. They recently obtained temporary registration for abamectin as a toxicant in fruit fly bait at 25 ml abamectin per 100 L. The addition of a thickener (Xanthan gum) does help prolong the attractiveness of the bait but they add it to water the day before at 2.5 g per L water because it is so difficult to dissolve. This is then mixed with the lure and toxicant the next day.

At a local ag-chemical distributor there was a presentation on Fruition: Dick Drew's new fruit fly trap being sold by AgNova www.agnova.com.au. The lure contains fruit volatiles that are more attractive to gravid females. These are put into a gel that lasts 8 weeks. The blue colour of the gel changes with time so that when it is transparent it needs to be replaced. Two interlocking circles of sticky blue Corex provide a substrate for the trap. Cobalt blue was found to be an important colour for this species. e.g. 92% females attracted and of these 90% were egg laying. But these traps must be used with protein baits to kill the immature adults and males. They did not have good results with GF120 for QFF. Xanthan gum makes the protein 3x more attractive. Packs of 15 or 2 traps. For monitoring use 15 per ha at 1.5 m high, just inside the canopy. For control use 30 to 50 traps per ha for moderate to high susceptibility. After 8 weeks replace whole trap. $20 each. $600 per ha for 8 wks. Would be useful to determine what the female levels are. Dan Papacek recommends continual baiting throughout the season without breaks.

Netting

I visited groves of citrus under net with Craig Shrewsbury of Fruit Doctors in Queensland who reckoned that UV-B levels were 30% less under net and less phenolics are produced in citrus plant tissue. The increased populations of mealybug under net in both Australia and South Africa suggest that this may be due to the presence of less phenolics, which are normally produced for sunscreen purposes. Under net they also see more flattids and more *Pezothrips kellyanus*, a species attracted to pollen. Worse residues under net may also be due to less UV-B. The mealybug here is mainly citrophilus. Apart from mealybug, mites are also worse under nets and they have recently found a *Heliothis* sp. under nets that has not been considered a pest before. Under nets they had to change their management and monitor water use more closely. They claimed a saving of 25 to 30% water and trees were less stressed, but if too much water was applied they got vegetative growth and less flowering. Due to increased vegetative growth they topped the trees with a machine twice a year, keeping the trees at about 4 m high for picking purposes because the nets were 6 m high. If drape nets were used, they would top the trees just before the spring flush so that the resultant new growth would act as a soft surface for pulling the nets over. Their main reason for using nets was improved packout with less wind damage and sunburn (talked of +20%). Nets were considered an advantage in reducing frost down to a temperature of 0°C when they were generally 1°C warmer, but below freezing the nets could have an adverse effect and

cause more freeze damage. Fruit colour under nets was not considered as good as in the open. The cost of installing nets in Australia is around R600 000 per ha so one grower who had bought a farm with nets thought he would rather buy more land at that price. They do not see a size improvement of Nadorcotts under net. The best rootstock for use under nets is *Poncirus trifoliata* which results in better Brix than Citrange. Overhead sprinklers were being used by one grower under nets to protect young Nadorcott trees from frost (Fig. 3). Only white overhead nets were seen, though the sides were sometimes black. One grower had a mixed block of mandarins under nets with rolled up black nets attached below the white net that could be dropped as curtains around Nadorcotts during flowering to separate them from the other mandarins and prevent bees from causing seeds (Fig. 4).

Figure 3. Young Nadorcott trees under net protected from freeze damage by overhead sprinklers.

Figure 4. Nadorcott trees (by short ladders) that can be curtained off from other mandarins with the black net during flowering.

Cultural practices

Some citrus farmers grow lucerne or white clover in the interrows to reduce the heat. These can also be used as an indicator of whether there is enough water present because the clover wilts at 30 kPa and citrus at 60 kPa. Spotted gums *Corymbia maculata* (Fig. 5) are used for windbreaks on some farms with one tree planted at the end of every second citrus row which are 6.5 m apart. It is considered less competitive than *Casuarina*. NAA 200g/L at 60 ml per L with PVA paint was being used in a trial to reduce regrowth but they found it almost as expensive as removing suckers manually. Ian Garden said they should add mineral oil to the PVA for better uptake. They use leather gloves to break off suckers manually. If they cut close to the main branch they do not get shoots afterwards so they intentionally leave a 20 cm stub (dirty cut). In Spain when Sunny is used to stop shooting they have to adjust their fertiliser rates. In a new orchard, young trees were planted in holes in black plastic mulch using a machine at the rate of 1000 trees per day. Every two rows of citrus they had a row of sorghum (called Sudax) to serve as a mini-windbreak while the trees were very small (Fig. 6). One farmer preferred microsprinklers to drip and claimed to get 20 tons more per ha. His annual pruning method for navels was to cut a door in the NE side of the tree one year and put a door in the SW side the following year.

Sometimes he would also make a window in the top. For mandarins, some growers used a vase shaped tree by taking out the centres and leaving four main branches.

Figure 5. Spotted gums planted at the end of every second row as a windbreak

Figure 6. Young citrus trees planted in plastic mulch with one drip line and sorghum windbreaks

Notes from talks given at the Citrus Australia Technical Forum attended by 370 people

<u>John Chavarria – From plate to tree: World citrus trends</u>

Markets drive the varieties and their profitability. Nules – returns are below the cost of production but Nadorcott returns are over 1:4. Australia is a high cost of production country. There is a need for differentiation between China, Philippines, Vietnam, Thailand and S. Korea markets. There are growth opportunities in China. Demand for navel oranges is declining but pigmented oranges sell well e.g. Cara Cara + blood type. Asia and US/Canada market wants a Cara Cara with external blush. Need better size and colour in bloods. Tango and Nardorcott seed counts are important in Western markets but not yet in China. In Australia, Imperials are important for the local market but Nardorcott may replace it. Lemons are popular for new health requirements so try planting some seedless lemons. However, cold treatment for exports is a challenge.

Red oranges are not yet imported by the EU. Kirkwood red – peel is stronger than Cara Cara. M7 navel has a very good taste profile. FJ navel is early. Brown navel is very sweet. Mandarins in Western markets; seedlessness is important. In the US 10% of fruit with 1 seed or 4% with 2 or more. Must be easy to peel and soft. Good brix 11.5 perfect but not too high! Tango 2 – 3 weeks earlier than Nardorcott seedless (SA) similar to Tango. Afourer SL is from Queensland. The Asian market must have 13+ Brix and can accept low acid. May have 2 standards for local fruit of export. Mustn't have Chinese on the carton as this makes it look cheap. Clementines are too soft for China. Smooth skins are preferred. Varieties for China include Daisy LS from US and Orri. Orri is difficult to grow with bearing problems. Spanish growers had to pay US$60 per tree to legalise Orri trees that were planted illegally. Kinnow LS is also of interest to Asia. Summerina is late mandarin. Local market: Decopon + Gold nugget. 2 PH SL lemon bears well. Summer Prina is late Spanish Lemon. Orri needs girdling.

There is hope for rootstocks resistant to HLB but the market does not want GM rootstocks. Rootstocks: C22 for original soils. Patricia Barkley rootstock. IVIA selections contact Maria Forner.

Nathan Hancock – 2016 Citrus tree census & Production Forecast 2017 to 2032

Citrus production in Australia now covers 24 000 ha and has increased by 8% since last year, but some orchards were just not included before. 28% of businesses comprise 79% of the area. Riverina still largest production area - also processing there. New plantings average 750 ha / yr. Lemons, mandarins and navels have increased. Imperial, Murcott and Nadorcott comprise 80% of the mandarins and 23% are Nadorcotts which is an open cultivar in Australia. They expect a 30% increase in mandarins over the next 10 years. Will be 20 000 tons by 2027. Red navels form most new orange plantings. Mid- and late-season navels are declining and need to be supported. Most lemons are planted in Queensland.

Geoff Cornwell – Exirel by DuPont: new chemistry for pest control in citrus

Expecting registration of cyantraniliprole (Exirel) shortly for chewing and rasping insects on citrus. Target pests are Thrips, light brown apple moth (LBAM) and Fullers rose beetle. Coragen, the sister product, is just for chewing insects. Thiamethoxam is good against Kelly's citrus thrips (KCT). Exirel stops insects from feeding very quickly. But they take longer to die. Movento has a good impact on KCT but the dosage is double that in South Africa. Exirel kills both larvae and adults. For LBAM it can kill larvae as they chew out of the egg. Exirel is translaminar and they get local translocation in the xylem. Exirel at 75ml/hl = Lorsban 100ml/hl against LBAM. Exirel targets muscle fibres and interferes with Ca ion flow so the insects end up starving. Can help manage FRB adults for 2-3 weeks. IPM compatible. Good information on a range of lab tests including parasitoids. 75ml/hl is used for all 3 pests with 2 applications per crop per season permitted. No preharvest interval required but maximum application volume is 4000 L/ha. FRB would apply December – January. Other trunk band sprays are usually applied late in season.

David Daniels – Agrichemical use in the Australian citrus industry – implications for trade, practises, permits & reviews

HIA publishes MRLs every 6 months. They test fruit samples with about 26 chemicals being picked up in their generic tests. Chlorpyrifos was the highest preharvest residue detected on 25% of all samples. Australian MRL for dithiocarbamates went from 0,2 to 7 ppm. Bifenthrin is used as a soil drench for weevils. Methidathion is going out. Minor use permit has been approved for abamectin against fruit fly in bait applied 6 times per year (Aruna showed that this does not work for *Ceratitis* spp.). Omethoate has gone. Clothianidin soil drench permit expires 31 July 2017 but may be renewed. Fluopyram and fluopyroxad fungicides are being used in Queensland.

Jinhua Mo – Gall wasp R&D update

Gall wasp has now been found in gardens in Perth. Chlorpyrifos is most active against adults. Success Neo (=Delegate) and Talstar very effective against larvae. Surround prevents adults from ovipositing: 5 kg/hl 1st spray and 2.5 kg/hl 2nd spray at 4000 L/ha. Systemics: Confidor and Samurai (clothianidin drench). Samurai at 8g and 12g /hl are effective. Movento kills larvae inside the twigs and prevents emergence. Movento used with Hasten at 50 ml/hl rather than mineral oil. Emergence from different cultivars is at the same time.

Craig Swanbury - Best practice control of mealybug — life after chlorpyrifos

Chlorpyrifos run-off kills soil natural enemies for thrips. Prodigy (methoxyfenozide = Runner) works for LBAM. 0.7% oil plus Chlorpyrifos still controls red scale. Perhaps Exirel will work for katydid. Movento plus oil will suppress mealybug. Mealybug is worst in grapefruit where chlorpyrifos is most often used. Spray coverage of oil with multifan machines is very good and can control mealybug. Talstar is only used if there is no fruit on the tree but it probably won't be registered. Samurai must be used after bloom – because of the bees. Navelina is particularly problematic for gall wasps.

Nerida Donovan - Graft transmissible diseases, Auscitrus, diagnostics (NSW – DPI)

GTD – no cure so now working on prevention. Exocortis can reduce yield by 50% on Troyer and 70% on trifoliata.
Must use bleach for viroids. Secateurs can be infected for 12 months. Have a pathogen collection for positives in tests. They clean up new cultivars and send them to the repository. Most nurseries do not disclose where the budwood comes from. Their budwood scheme is not compulsory. California floods their system with clean buds so no incentive to get buds elsewhere. Australia doesn't have enough buds to supply at the moment. Citrus trees cost about $15 each but buds only about 50c. People are probably the worst vectors of GTDs. Captan doesn't work for CBS. Post ACP/HLB arrival will be crucial. Must make CIP compulsory. Would need some aerial sprays to get around farms in 48 h. Much of HLB prevention involves psychology. Organic farmers will have a problem.

Stuart Pettigrew – Biosecurity

Raising awareness of risks. Need to work with pest scouts. Tomato psyllid has just been found in retail nurseries in Perth. HLB in Florida now has cost the US $9 billion. Canker is easier to identify from fruit lesions. Have done some trapping in metropolitan areas. Works with Federal Department of Agriculture funds and does urban surveillance based on a risk-based strategy.
Who does it and who pays for it? One survey can cost $35 000. About $1 000 per site in peri-urban areas.

Judith Damiani - Chief Executive Officer, Citrus Australia

Only 150 km between Papua N. Guinea and N. Queensland. In supermarkets local Valencias are more expensive than Californian navel oranges. Citrus Australia offers an Emerging Leaders scholarship. This covers a PMA course and a visit to PMA USA.

Andrew Robson - Satellite based remote sensing (GIS)

Healthy leaves reflect a lot of NIR. NDVI is the ratio between NIR and visible red light. Satellites can provide down to 30 cm resolution at a cost of $3 800 for 100 km^2. Drones are too accurate for this purpose.

James Underwood – Robotics

Tree volume calculations are being provided by a mobile robot that scans trees. This can show decreasing yield in an orchard over time. With orange trees you only see 1/3 of the fruit with the naked eye. It can see whether the fruit is all on the top of the tree or better distributed.

Craig Shepherd - Capturing images of all horticultural crops 2 ha & above

Can access maps on the internet. Landsat images have been translated. Land use survey app uses GPS on phone. Also have peer review of draft maps. Commodity maps will be very important for biosecurity.

Lyn O'Connell – Department of Agriculture and Water Resources

Has primary responsibility for biosecurity. Citrus is the largest fresh fruit export industry. The Torres strait is 150 km gap north of Queensland. China recognises fruit fly free areas in South Australia for nectarines. The government is giving $200 million to strengthen surveillance, scientific capability and facilities.

Steve Burdette - What is being done to protect the Australian citrus industry? A growers' perspective

HLB – considered the most serious citrus threat. It will be a long time before we will have resistant trees. *Xylella fastidiosa* (CVC) has a lot of hosts and suitable vectors are present in Australia so priority is high. The North Australian Quarantine strategy is doing a good job but there is not enough awareness. They have a

Biosecurity levy and an HLB contingency plan. The EMAI are well prepared to react but they need to include the local community more. There is a need to prioritise both the vector and HLB disease. We need centres of excellence.
We also need to review legislation for abandoned orchards. On farm BS awareness. AusCitrus is putting insect-proof netting on trees to see if it is viable. We need to provide the plants that people are tempted to import. Post entry quarantine: Mickleham facility – 144 ha property north of Melbourne airport with 2000 m^2 greenhouse space and 1200 m^2 shade house. agriculture.gov.au Budwood quality imported via the Melbourne facility is poor which encourages people to find alternative routes.

Darryl Barbour, Dan Ryan and Penny Measham - Sterile insect technology & the national strategy for fruit fly control

SIT –National fruitfly strategy includes action plan. The peri-urban interface is important. Email address for fruit fly at Plant Health Australia fruitfly@pha.com.au Have a consortium approach. $45 million being shared for SIT. Developing techniques for QFF but still need a male only fly. Started doing SIT in 1957 in this area. Field trials in QLD, NSW and VIC. Social research and community involvement is good.

Dick Drew (Griffith University)– Fruition trap

20% of female *Bactrocera tryoni* are considered to have eggs. He worked with Prokopy on trap colour and found that cobalt blue was the best for this species (like the lid on the Sensus trap). Agnova – the Australian company that supported them. Have tried to use the lure in a bucket trap but could not catch enough flies. Used sheets under tree for bait evaluation. Natflav has more attraction for females than AY50 (disputed by Dan Papacek after conducting trials that showed the opposite). Sticky blue traps are $25 each and 40/ha required, plus $10/ha per bait spray for the non-gravid females and males. Has recovered released flies up to 100 km away.

6.6 **5th International Research Conference on Huanglongbing in Orland Florida**
Glynnis Cook

Aim: 1. Visit Prof Dawson Laboratory to see facilities where *Diaphorina* citri is reared and how HLB challenge studies are conducted.
2. ICRHLB conference attendance.

Delegates & attendance: Over 400 delegates and representatives from 24 countries attended the ICRHLB.
Conference Theme: Innovation & breaking the boundaries of convention
HLB impact in Florida: $466 million per year in losses in Florida, $125 million in consumer losses and $341 million in producer income losses

Visit to Bill Dawson in Lake Alfred
Dr Hano Maree and I were hosted by Prof Dawson and we met with various technicians and researchers. Hano spent time with them on his previous visit and has developed good working relationships. Prof Dawson showed us the plant production, ACP rearing facilities and the various glasshouse trials including HLB challenge studies. One room uses metal halide lighting, but most of the rooms use normal fluorescent lighting. The systems are not complex and can easily be duplicated.
Prof Dawson shared with us that the CTV clone approach is going to be implemented in a large scale roll-out to deliver the Spinach defensin antimicrobial peptide. This is being done by Southern Gardens and they plan to plant 400 000 acres. Prof Dawson is not involved apart from assisting in inoculum supply. He is concerned that should this fail, this will discredit the approach. He said the defensin peptide apparently worked well in low pressure trials and less so in high pressure environments. According to him the CTV delivery approach should not be seen as a silver bullet, but should be used in an integrated control approach.

Summary of Introductory Talk on Vector/Disease Management:
Insecticides/Biocides to manage the vector or pathogen
- Governments, universities and multi-nationals are all role players
- Efficacy trials are necessary
- Adding new pest/crop to an existing labels
- Resistance management important

Biological Control
- Difficult to commercialize
- Practical application require management from farmer and consultants

Citrus under protective screen (CUPS)
- Many unknowns
- The real innovation will be by farmers and private companies to see if they can make it commercially viable

Biotechnology
- RNAi: Species-specific RNAi sprays or PIPS for HLB vector
- Gene Editing: introgress desirable genes into populations
- Transgenic trees: immune, resistant or tolerant to HLB (Spinach defensins (SoD2) in process)
- Transgenic/Cisgenic/Gene Edited insects that no longer transmit the HLB pathogen, then add a gene drive to eradicate HLB insect vectors

Breeding
- Rootstocks that convey immunity, resistance or tolerance to HLM in the whole (grafted) tree

Biotechnology hurdles (lessons from the potato industry)
- Meeting regulatory hurdles takes a long time: years
- Must follow regulatory processes (they are there to help)
- Inform industry stakeholders of commercial intentions and timelines: communicate early
- Consider trait stewardship and trait durability for commercial businesses, how to extend trait failures, resistance or other?
- Biotech will take time to be developed, registered and commercially used –years

Insect and Pathogen Biology
- Helps improve chemical and biological control

Traditional insect vector and pathogen management, CUPS, etc will be needed for years –they may be the only solutions we ever have.
We never “solve” pest problems, just manage them. Resistance build-up is always a concern.
If we eradicate one pest, another will take its place.
Sustainable pest management means using multiple methods: biotech is not silver bullet, just another bullet to use in the attack.

Cultural control
The application of epibrassinolide to HLB infected citrus plants reduces the titres of CLas. The results suggest that eBL treatment upregulates defence genes in citrus against CLas and might be useful within an integrated management program.

Growers are adding bactericides (antibiotics) to their disease management including oxytetracycline and streptomycin. Questions remain regarding the level of suppression of CLas that these and other bactericides provide and the level of improvement in tree health that can be expected. Some presentations however reported that trunk injection of oxytetracycline reduced CLas titres, improving citrus yield and decreasing fruit drop, whereas another trial reported poor suppression of CLas.

For potential control of HLB, 46 antimicrobials (non-antibiotics) were screened for effectiveness and phytotoxicity. Despite different modes of actions, 15 antimicrobials, such as Alliette, Carvacrol and P-Cymene, were all effective in eliminating or suppressing Las with both the lowest Las infection rate and titres of the treated scions and inoculated rootstock. The effective antimicrobials identified are potential candidates for control of HLB, either for the rescue of infected citrus germplasm or for restricted field application. Various research groups report screening many antimicrobial peptides for efficacy.

A compound, Zinkicide™ is being tested for potential curative capability against CLas. It is a plant nutrient-based nanoparticle with antimicrobial activity. It can translocate into plant vascular tissue through foliar sprays, trunk sprays or soil drenches. MS3T (T-SOL) is micronutrient based (Zn-chelate) for systemic activity.

A useful screening bioassay for *in planta* evaluation of bactericidal compounds active against CLas was presented. It is a quick assay using detached leaves which can evaluate translocation in phloem, toxicity and bactericidal effectiveness.

Vector control
Kaolin is a white nonabrasive fine-grained mineral that is sprayed onto plants and forms a particle film. It suppresses several species of insects by host selection interference (repellent) and interferes with feeding of ACP. Two kaolin formulations were found to be effective. Since kaolin reduces the number of psyllids and protects citrus plants from insect feeding, application on young citrus planting is recommended as an HLB management strategy, mainly on the edge of the farms.

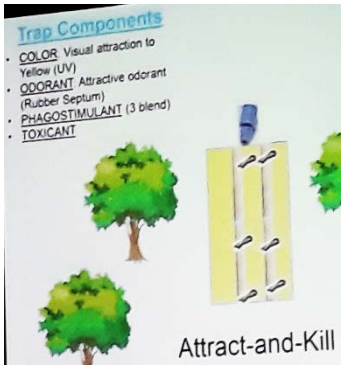
The use of a ground cover of metalized plastic mulch as a repellent is reported to have additional horticultural benefits. The plastic can remain effective for 3 years, but oil containing sprays should be avoided as they cause polyethylene degradation. Costs include the plastic itself and a drip irrigation system. Metalized mulch reduced ACP populations and HLB incidence compared to whiteface mulch or bare ground. In addition, the mulch plus drip irrigation system increases soil moisture, reduces weed pressure, and accelerates growth. Increased growth rate, yield and brix are claimed.

Control of ACP and HLB in urban settings might need to rely on biocontrol. A novel system for infecting adult psyllids with a commercially available pathogenic fungus, *Isaria fumosorosea* (PFR-97) was developed and field-tested. Individual ACP infected with PFR-97 can start epizootics (disease outbreaks) that will decimate psyllid populations on residential citrus and thus lower the risk of immigrating adult psyllids spreading HLB to commercial citrus groves.

Vector control treatments used in São Paulo State, Brazil:
Chemical control
- Nursery (1-5 days before planting) Systemic insecticides (drench application)
- New planting (0-3 years) Spray application (frequency 7-14 days)
- Application of systemic insecticides by drench or trunk (3-4 applications/year)

External actions and regional management (Psyllid Alert System)
Eradication of psyllid plant hosts (citrus or orange jasmine)
Release of *Tamarixia radiata*
Entomopathogenic fungi *Isaria fumosorosea* –adult psyllid mortality >80% under field conditions
Use of kaolin sprays

An attract-and-kill device (sticky trap) which mimics citrus flush colour (lime-green) and is impregnated with a potent pyrethroid was developed. The deployment of 20 to 50 traps per tree reduced ACP population by 60%. Currently it is effective for 12 weeks, but they are improving the stabilizer. At $1.20 /trap this is expensive, but can be used for control on the border rows.



The development of another attract-and-kill device is underway. Adult psyllids are attracted to the trap by colour and odour; after alighting, psyllids are induced to probe and feed by using phagostimulants in a wax matrix attached to the surface of the device. Several chemical blends only demonstrated modest attraction in the field. Acetic and formic acids which originate as breakdown products of plant volatiles including *β*-ocimene and citral (geranial and neral) that oxidize spontaneously in air were found to be highly excitatory to antennae of male and female adult ACP. A mixture of these induced increased probing by ACP. Addition of a third compound (para-cymene) further increased probing in a 3-component blend. Psyllids can then be killed by a contact insecticide or other agent contained in the wax matrix. This system is proposed to be used in new plantings together with kaolin sprays.

Smart traps: 3D printed and developed to trap when >20 ACP on yellow sticky trap and used to collect ACP to test for CLas infectivity. Additional inclusion of LED lights & lures can improve trapping.

Systemic insecticides for young tree management

Soil applied systemic insecticides are most effective to protect frequent flushing of young trees.
Dependence on neonicotinoids (need for additional systemic insecticides and non-chemical strategies).

Rotation of soil applied neonicotinoids and cyantraniliprole most effective at reducing ACP numbers, HLB incidence and increasing growth and yield compared to untreated trees receiving only one mode of action. Cost of cyantraniliprole still prohibitive and toxic to bees. Registered in US, Canada, China and India. Chemigation with drip improved control after first year. Control insufficient under conditions of high ACP and HLB incidence. Additional practices such as UV reflective mulch and area-wide management to reduce ACP numbers and bring trees to bearing age in healthy condition.

Citrus for retail sale in Florida is required to be treated with an imidacloprid-based soil drench prior to sale to prevent colonization by the pests. Data presented suggests that some treatments were failing in the second month after treatment.

Many contact insecticides are effective against ACP, but psyllid eggs or nymphs may be missed if present in or under leaves. The residual life of the insecticide is important for control as eggs hatch and nymphs move onto treated leaf surfaces. The residual impact of weathered residues of 19 insecticides on nymph survival was investigated: Thiamethoxam and the mixtures imidacloprid+beta-cyfluthrin, chlorantraniliprole +thiamethoxam, abamectin + thiamethoxam were found to have longer residual control (45-54 days of greater than 50% mortality) compared to cyfluthrin, fenpropathrin, beta-cyfluthrin, zeta cypermethrin and spinetoram, flupyradifurone, spirotetramat, abamectin, cyantraniliprole, diflubenzuron, fenpyroximate, and abamectin + cyantraniliprole. The pyrethroid insecticides tested had no statistically detectable activity 25 days after application. Of the organic insecticides, spinosad + oil showed greater residual control of *D. citri* compared to pyrethrins + oil, but the effects of organic insecticides were short lived; by ten days' post treatment there was no statistically detectable residual control of nymphs.

Biotechnology approaches

Transgenic citrus rootstock lines that are silenced for a citrus gene that acts as a brake on programmed cell death and defence responses. The silencing signal was confirmed to move into non-transgenic citrus scions, resulting in curing of HLB and possibly enabling long lasting, natural resistance.

The use of the CRISPR-Cas technology as a programmable antimicrobial is being investigated, but delivery also poses a significant barrier. A virus was engineered to deliver the antimicrobial system to bacteria under simulated phloem conditions and evaluate the ability of the virus to persist within the plant host.

RNAi experiments: root application appeared to be dose dependent, desired phenotypes were observed in some, but not all plants.

Breeding

Evidence for dominant gene for HLB tolerance in *Poncirus trifoliata* was presented.
Citron was also found as a common denominator in HLB in pedigrees of tolerant cultivars.
Field trials provided evidence for field tolerance to HLB in some rootstock selections, including US-942, US-1516, and US-802

HLB Detection

Identification of appropriate diagnostic samples in non-symptomatic trees remains challenging. The earlier the detection of CLas infections, especially when asymptomatic or subclinical, the more effective infected-tree removal can be. In a study, HLB symptoms were visible ca. 4 months following exposure to CLas infected ACP, but already detected by qPCR in citrus shoots ca. 21 days following inoculation.

Current HLB diagnostics are dependent on leaf sampling and uneven distribution can result in false negatives. Root infection appears to occur prior to foliar symptom development and CLas is more evenly distributed in this tissue, making roots more appropriate to sample.

A unique CLas gene, *nrdB*, was identified. CLas has five copies of *nrdB*, contrasting to the three copies of 16S rRNA gene. Therefore, PCR using *nrdB*-based primer set is more sensitive than the 16S rRNA gene-based primer set. Single nucleotide polymorphisms (SNPs) were found in *nrdB* sequences between CLas and CLaf. Thus *nrdB* sequence is more discriminative and more specific than the 16S rRNA gene. The *nrdB*-based PCR provides an alternative detection of CLas.

Viability qPCR (V-qPCR) was used to estimate live CLas populations in grapefruit leaves at different maturity stages and leaves with varying levels of symptoms collected from naturally infected field trees. Lowest CLas titres were found in immature leaves during the summer followed by a significant increase during the fall. During the summer, mature leaves harboured more live CLas than immature leaves.
Antibodies against the pathogen that causes HLB have been developed and used in a relatively simple assay to detect the pathogen. Because the new assay is based on protein detection using antibodies as opposed to DNA detection with PCR, it is a good complement to DNA based assays.

Canine assisted detection

Dogs trained to detect HLB-infected trees will be used as an early detection method for commercial and residential survey in all US citrus producing states. Dogs detected ACP inoculated plants 2-3 weeks post inoculation compared to 2-12 months for qPCR.
Canines were tested against infections within different cultivars, roots, infections from both grafting and psyllids. In addition, dogs were tested against several different citrus diseases and isolates of HLB. In all trials, the dogs had no problems identifying the HLB infected plants with a high degree of accuracy, regardless of species or isolate. Tests on different aged infections, different cultivars, roots, and infections showed the dogs abilities to detect and differentiate an HLB infected tree from clean trees at accuracies exceeding 99%. Dogs trained on CLas were also intrinsically capable of detecting CLaf and CLam infected trees without additional sensitization or training.

Statistical models developed to evaluate surveillance systems. Effective detection is dependent on the sampling approach used and should be based on the pathogen prevalence in the area and can be optimised by both vector and host sampling.
Models are available as web-based tools:
For effective early detection surveillance activities, the epidemiology of a pathogen (in particular, the rate of epidemic growth) should be considered when developing surveillance strategies. The presence of insect vectors as well as host plants in the HLB pathosystem adds another layer of complexity to the issue of surveillance, since it leads to the question of whether to sample from hosts, vectors, or both. Although this question has considerable implications for the design of the surveillance strategy, many surveillance schemes focus on sampling from host plants, with vectors sampled more opportunistically. Statistical model of host and vector sampling linked to a mathematical model of pathogen transmission. The resultant model allows us to quantify the relative sampling efforts and/or costs required from hosts and vectors in order to detect a specified incidence of infection in either. They demonstrate that the overall incidence at first detection is minimised when samples are exclusively collected from either hosts or vectors but not from both. As well as identifying whether hosts or vectors should be sampled, the method gives a numerical output which indicates how robust this decision is to changes in sampling costs. This has potential for use as a simple tool to determine where best to place sampling resources whilst accounting for both epidemiological issues and economic constraints.

Surveys

In **Texas** HLB incidence appears to be increasing more in residential sites where ACP control is less aggressive. The disease has spread to all three counties in the commercial citrus area of South Texas, and to two counties around Houston. There is little evidence of severe tree decline thus far, except in some residential sites where tree care is minimal, and in orchards where other issues such as *Phytophthora* infections are affecting tree health. The voluntary area wide management of psyllid combining two dormant sprays and aggressive ACP management during the active growing season implemented in > 92% of citrus acreage has resulted in significant vector population suppression which may be slowing HLB spread in Texas. Other factors which may be affecting the slower development of HLB in Texas include the discrete flush cycles that restrict ACP reproduction, and the long (4 to 5 months), dry and hot summers with mean daily temperatures exceeding 32°C from May to September.

California:
ACP was first found in a residential area of Southern California in 2008. ACP remained restricted to urban and suburban areas until 2011 when it was found in commercial citrus groves. Grove characteristics, such a size, influence how quickly they were invaded by ACP. However, the strongest effect was from groves being located near either residential infestations or other already-invaded groves. Results suggest that ACP spillover from residential areas contributed to early infestations in citrus groves, but then as more groves became infested, grove-to-grove spread of ACP became more important.

Brazil- The spread of CLas from the initial detection region to other regions is not uniform. Regions of drier winters and warmer summers have lower disease incidences compared to regions with milder climates.
A large scale assessment of single psyllid CLas infectivity was done in several regions, showing that the increase of ACP populations is during spring and summer, but psyllid infectivity trends differed between regions.
São Paulo state, Brazil: Cumulative disease incidence (per plot) varied from <4.0% to 80%. ACP were poorly detected by shoot evaluation conducted 2 to 5 times/month in 1% of trees in each plot. In contrast, *D. citri* was more effectively identified in yellow sticky cards, even though ACP were infrequently detected on the yellow cards. Plots with similar disease management varied from 7.1% to 25.9% in disease incidence. Surveys around those farms were done to identify neighboring citrus and *Murraya* sp. that could harbor HLB and ACP. Results suggest that isolated residential trees are sources of HLB infection.

Host pathogen interaction
Bacterial pathogens secrete proteins called effectors, which contribute to disease development. One effector, SDE1, has 40-fold higher expression in citrus relative to in psyllid, implicating a possible role in HLB disease progression. A secreted pathogen effector, named SDE1, associates with a family of citrus proteases that are potentially vital to defend against HLB.

6.7 **Visit to Steritech, a commercial gamma irradiation plant, in Narangba, Queensland, Australia (Feb 2017)**

Tim Grout

I was hosted by Ben Reilly, Export Business Development Executive and Seth Hamilton, the Quality Assurance Officer. Steritech is a family owned business with three gamma irradiation facilities in Victoria, New South Wales and Queensland (http://www.steritech.com.au/contact/). The facility I visited is successfully treating pallets of mangos, summer lemons and mandarins for export and this is why it was of interest. On asking about other sources of irradiation that would be less problematic in terms of radioactive waste they said that gamma rays were still more attractive than eBeams and X-ray technology which both had labour, health risks and high electricity costs. Even if X-ray systems could be installed in packhouses the capital outlay would be enormous and it would only be used during the season.

They stressed that maintaining the cold chain was extremely important to minimize fruit damage and that condensation should be avoided. Fruit entering the facility was off-loaded into cold rooms at 2°C (Fig. 1) then moved into the irradiation room which was at about 16°C, but the treatment only lasted 54 minutes so the fruit temperature would only rise to about 3.5°C before it was moved to 2°C again for storage before loading back into refrigerated trucks. They claim that much of the damage observed in research work was because the fruit was treated at ambient temperature and the cold chain was broken. However, they are also not evaluating the quality of fruit later than 21 days after treatment because they are flying the fruit overseas within a day or two of treatment.

The pallets of fruit cartons move on rails from the cold room into the treatment chamber, entering the chamber at ground level (Fig. 2). The radioactive source is a wall of rods enclosed by stainless steel around which the pallets move (Figs. 3 and 4). Each pallet is exposed to irradiation for 3 minutes in one of 18 positions before exiting the chamber. Pallets are treated on one face on the ground level before moving around to the opposite side of the wall where they are treated on another face. The pallets are then lifted up to a second level and treated on the third and fourth faces before leaving the chamber at the upper level (Fig. 5).

They work with a dosage ratio of 2.2 between the inside fruit and outside fruit on a pallet. For mandarins treated for export to Vietnam the minimum dose required is 150 Gy (generic fruit fly dose for *Bactrocera tryoni*) so the outside cartons are receiving 330 Gy. Summer lemons from Queensland (hot climate) seem to be more tolerant than other sets in the year or other citrus types and can tolerate 800 Gy on outside cartons. When methyl bromide is used on lemons they lose 5% so they can tolerate losses of 1-3% with irradiation. They are treating 30% of Queensland export mangos and these are treated when they are ripe, then flown to their markets. The dosage depends on the importer. The department of Agriculture clears the consignments for export after checking dosimeter records. When not irradiating fruit, the facility is used for various other purposes including the irradiation of bee hives for European foul brood disease at dosages of up to 15 kGy.

Fig. 1. Cold rooms with racks for multiple tiers of pallets.

Fig. 2. Pallets entering the treatment chamber at ground level, observed by Seth Hamilton and Ben Reilly.

Fig. 3. The Cobalt 60 source rises from a pool of water into the stainless steel wall with pallets on either side at both ground level and the level above.

Fig. 4. Rows of pallets moving around the gamma source.

Fig. 5. Pallets exit treatment chamber at upper level and are then dropped to lower level for movement to cold room

7 EXTENSION / VOORLIGTING April 2016 - Maart 2017

By/Deur Hennie le Roux, Hannes Bester, M.C. Pretorius, Keith Lesar, Dawid Groenewald, Andrew Mbedzi en Melton Mulaudzi (CRI)

7.1 VOORLIGTINGOORSIG

7.1.1 Die 2016 Seisoen

Die knellende droogte oor groot dele van die land was en is steeds ŉ groot bron tot kommer. In sekere van die produksiestreke was daar net genoeg water tot begin van die lente, met sommige damme se vlakke wat op ŉ vroeë stadium kritiese vlakke begin bereik het. Die meeste produsente het drastiese maatreëls in plek gekry om hul beskikbare water meer effektief te gebruik. Van hierdie praktyke sluit die verwydering van onproduktiewe boorde in, asook ŉ uitbreiding van herplant programme. Die oespotensiaal in meeste van hierdie streke is wel laer maar die laat herfsreëns het wel bygedra dat daar baie minder kleiner vruggroottes op die Navel en Valencia kultivars voorgekom het.

Die totale aanvanklike oesskatting is op ŉ vroeë stadium afwaarts aangepas na 108.4 miljoen kartonne, hoofsaaklik a.g.v kleiner vrugte en swakker uitpakke. Bydraende faktore tot swakker uitpakpersentasies was hoë vlakke van kraakskil, veral in die Oos-Kaap, asook chimeras op sekere Valencia-seleksies in die Noorde. Verskeie sitrustipes, veral sagtesitrus en nawels, het gesukkel om behoorlik te kleur. Die vruggrootte was in sekere areas effens aan die klein kant, maar ten spyte daarvan was verdienste uit die markte goed. Die totale volumes in meeste van die streke was aansienlik minder as die vorige seisoene. Daar was aanvanklik ŉ vrees dat daar heelwat kleiner vrugte in die noorde sou wees weens min water maar laat somer reëns en minder vrugte het gekompenseer en daar was ŉ tekort aan kleiner vrugte in die mark. Die totale uitvoervolumes vir 2016 was uiteindelik 109 miljoen kartonne. Die volumes van sagtesitrus en nawels was ietwat op teenoor 2015, terwyl dié van Valencias en pomelo's beduidend laer was. Suurlemoene was soortgelyk as die vorige seisoen. Ten spyte van die laer volumes, beskryf verskeie produsente dit as hul beste jaar ooit.

Bederf was vroeg in die seisoen reeds hoog, veral suurvrot a.g.v die afwesigheid van guazatine, maar ook groen- en blouskimmel. ŉ Positiewe uitvloeisel hieruit is dat die Sondagsriviervallei ŉ pakhuisforum gestig het wat gereeld vergader om inligting te deel en mekaar met bederfbeheer te ondersteun. Heelwat klagtes oor bederf uit die markte het voorgekom en kan waarskynllik aan verskeie faktore toegeskryf word, soos groot veranderinge t.o.v swambeheerprodukte, weerstand teen sommige produkte, en natuurlik bestuur in die pakhuis. Hierdie probleme is tydens die CRI Na-oes werkswinkels aan die begin van 2017 aangespreek. Die beperkte na-oes produkreeks se uitdagings was duidelik met heelwat fito wat gerapporteer was vanaf verskeie pakhuise uit verskillende areas.

Valskodlingmot (FCM) en vrugtevlieg (VV) was ŉ besondere groot probleem in meeste areas. Ongekende vlakke van veral FCM is in die Wes-Kaap gerapporteer, en dis onverklaarbaar wat daartoe aanleiding gegee het, veral as in ag geneem word dat FCM-aktiwiteit vroeg in die seisoen bykans afwesig was. Verskeie werkswinkels oor FCM en VV is aangebied om produsente te help om die regte strategieë in plek te sit. In areas met gemengde vrugteverbouing, soos die Wes-Kaap en Benede-Oranjerivier, is daar ŉ toenemende behoefte vir ŉ geïntegreerde benadering vir areawye plaagbeheer onder die uitvoerprodusente, maar dis veral rosyntjie- en wyndruiweprodusente wat samewerking vir areawye beheer besonder moeilik maak, aangesien hulle geen nut daarby vind nie. ŉ Aansienlike toename in witluis en rooidopluispopulasies was in meeste areas sigbaar.

Tydens DAFF en die EU FVO afvaardiging se besoek vroeg in die seisoen is heelwat klem op die Swartvlek prosedures vir beide produsent en DAFF amptenare gelê. Prosedures is in praktyk geoudit. By elke geleentheid is navrae gerig oor vrugtevlieg en valskodlingmot beheerstrategieë. Die afvaardiging was heel tevrede met die standaarde op plase. Produsente is gekomplimenteer met hul positiewe ontvangs van hierdie afvaardiging, asook hul rekords wat op datum is.

Studiegroepe in die noorde van die land het weer mooi op dreef gekom a.g.v die betrokkenheid van CRI Voorligting en Navorsing. Dit is duidelik dat daar steeds ŉ behoefte aan gereelde tegnologie-oordrag is. Die uitdaging is om die studiegroepe in al die produksiestreke weer aktief aan die gang te kry, ten spyte van onvoldoende hulpbronne.

Oor die algemeen gaan dit uistekend met die Sitrusbedryf en die tekens dui op optimisme onder die produsente. Boombestellings by die kwekerye is almal vol bespreek tot ten minste 2018, talle nuwe plase is aangekoop en verskeie nuwe pakhuise word gebou. Daar is ook groot belangstelling by nuwe toetreders tot die bedryf.

7.1.2 **Die 2017 Seisoen**

Vanuit ŉ produksie-oogpunt lyk die vooruitsigte vir die 2017 seisoen belowend. Volumes lyk op hierdie stadium beduidend hoër as 2016 en die eerste oesskattings dui op ŉ rekord uitvoervolume van 122 miljoen kartonne vir Suider-Afrika, waarvan 119 miljoen vanuit Suid-Afrika. Die skatting vir Valencias is 50 miljoen kartonne, nawels 26 miljoen, suurlemoene 17.5 miljoen, sagte sitrus 13.2 miljoen en pomelo's 13.8 miljoen.

Die vruggrootte mag moontlik effens kleiner wees as 2016 a.g.v die hoër oeslading. Markte is ietwat meer onder druk as verlede jaar hierdie tyd. As net die verandering in die wisselkoers in aanmerking geneem word, kom dit op hierdie stadium neer op ŉ afname van ongeveer R25 per karton. FCM-druk is hoog in veral die Oos- en Wes-Kaap en produsente doen alles moontlik om die vlakke af te bring. Stinkbesie (Nezara viridula) in die Benede-Oranjerivier het getoon dat dit binne die bestek van ŉ week groot vrugval tot gevolg kan hê indien dit nie dringend bestuur word nie.

Marktoegang na die EU gaan hierdie jaar groter uitdagings aan die bedryf stel. Die EU gaan ongetwyfeld nuwe fitosanitêre vereistes t.o.v valskodlingmot (FCM) aankondig, vir implementering in 2018. Onderskeppings van FCM gedurende hierdie seisoen, veral vroeg in die seisoen, sal ŉ bepalende rol speel in die tipe maatreëls wat ingestel gaan word. Daar is dus geweldig druk op alle skakels in die waardeketting om FCM met die nodige erns te bestuur, voor-oes sowel as na-oes.

7.1.3 **CRI-PTF**

Druktoetse van geakkrediteede kartonvervaardigers se kartonne is deurlopend gemonitor. Enkele gevalle waar die kartonne nie aan die spesifikasies voldoen nie, is met die betrokke vervaardigers opgeneem en ŉ dringende beroep is op hulle gedoen om aandag daaraan te gee. Op versoek van verskeie produsente en uitvoer-agente is kartonne ook getoets en verslae is aan hulle gestuur. Op versoek van twee groot uitvoerders is daar ondersoeke gedoen om te bepaal wat die oorsake was van kartonne wat inmekaar gesak het. Oorsese verslae is bestudeer en dit is opgevolg deur laboratorium toetse. Die basiese massa van al die betrokke "liners" sowel as die "fluting" is bepaal. Die belangrikheid van die regte papier en basiese massa kan eenvoudig nie oorbeklemtoon word nie. Die grootste probleme met verpakkingsmateriaal gedurende die 2016 seisoen was die basiese massa van die papier wat vir kartonne gebruik is, spasiëring- en afmetings van bo-dek planke op die sitruspalette, swamgroei op palette, asook palettiseringsprotokolle wat nie deur pakhuise gevolg is nie.

Enkele gevalle van temperatuur probleme met vrugte wat bestem was vir China is ondervind, veral met oop vertoon-kartonne en die E15C kartonne. ŉ Proef met T64 (E15C) Supervent kartonne is herhaal en addisionele ventilasie-gate op oop vertoon-kartonne is ook aangebring. Een baie ernstige probleem was palette wat nie aan die spesifikasie voldoen het nie en vertikale lugvloei belemmer het. Paletvervaardigers is ernstig vermaan om seker te maak dat alle sitruspalette wat hulle vervaardig in alle opsigte aan die Verpakkingswerkgroep se spesifikasie moet voldoen. Vertikale lugvloei deur skeepshouers, die voorverkoeling en kouesterillisasie, veral in Durban, was ook nie altyd na wense nie en is ook aangespreek.

Navorsingswerk op meer koste-effektiewe pakmateriaal is voortgesit en voorlopige resultate met nuwe verbeterde papier en ligter kombinasies, asook ŉ nuwe oop vertoon-karton ontwerp lyk baie belowend. Dubbelsyfer papierprysverhogings is vanaf 1 Oktober 2016 aangekondig. Op versoek van een van die grootste kartonvervaardigers is na al die opsies gekyk wat gebruik kan word om die prysverhogings laer te kry. Deel daarvan was ook ŉ strategie om die papierkombinasies te verander. Dit is suksesvol gedoen en die beplande prysverhogings is afwaarts aangepas. Mpact Paper wil ook baie nouer betrokke raak by die toets van kartonne, soortgelyk aan die toetse wat by die Sappi Technology Centre gedoen word. Opvolg gesprekke is in die vooruitsig.

Daar was weer versoeke van sekere oorsese kopers dat openinge ("hand holes") weer op die kopkante van oop vertoon-kartonne aangebring moet word. Die oorsponklike oop kartonne in die laat negentiger jare het sulke "hand holes" gehad maar om baie goeie redes is die gate verwyder. Op versoek van die uitvoeragente is motiverings geskryf waarom die gate nie terug gebring moet word nie.

Op versoek van Sappi is ŉ "mulching" proef met ŉ baie ligte ingevoerde papier op ŉ plaas in die Brits omgewing gedoen. Die doel van die "mulching" is om die vog-inhoud in die grond te verleng en onkruid te beheer. Die papier was beslis nie sterk genoeg nie en het na drie weke begin skeur en verrot. ŉ Opvolgproef met papier met ŉ hoër basiese massa is gedoen en na ses weke het die papier nog uitstekend vertoon. Die proef is steeds aan die gang om die leeftyd van die papier "mulch" te bepaal.

Paletvervaardigers is bekommerd oor die langtermyn beskikbaarheid van hout. ŉ Paletvervaardiger in die Oos-Kaap het versoek dat die houtblokke met saamgeperste houtskaafsels vervang word. Tydens die vervaardigings-proses word Ureaformaldehyde gebruik. Die ondersoek om te bepaal of dit op palette gebruik mag word, is aan die gang, maar daar is nog nie ŉ finale antwoord vanaf die betrokke paletvervaardiger gekry nie. Uit ŉ sterkte- en blootstelling-aan-vog-oogpunt gesien lyk dit belowend. Voorlopige proewe met E-Dek (deel van Omega Bins) op palette wat van "mild steel" vervaardig word, is begin. Soos met plastiek sal hierdie palette net ekonomies wees as die palette hergebruik kan word. Vir etlike jare word daar op beperkte skaal gebruik gemaak van Chep palette na die VK en sekere Europese lande. Sitrus word op Chep palette uitgevoer en die palette word dan deur Chep op die onderskeie markte versamel en daarna in "Chep Poele" opgeneem. Chep SA ondersoek tans die moontlikheid om palette soortgelyk aan ons standaard sitruspalette te vervaardig. Onderhandelinge met Chep het begin en die eerste palette vir ŉ reeks van toetse en proewe sal teen Mei 2017 beskikbaar wees. Op versoek van Pallet Warehouse is daar begin met samesprekings om palette by produsente se pakhuise te vervaardig. Dis nog in die beginstadium, maar een van die toonaangewende sitrusprodusente het aangedui dat hy belangstel. Die eerste gesprekke word beplan vir die 3de week in April 2017.

Tydens die Verpakkingswerkgroep se vergadering in November het een van die geakkrediteerde vervaardigers ŉ versoek gerig dat die stapelsterkte toetse onder "droë" toestande (50% RH en 23°C) gedoen moet word. Sedert die begin van die akkreditasie stelsel in 2007, word die stapelsterke (BCT) teen 87% RH en 32°C getoets. Dit is ondersoek en die besluit is geneem dat die status quo beslis gehandhaaf moet word. Alle uitvoersitrus word aan hoë humiditeit blootgestel en dit sal fataal wees om die stelsel te verander.

Die SA Sitrusbedryf voer jaarliks 120 000 ton papier uit in die vorm van kartonne. Wanneer hierdie kartonne herwin word staan dit bekend as K2 en K3 "waste paper". Instansies wat papier in SA herwin betaal tans R1250 per ton vir K2 en K3, met ŉ totale waarde van R150 miljoen. 'n Ondersoek hieroor is gedoen om vas te stel of of daar ŉ manier is waarop die produsente ŉ gedeelte van hierdie waarde terug kan kry. Die uiteindelike slotsom van die ondersoek was dat die koper die karton saam met die vrugte koop en hy dit dus kan verhandel na sy goeddunke.

Die Pakmateriaal Spesifikasies en Palettiserings Protokol Handleiding vir 2017 is gedurende die eerste week in Maart uitgestuur.

Na verskeie samesprekings met Sappi se senior bestuur het hulle goedgunstelik ingestem om vir die sewende agtereenvolgende jaar sitruskartonne van al die geakkrediteerde kartonvervaardigers by hulle SANAS geakkrediteerde laboratorium op hulle kostes te toets. In samewerking met Sappi se personeel is ŉ skedule opgestel en gedurende die laaste week in Januarie aan alle geakkrediteerde kartonvervaardigers gestuur. Daar is ook besluit om gedurende 2017 meer kartonne by pakhuise te trek. Opregte dank en waardering is teenoor Sappi uitgespreek. ŉ Dokument waarin wysigings aan die Akkreditasie Stelsel uiteengesit is, is opgestel en aan alle geakkrediteerde kartonvervaardigers gestuur.

Gedurende Januarie 2017 is die eerste in ŉ reeks proewe met meer koste-effektiewe A15C kartonne by Houers in Letsitele vervaardig. Die kartonne is met Sappi se nuut ontwikkelde 150g/m²Ultraflute+ gedoen. Die kontrole was 165g/m² Standaard Ultraflute. Suurlemoene is by Alicedale Estate in Tshipise gepak en per pad na Durban vervoer. Stapelsterkte laboratorium toetsresultate asook die eerste evaluering van die kartonne by aankoms in Durban was uiters belowend. ŉ Finale verslag sal na die oorsese evaluering opgestel word.

CRI Nelspruit het verlede jaar begin met die toets van vrugtevlieg lokvalle wat van ingevoerde gebleikte pulp vervaardig is. Die pulp is moeilik bekombaar en ook duur. In samewerking met CRI Nelspruit en Sappi, Ngodwana, is plaaslik vervaardigde "pulp sheets" bekom. Hierdie plaaslik vervaardigde "pulp sheets" is aan CRI, Nelspruit beskikbaar gestel en daar is begin met proewe om te bepaal of die plaaslike produk suksesvol is.

Samesprekings met NNZ, ŉ Nederlandse Maatskappy, oor die verskaffing van toedraaipapier (wrappers) vir sitrus het plaasgevind. Hierdie papier word nie meer in SA vervaardig nie en word alles ingevoer. Opvolggesprekke sal gedurende April 2017 plaasvind met die doelwit om toedraaipapier goedkoper vir die produsente te kan bekom.

Daar is ŉ behoefte vir volledige standaarde vir gelamineerde papier hoekstukke. Pride Pak beskik oor volledige toerusting om verskeie toetse te doen en het aangebied om betrokke te raak by die daarstelling van standaarde. ŉ Verskeidenheid van hoekstukke sal deur die loop van die 2017 seisoen by pakhuise getrek word en aan Pride Pak gestuur word om te bepaal of dit moontlik is om verbeterde standaarde vir hoekstukke op te stel. Ondersoeke na nuwe plastiese hoekstukke gaan voort. Dis baie belangrik dat dit tot die finansiële voordeel vir die produsent moet wees en dat dit vir oorsese kopers aanvaarbaar moet wees.

7.1.4 **Na-oes voorligting**

ʼn Suksesvolle reeks sitruspakhuis besoeke/konsultasies is vanaf die begin van die 2016 pakseisoen afgehandel. Die pakhuise is weer op ʼn een tot een basis besoek en die houding en terugvoering is weereens baie positief, met goeie interaksie en samewerking met die pakhuise. Pakhuisbestuur is meer tegemoetkomend en gewillig om hulle idees en vertroulike informasie t.o.v terugvoering oor bederf, residu-resultate (baie residumonster ontledings is gewys), ens. te bespreek, en is ook bereid om die nodige aanbevole veranderinge aan te bring. Die een tot een pakhuisbesoek interaksie bly ʼn wenner wat samewerking betref. Pakhuisforums is reeds in SRV, Patensie en Letsitele op die been gebring.

Voor en tydens die 2016 seisoen was daar weereens wisselvallige omgewingstoestande wat kommer oor vruggehalte veroorsaak het, veral rakende die skilgehalte van die sagtesitrus, suurlemoen, en nawel kultivars. Dit het met die satsumas oor die algemeen egter goed gegaan, behalwe vir ʼn bietjie brand hier en daar. Brand is wel ook op nawels, clementines en suurlemoene waargeneem a.g.v die gebruik van guazatine op nat vrugte tydens ontgroening.
Brand is ook op sekere kultivars gesien a.g.v die moontlike verkeerde gebruik van nuwe chemikalieë, veral in die voorpakhuis ‘’drench’’ mengsel voor ontgroening. Sporadiese bederf, veral suurvrot, was meer van ʼn probleem in beide die Noord- en Oos-Kaap gebiede, veral op die vroeë kultivars (satsumas en clementines) en op die nawels. Verskeie gevalle van Diplodia stingel-ent verrotting is ook waargeneem, al was dit ʼn droë seisoen oor die algemeen. Alternaria kern- en nawel-ent verrotting is ook op sekere kultivars waargeneem.

Dit was duidelik dat daar ʼn opbou van swamspore in die was-stelsels, veral die dompelbaddens, was. Chloor vlakke, wat in hierdie stelsels gemeet word, bly laag, en soms is geen konsentrasie meetbaar nie, en die pH vlakke is ook nie reggestel nie. Al hierdie probleme dra by tot swak pakhuissanitasie en dit bly steeds ʼn resep vir bestandheid en ʼn toekomstige ramp. Baie monsters van vrugte met Penicilliumspore is by al die Oos-Kaap en Burgersfort pakhuise geneem en vir bestandheid ontleed. Tydens die 2015 pakhuisbesoeke is swamspoor pluise van Penicillium-besmette uitskot vrugte, retensie monsters en van besmette vrugte in die vrugwas stelsels geneem en die eerste tekens van bestandheid het toe al voorgekom. Tydens die 2016 seisoen is hierdie praktyk by die pakhuise weer toegepas, en die pakhuise is ook aangemoedig om hulle eie monsters in te stuur vir moontlike bestandheid ontleding. Drie en twintig uit vyf en vyftig spoormonsters wat deur die DC getoets is, het ʼn mate van bestandheid (verlies van beheer) teen imazalil en pyrimethanil gewys. Dit is 42% van al die monsters. Monsters wat van die wande van skeepshouers geneem is toon hoë vlakke van besmetting van verskeie na-oes patogene. Hierdie probleem is onder PPECB se aandag gebring om dit aan te spreek.

Waksaanwending is ook nog ʼn kritiesebeheerpunt wat baie wisselvallig in pakhuise is a.g.v nat vrugte in die waksaanwending. Daar is duidelik min waks op die vrugte te sien na aanwending, en dit is heel waarskynlik die oorsaak van koueskade en vogverlies simptome wat te sien is in foto’s in terugvoering van die markte af. Die vogverlies simptome lyk soos skilafbraak, in en om die blomkelk weefsel waar daar heelwat minder waks aangewend word. Hand aan hand met die wisselvallige bestuur van die waksaanwending is die ooraanwending van waks op die vrugte, en die gevolglike oorskryding van die MRL’s van die na-oes middels.

By verre die grootste probleem tydens die 2016 seisoen was kraakskil, meestal op nawels, en dan ook op Clementines en ander kultivars. Die waarneming is dat kraakskil vir die afgelope vier seisoene toegeneem het. Vruggrootte en vrugkleur was ook ʼn geweldige probleem tydens die 2016 pakseisoen. Baie klein en ook uitermatige groot vrugte, veral nawels, kan op die pakhuislyne gesien word. Die warm nagtemperature het bygedra tot vrugte wat nie vining genoeg opgekleur het nie, en dit het veroorsaak dat pakhuise hierdie vrugte te lank in ontgroening laat staan het, tot nadeel van die vruggehalte.

Die onttrekking en opheffing van enige verdere gebruik van die na-oes swamdoder guazatine in die EU markte, en ook die gebruik van die nuwe geregistreerde swamdoder propiconazole, en die propiconazole/pyrimethanil formulasie in die ander markte, was ʼn groot ontwrigting vir die meerderheid pakhuise. Dit het veroorsaak dat baie van die pakhuise verkeerde aanbevelings toegepas het. Die meerderheid pakhuise wat suurvrot probleme hierdie seisoen ondervind het, het oorgeskakel na die gebruik van die nuut geregistreerde ‘’Imaculate’’ in die waksaanwending. Meeste pakhuise poog steeds om hulle kritiese beheer punte/stelsels goed te bestuur en die sanitasie komponent het in baie van die pakhuise verbeter.

ʼn Groot bekommernis is die aantal chemiese maatskappye/verskaffers wat verkeerde pakhuisbehandelings aanbeveel, en verskeie pakhuise wat hierdie aanbevelings aanvaar en toepas. Verkeerde aanbevelings is gunstig vir die ontwikkeling van moontlike Penicillium bestandheid teen die na-oes swamdoders. Die gebruik van ongeregistreerde middels in die sitruspakhuise is kommerwekkend. Dit sluit hoofsaaklik die “saniteermiddels” in, en soms ook die benatters, wat deur die chemiese verskaffers versprei word, sonder om eers vir CRI te raadpleeg of die middels goedgekeur is al dan nie.

Te veel pakhuise versuim nog om vrugretensiemonsters van elke besending op te berg. Probleme wat verliese tot gevolg kan hê en tekens van die moontlikheid van bestandheid sal vinnig in die retensiemonsters waargeneem kan word.

Die jaarlikse CRI Na-oes werkswinkels is weer gedurende Januarie en Februarie 2017 in die ses groot produksiestreke aangebied. Die formaat van die werkswinkels is suksesvol aangepas om voorsiening te maak vir die groeiende bywoningsgetalle. Minder en korter praatjies is aangebied om tyd vir paneelbesprekings toe te laat. Geen bemarkingspraatjies deur die borge is aangebied nie. Die terugvoer hieroor was baie positief. ʼn Wye reeks onderwerpe is aangespreek wat strek van voor-oes regdeur tot die logistieke ketting. Vir die eerste keer in die geskiedenis van die CRI Na-oes werkswinkels is die 1000 kerf tov bywoning oorskry, met ʼn totaal van meer as 1100 persone wat bygewoon het.

Die eerste Satsuma besending van die 2017 seisoen vanaf die Noordelike gebiede het goed verloop. Die interne gehalte was aanvaarbaar, maar daar is ook ʼn bietjie fitotoksisiteit (brand) op ʼn klein persentasie vrugte na ontgroening waargeneem. ʼn Residu oorskryding (>10mg/kg) van pyrimethanil op Satsumas in ʼn opgegradeerde voorontgroening storting ("drench") is ook gerapporteer. Die eerste Satsumas vanaf Citrusdal, en die eerste Satsuma besending vanaf Clanwilliam se algemene gehalte is as ''goed'' gerapporteer. Nogtans het die omgewingstoestande weer ʼn rol gespeel. Die dag temperature het van 36°C tot > 40°C gestyg, en die nag temperature het slegs tot 23-25°C gedaal. Dit het veroorsaak dat die produsente opgehou pluk het en dat die vrugte nie op die bome behoorlik opgekleur het nie.

Die eerste suurlemoene vanaf die Noorde, en die eerste Star Ruby pomelo's en suurlemoene vanaf Tshipise, se algemene gehalte is ook baie goed, maar opvolgreën het pluk bemoeilik. ʼn Paar gevalle van endokserose op suurlemoene uit die Noorde, as gevolg van die uitermatige hoë temperature, is ook waargeneem. Oleo het ook sporadies voorgekom uit die Noorde as gevolg van selwandspanning tydens die nat toestande. Die eerste latentepatogeen infeksies (Diplodia stingelent verrotting) op suurlemoene is ook in die Noordelike gebiede waargeneem, as gevolg van die hoë swamspoorlading op dooiehout in die bome en weereens die nat toestande. Bome was in baie plekke nie voldoende afgerand nie en as gevolg van die laaghangende vrugte en deurdrenkte boorde is die eerste geval van Phytophthora bruinvrot op suurlemoene in ʼn boord waargeneem. Erge roetskimmel op vrugte a.g.v ernstige witluisbesmetting veroorsaak probleme in die pakhuise en dit sal so spoedig moontlik op boordvlak aangespreek moet word.

7.1.5 **9de Sitrus Navorsings Simposium**

Die CRI Sitrusnavorsingsimposium, wat van 21-25 Augustus 2016 by Champagne Sports Resort aangebied was, was baie goed bygewoon. Die totale bywoning was ʼn rekordgetal van 580 persone. Hoewel die primêre teikengroep die tegniese mense is, wat die inligting kan verwerk om praktiese aanbevelings te kan maak, soos bv. konsultante, chemiese maatskappye, bemestings-maatskappye, tegniese persone verbonde aan uitvoemaatskappye en verpakkings-instansies en selfs van die borge, was 26% van die bywoning afkomstig uit produsente-geledere. Tydens die drie dae is 77 praatjies aangebied, waaronder verskeie "key note speakers". Die besluit vooraf om slegs praatjies met ʼn "take home message" aan te bied, het goeie vrugte afgewerp en uitstekende terugvoer ontlok.

BASF was die hoofborg gewees, Arysta LifeScience het die "Welcoming Dinner" geborg, RiverBioscience die "Happy Hour" en Noordchem die gholfdag. Die Gala Aand is weereens deur Sappi geborg, met Villa Crop Protection wat die vermaak geborg het. Die Gala Aand was ʼn groot geleentheid vir Sappi aangesien dit vanjaar hul 80ste verjaarsdagvieringe was en hulle hierdie geleentheid ten beste wou benut. Verskeie Goue, Silwer en Brons borge was ook betrokke. Dassie Smit, Tian Schutte en Mike Holtzhausen het elk ʼn tegniese merietetoekenning ontvang vir hul onderskeie uitstaande bydrae tot die sitrusbedryf.

7.1.6 **Biosekuriteit**

Gedurende 2016 het dit aan die lig gekom dat produsente in die noorde van Namibië belang stel om sitrus te vestig. Daar loop ook gerugte rond dat die Chinese, in samewerking met die Namibiese regering, beplan om 8000ha sitrus te vestig. Binne die Namibiese regering is ʼn struktuur, genaamd AMTA (Agro Marketing and Trade Agency), gestig om nuwe ontwikkelinge in die landbou en ander nywerhede te ondersteun. Die risiko tov die bedreiging wat eksotiese siektes en plae vir Suider-Afrika inhou, veral HLB, indien plantmateriaal van veral China en Brazilië ingevoer sou word, het daartoe gelei dat ʼn besoek aan Namibië einde 2016 weer opgevolg is met ʼn besoek deur Hannes Bester en Dr Hoppie Nel gedurende Maart 2017. ʼn Vergadering is met AMTA gehou om samewerking van die Namibië-regering met die Suid-Afrikaanse Sitrusbedryf te bespreek. Die CGA het vooraf aangedui dat Namibië op dieselfde basis as Zimbabwe by die strukture en werksaamhede van die CGA kan inskakel. Die ekonomiese faktore van sitrusverbouing en die risiko t.o.v biosekuriteits-

aangeleenthede is deeglik onder AMTA se aandag gebring. AMTA het ŉ verslag aangevra wat die lewensvatbaarheid van sitrus in Namibië omskryf, sodoende stel dit hulle in staat om strategiese besluite rakende hul betrokkenheid by sitrus-ontwikkeling te neem. Die doel van samewerking met AMTA is om te verseker dat hulle met die Suid-Afrikaanse Sitrusverberingskema saamwerk om sodoende die sitrusbedrywe van die onderskeie SADEC lande teen enige eksotiese siektes te beskerm.

Vergaderings is ook met produsente-groepe in beide Outjo en Tzumeb gehou waar dieselfde inligting bespreek is. Produsente wil graag sitrus vestig, maar besef dat hulle dit nie sonder die hulp van die regering kan doen om infrastruktuur te skep nie, en dit skep onsekerheid oor die omvang van hoeveel hektaar sitrus moontlik gevestig kan word. ŉ Vergadering met Mpact in Walvisbaai is gehou om hulle moontlike betrokkenheid by sitrus-ontwikkelings te bespreek. Mpact het aangedui dat hulle die moontlikheid om terugwaarts te integreer beslis sal ondersoek wanneer die tyd reg is. Koelgeriewe en hawefasiliteite behoort nie ŉ wesenlike probleem vir sitrusuitvoere te wees nie.

7.1.7 **Sitrus-koueketting**

Toename in uitvoervolumes, en veral toename in die volumes na koue-steri markte, plaas geweldig druk op die kapasiteit en doeltreffendheid van die koueketting. Daar is veral kommer oor die sterk moontlikheid dat Europa reeds volgende seisoen gaan aandring op koue-steri op sitrus uit Suid-Afrika, wat die Sitrusbedryf in ŉ kritieke situasie t.o.v koue-steri kapasiteit en kundigheid gaan dompel. ŉ Ondersoek het bv. getoon dat ondoeltreffende voorverkoeling die oorwegende rede vir meeste koue-steri “failures” na China is. Voorverkoeling is ŉ baie gespesialiseerde en belangrike funksie in die koueketting. Die gebrek aan kundigheid en koue-steri kapasiteit is reeds welbekend. Die geleidelike agteruitgang die afgelope klompie jare in die doeltreffendheid van die sitrus-koueketting is ŉ groot bron tot kommer.

Die konvensionele skepe is oud en raak onvoldoende vir koue-steri. Daar is geen vooruitsigte dat nuwe konvensionele skepe gebou gaan word nie. Dit kom daarop neer dat meer en meer koue-steri verskeping in houers gedoen moet word, terwyl houers nie werklik die mees geskikte opsie vir hierdie doel is nie.

Die uitdagings in die koueketting raak gevolglik eksponensieël meer namate die totale uitvoervolumes toeneem, volumes na koue-steri markte toeneem, konvensionele verskeping uitfaseer en verskeping in houers toeneem, en ook as gevolg van ontoereikende voorverkoelingskapasiteit en kundigheid. Die realiteit is dat hierdie uitdagings net meer en meer gaan word, nie minder nie.

Sedert 2015 is ŉ ernstige behoefte deur die Exporters Technical Panel en die Cooling Working Group geïdentifiseer om ŉ Koueketting-spesialis vir die Sitrusbedryf aan te stel om verantwoordelikheid oor die sitrus-koueketting te neem. Hierdie behoefte is tydens die afgelope vergaderings van beide hierdie twee lidmaatskap-groepe van die CRI-PTF weer sterk op die voorgrond geplaas. Die fokuspunte wat vir hierdie spesialis bepaal is, sluit in operasionele navorsing in koueketting-innovasie om verkoelings en koue-steri protokolle in lyn met fisiologiese navorsing en vrugkwaliteit te bring, om toesig te hou oor alle operasionele aktiwiteite en tegnologie in die koueketting, en om ondersteuning aan produsente te verleen tydens beplanning en oprigting van hul privaat voorverkoelings-fasiliteite.

7.1.8 **Voorligtingsbehoeftes**

Dit het die afgelope seisoen duidelik geword dat daar ŉ baie sterker fokus op na-oes voorligting geplaas moet word om in die bedryf se behoeftes te voorsien. Die CRI Na-oes werkswinkels wat jaarliks voor die aanvang van die pakseisoen aangebied word, en die beperkte jaarlikse aantal pakhuisbesoeke, is nie voldoende nie. Die Sondagsriviervallei het met die inisiatief van Die Koöperasie ŉ baie suksesvolle pakhuisforum gestig waar inligting uitgeruil en besluite geneem word. Die sukses van hierdie forum is ŉ sterk aansporing om soortgelyke forums in ander areas ook op die been te bring. Addisionele kapasiteit vir na-oes voorligting, veral pakhuisbesoeke, moet geskep word.

Nadat die onderskeie CRI Streekswerkswinkels destyds begin is en CRI nie meer die studiegroepe aktief gedryf het nie, het meeste van die studiegroepe, veral in die Suide, onaktief begin raak. Die feit dat die meeste studiegroepe in die Noorde weer suksesvol aan die gang gekry is, wys daar is steeds ŉ behoefte aan studiegroepe in die areas. ŉ Hernude poging hiervoor gaan weer in die nuwe jaar van stapel gestuur word. Ook hiervoor word addisionele kapasiteit benodig.

Opsomming van aktiwiteite deur Hannes Bester, MC Pretorius, Dawid Groenewald en Keith Lesar vir die periode April - Junie 2016

Datum	Studiegroep/Aktiwiteit	Onderwerpe/Aksies	Betrokkenes / Sprekers
10 Apr 16	Dr Schalk van Wyk: Hoopstad	Organiese produkte: Restore & Crop Candy	Hannes Bester
11 Apr 16	Hans Bence: Kroonstad	Organiese produkte: Restore, Crop Candy en Kelp	Hannes Bester
11 Apr 16	Weipe	Boorde besoek en kultivarblok besoek	MC Pretorius Johan Joubert
12 Apr 16	Suid Zimbabwe	Studiegroep vergadering en Maturity tabelle	MC Pretorius Johan Joubert Graham Barry
13 Apr 16	Letaba: Chimera werkswinkel	Chimera probleme op Valencias	Hannes Bester MC Pretorius Paul Fourie Johan Joubert Faan van Vuuren Hennie le Roux
14 Apr 16	Nelspruit: Knittex – William Roberts	Knittex en CRI samewerking	Hennie le Roux Hannes Bester
	Simposium vergadering	Simposium reëlings	Hennie le Roux Hannes Bester MC Pretorius Liezl vd Linde Christine Stoppel-Grove
15 Apr 16	Robertson	FCM & FF werkswinkel	Sean Moore Aruna Manrakhan Martin Gilbert
26 Apr 16	Kakamas: Benede-Oranjerivier studiegroep	DAFF / EU CBS vergadering	Hannes Bester
27 Apr 16	Kakamas: Pieter Botha Piet Dykman: Triple D	Snoei Sitrus vestiging	Hannes Bester
28 Apr 16	Upington Landbou Expo	Lesings: Nico Kelder Tracy Davids Trevor Tuft Peter Johnson	Hannes Bester
	Stihl: Kevin Richards Van Zyl Agri: Johan v Zyl IDC: Mandy Bothma	Simposium borgskappe en uitstallings	Hannes Bester
28 Apr 16	Groblersdal	De Wagendrift studiegroep beplanning	MC Pretorius
29 Apr	Marble Hall	Marble Hall sitrus	MC Pretorius
29 Apr 16	Ganspan: Danie Mathewson	Boordbesoeke	Hannes Bester
30 Apr 16	Hartswater:	Oase Kwekery	Hannes Bester
5 & 6 Mei 16	Houers en Noorchem, Letsitele	Proef met meer koste-effektiewe kartonne en vergadering met Noordchem oor borgskap vir symposium gholfdag	Dawid Groenewald
10 -12 Mei 16	SASRI - KZN	NSSA vry-lewende werkswinkel	MC Pretorius Bheke Chele
16 Mei 16	2 Rivers Pakhuis Kirkwood	Pakhuisbesoek/konsultasie	Keith Lesar
16 Mei 16	Mandaryn Pakhuis Kirkwood	Pakhuisbesoek/konsultasie	Keith Lesar
16 Mei 16	Golden Ridge Pakhuis Kirkwood	Pakhuisbesoek/konsultasie	Keith Lesar
16 Mei 16	Sitrus Rand Pakhuis Kirkwood	Pakhuisbesoek/konsultasie	Keith Lesar

16 Mei 16	Panzi Pakhuis Kirkwood	Pakhuisbesoek/konsultasie	Keith Lesar
16 – 17 Mei	Zebediela	Bestuursvergadering – Toekoms van Zebediela	MC Pretorius Andrew Mbedzi
17 Mei 16	SRCC Pakhuis Lyn 2 Kirkwood	Pakhuisbesoek/konsultasie	Keith Lesar
17 Mei 16	SRCC Pakhuis Nuwe	Pakhuisbesoek/konsultasie	Keith Lesar
17 Mei 16	SRCC Hermitage Pakhuis Kirkwood	Pakhuisbesoek/konsultasie	Keith Lesar
17 Mei 16	Ponders End Pakhuis	Pakhuisbesoek/konsultasie	Keith Lesar
17 Mei 16	SRCC Summerville Pakhuis Kirkwood	Pakhuisbesoek/konsultasie	Keith Lesar
	Lemoenkop Citrus	Pakhuisbesoek	Hannes Bester
17 Mei 16	Sun Citrus Pakhuis Kirkwood	Pakhuisbesoek/konsultasie	Keith Lesar
19 Mei	Oorlogspoort Pakhuis	Pakhuisbesoek/konsultasie	Keith Lesar
19 Mei 16	Vensterhoek Pakhuis Patensie	Pakhuisbesoek/konsultasie	Keith Lesar
19 Mei 16	Patensie Koop x 2 paklyne	Pakhuisbesoek/konsultasie	Keith Lesar
23 Mei	Karino	Karino produsent besoeke	MC Pretorius Hannes Breedt
24 Mei 16	Onderberg	GFC- Droogtebestuur en Midknight bespreking en boord besoeke	MC Pretorius
25 Mei 16	Kakamas: Kobus van Zyl Groenheuwel Bdy Zwartbooisberg	Boordbesoeke	Hannes Bester
	Oranjerivier Kwekery	Kwekerybesoek	Hannes Bester
30 Mei 16	Weipe	Noordgrens – Boord en kultivar boord besoek	MC Pretorius Johan Joubert
31 Mei 16	Tshipese	Alicedale – Algemene bespreking Peter Nickolson	MC Pretorius Johan Joubert
31 Mei 16	Wes-Kaap:	Maxi-Yield: Graeme Coomer	Hannes Bester
1 Jun 16	Stellenbosch	Johan Janse v Rensburg: DOW Jaco Theron: JBT	Hannes Bester
2 Jun 16	Tom Burke Waterberg	Boordbesoek – Rolemsha en fito probleme; Kultivar blok besoek. Bufland sitrus – boord en pakhuis besoek en droogtestrategie bespreking	MC Pretorius Johan Joubert
2 Jun 16	Somerset-Wes	Vaughan Hattingh	Hannes Bester Vaughan Hattingh
3 Jun 16	Marble Hall en Groblersdal	Piet Engelbreght trust Schoonbee landgoed Roslee; kultivar blokke en pakhuis fito probleme	MC Pretorius
6 Jun 16	Lemoenkop Pakhuis Vaalharts	Pakhuisbesoek/konsultasie	Keith Lesar
7 Jun 16	Saamfarm Pakhuis Jan Kempdorp	Pakhuisbesoek/konsultasie	Keith Lesar
8 Jun 16	Mosplaas (Karsten) Pakhuis	Pakhuisbesoek/konsultasie	Keith Lesar
8 Jun 16	Winterton: CSR	Simposiumvergadering	Hannes Bester Hennie le Roux MC Pretorius

			Dawid Groenewald Liezl vd Linde
9 Jun 16	Renosterkop Pakhuis Augrabies	Pakhuisbesoek/konsultasie	Keith Lesar
13-14 Jun 16	Witrivier	Villa/CRI werkswinkel	Hannes Bester MC Pretorius Sean Moore Aruna Manrakhan Wilma Du Plooy Martin Gilbert
14 Jun 16	Letsitele	EU FVO oudit besoek	MC Pretorius
15 Jun 16	Letsitele	EU FVO oudit besoek	MC PRetorius
15 Jun 16	Naranja Pakhuis Burgersfort	Pakhuisbesoek/konsultasie	Keith Lesar Wilma du Plooy
15 Jun 16	Nelspruit	IPM workshop	Hannes Bester Hennie le Roux Sean Moore Aruna Manrakhan Tim Grout Martin Gilbert Wayne Kirman John-Henry Daneel Peter Stephen
21 Jun 16	Ryton Pakhuis Nelspruit	Pakhuisbesoek/konsultasie	Keith Lesar
21 Jun 16	Barberton	Suurlemoen aanplanting beplannings vergadering	MC Pretorius Andrew Mbedzi
22 Jun 16	Oorsprong Pakhuis Malelane	Pakhuisbesoek/konsultasie	Keith Lesar Wilma du Plooy
22 Jun 16	Addo/Kirkwood	EU FVO afvaardiging	Hannes Bester
28 Jun 16	Kirkwood	Sundays River Technical Packhouse Forum meeting	Hannes Bester Wilma Du Plooy

Opsomming van aktiwiteite deur Hannes Bester, MC Pretorius, Dawid Groenewald en Keith Lesar vir die periode Julie - September 2016

Datum	Studiegroep/Aktiwiteit	Onderwerpe/Aksies	Betrokkenes / Sprekers
4 – 8 Jul	Groblersdal/ Marble Hall	Produsent en pakhuis besoeke: Roslee, Schoonbee, Piet Engelbreght trust, Hannes Hertzog, De Wagendrift sitrus	MC Pretorius
6 Jul 16	Oorsprong Pakhuis Malelane	Pakhuisbesoek/konsultasie	Keith Lesar
8 Jul 16	le Roux Famile plaas	Mulching Proef	Dawid Groenewald Jason Knock (Sappi)
11 Jul 16	Navorsingsprioriteite	Bemesting & besproeiing	Hannes Bester Tim Grout Teunis Vahrmeijer
12 Jul 16	Dawid Groenewald	Verpakkings-aangeleenthede	Hannes Bester Dawid Groenewald
13 Jul 16	Symington Pakhuis, Hectorspruit.	T64 SV Proef	Dawid Groenewald Frikkie van Wyk Gerhard Greeff
14 Jul 16	Tomahawk Pakhuis Lone Tree Jeppes Reef	Pakhuisbesoek/konsultasie	Keith Lesar
18 – 20 Julie	CRI Grondvesblok	Grondvesblok evaluasies	MC Pretorius
21 – 22 Julie	Port Elizabeth	DAFF vergroeningsopname in PE	MC Pretorius Makhosi DAFF

25 Jul 16	Fruit Star Afrifresh Nkwaleni	Pakhuisbesoek/konsultasie	Keith Lesar
26 Jul 16	Chennels (ex Farm Secure) Nkwaleni	Pakhuisbesoek/konsultasie	Keith Lesar
27 Jul 16	Katope Pakhuis (Vernon Tocknell) Nkwaleni	Pakhuisbesoek/konsultasie	Keith Lesar
27 Jul 16	Stellenbosch: Caren Jarmain	FruitLook	Hannes Bester
	DOW	Closer launch	Hannes Bester
28 Jul 16	Bolton Sitrus Pakhuis	Pakhuisbesoek/konsultasie	Keith Lesar
29 Jul 16	Fowler Farms Nkwaleni	Pakhuisbesoek/konsultasie	Keith Lesar
29 Jul 16	Carisbrooke Richmond/Ixopo	Pakhuisbesoek/konsultasie	Keith Lesar
4 Aug	Hoedspruit	Hoedspruit studiegroep vergadering	MC Pretorius
6 – 10 Aug	Nelspruit	Simposium beplanning en hulp aan Liezl	MC Pretorius
10 Aug 16	PLM Pakhuis Burgersfort	Pakhuisbesoek/konsultasie	Keith Lesar
10 Aug 16	Waterval (Bosveld) Pakhuis Burgersfort	Pakhuisbesoek/konsultasie	Keith Lesar
11 Aug 16	Marx Pak Pakhuis Ohrigstad	Pakhuisbesoek/konsultasie	Keith Lesar
11 Aug 16	JP Landgoed (Cobus Beetge) Pakhuis Ohrigstad	Pakhuisbesoek/konsultasie	Keith Lesar
11 Aug 16	OR Pack Ohrigstad	Pakhuisbesoek/konsultasie	Keith Lesar
12 Aug 16	Morone Sitrus Burgersfort	Pakhuisbesoek/konsultasie	Keith Lesar
11 Aug 16	Vaalharts met Sean Moore: Danie Mathewson Retha Greyling	IPM	Hannes Bester Sean Moore
11 Aug	Groblersdal	Loskop studiegroep: VKM beheerstrategië	MC Pretorius Wayne Kirkman
12 Aug 16	Vaalharts met Sean Moore: Leon Du Preez Michael van Niekerk	IPM	Hannes Bester Sean Moore
19-20 Aug 16	Winterton: CSR	Voorbereiding vir Navorsingsimposium	Hannes Bester MC Pretorius Liezl vd Linde
21-24 Aug 16	Winterton CSR	Sitrus Navorsingsimposium asook gholfdag op 21 Aug 16	Hannes Bester MC Pretorius Liezl vd Linde Dawid Groenewald.
26 Aug 16	Tzaneen	Paletvervaardigers/Verskaffers van hout en Houers	Dawid Groenewald. Frikkie van Wyk. Chris Human.
29 - 31	Nelspruit	Produsent besoeke: Karino produsente, Onderberg GFC en Nelspruit wes	MC Pretorius Hannes Breedt James Warrington Carlien Grobler
5 – 9 Sept	Nelspruit	Studies – veldwerk exp 1092	MC Pretorius Prof Nico Labuschagne Charl Kotze
6 Sept 16	Henley Pakhuis Letsitele	Pakhuisbesoek/konsultasie	Keith Lesar
6 Sept 16	Merite Pakhuis Letsitele	Pakhuisbesoek/konsultasie	Keith Lesar
6 Sept 16	The Plains Pakhuis Letsitele	Pakhuisbesoek/konsultasie	Keith Lesar
6 Sept 16	Group 91 Letsitele	Pakhuisbesoek/konsultasie	Keith Lesar

7 Sept 16	Mahela Boerdery Letsitele	Pakhuisbesoek/konsultasie	Keith Lesar
7 Sept 16	Laeveld Pakhuis Letsitele	Pakhuisbesoek/konsultasie	Keith Lesar
7 Sept 16	Rooister Boerdery Letsitele	Pakhuisbesoek/konsultasie	Keith Lesar
7 Sept 16	Namibia: Grootfontein Johan Louw Gerrit Engelbrecht Henk Botha	Produsentebesoeke	Hannes Bester
7 Sept 16	Namibia: Tsumeb Friedel Blume Leon van Molendorff Heinrich van Eeden Jurg Sproër	Produsentebesoeke & Studiegroepvergadering	
8 Sept 16	CP Minnaar Letsitele	Pakhuisbesoek/konsultasie	Keith Lesar
8 Sept 16	Letaba Pakhuis	Pakhuisbesoek/konsultasie	Keith Lesar
8 Sept 16	Namibia: Windhoek	AMTA: daag nie op nie	Hannes Bester
20 Sept 16	Rosle Boerdery Schoeman Boerdery	Vertikale lugvloei toetse op verskillende karton ontwerpe	Dawid Groenewald. Koos de Wet Christo de Jonge Marius Wessels
13-24 Sept 16	Brazil: Iguazu	International Citrus Congress	Hannes Bester MC Pretorius
30 Sept 16	Sappi Technology Centre.	Basiese massa en tipiese waarde toetse. APL en New Era kartonne.	Dawid Groenewald. Marilyn Naidoo.

Opsomming van aktiwiteite deur Hannes Bester, MC Pretorius, Dawid Groenewald en Keith Lesar vir die periode Oktober - Desember 2016

Datum	**Studiegroep/Aktiwiteit**	**Onderwerpe/Aksies**	**Betrokkenes / Sprekers**
5 Okt 16	PHI: Ryan Newborn	Onderhoud oor PHI	Hannes Bester
	Groblersdal	Roslee – Charles Rossouw	MC Pretorius
6 Okt 16	Groblersdal	Piet Engelbrecht trust	MC Pretorius
11 Okt 16	Loskop - Groblersdal	Studiegroep vergadering: Simposium terugvoer, CRI werkswinkels, swartvlek bestuur, spuittegnologie demonstrasie	MC Pretorius Tian Schutte
19 Okt 16	Johannesburg: IPM Research Committee meeting	Research priorities	Hannes Bester Tim Grout Sean Moore
	Johannesburg	Bayer CropScience: borgskap vir NSSA bespreking	MC Pretorius Mieke Daneel
20 Okt 16	Johannesburg: DM Research Committee meeting	Research priorities	Hannes Bester Tim Grout Paul Fourie
25 Okt 16	Stellenbosch: CFQM Research Committee meeting	Research priorities	Hannes Bester Tim Grout Paul Cronje Jakkie Stander
24 – 28 Okt 16	Stellenbosch	Universiteit – studies en Kontrakwerk vergadering vir aalwurmdoders	MC Pretorius
26 Okt 16	Stellenbosch: Cultivar Committee meeting	Cultivar evaluation	Hannes Bester Tim Grout Johan Joubert
	CMF Meeting	Agenda	Dawid Groenewald
27 Okt 16	Stellenbosch: Cooling Working Group	Agenda	Hannes Bester Dawid Groenewald

	Lean van Biljon	Tegniese aangeleenthede	Hannes Bester
	Francois Du Preez	Vakante pos	Hannes Bester
2-3 Nov 16	Nelspruit	Bayer met Turkse besoekers besoek CRI Nelspruit en boorde	MC Pretorius
7 Nov 16	Letsitele	Produsent besoek: Mahela	MC Pretorius Johan Joubert Jakkie Stander Wilma du Plooy
8 Nov 16	Letsitele	Produsent besoek: Algemende bespreking by Groepe 91 DuRoy kwekery Studiegroep vergadering	MC Pretorius Johan Joubert Jakkie Stander Wilma du Plooy Sean Moore
	Vereeniging: Hentie Park	Verkoelingstegnologie	Hannes Bester
	Sappi Technolgy Centre	Ondersoek probleme met kartonne	Dawid Groenewald
9 Nov 16	Nelspruit: CRI Na-oes werkswinkels beplanningsvergadering	Agenda	Hannes Bester Dawid Groenewald MC Pretorius Keith Lesar Liezl vd Linde Wilma du Plooy Catharine Savage
	Nelspruit	Karino studiegroep	MC Pretorius Johan Joubert Jakkie Stander Sean Moore Paul Fourie
10 Nov 16	Pretoria: Packaging Working Group meeting	Agenda	Dawid Groenewald Hannes Bester
	Nelspruit	CIS Virologie en Soilborne vergadering	MC Pretorius Paul Fourie Glyniss Cook Kobus Breytenbach Chanel Steyn Faan van Vuuren
11 Nov 16	Nelspruit: Voorligting Beplanningsvergadering	Agenda	Hannes Bester MC Pretorius Keith Lesar Liezl vd Linde
14 Nov 16	Nelspruit: Flip Welman	Palet templaat M'Pact borgskap	Hannes Bester
15 Nov 16	Pretoria: Dawid Groenewald	CRI-PTF beplanning	Dawid Groenewald Hannes Bester
16 Nov 16	Brits: Produsentebesoeke	Bemesting & besproeiing Snoei Plaagbeheer	Hannes Bester
17 Nov 16	Hartbeesfontein: Hesti le Roux	Logistieke projek: kapasiteits-behoefte	Hannes Bester
	Krugersdorp: Teunis Vahrmeijer	Bemesting & besproeiing navorsing en voorligting	Hannes Bester
18 Nov 16	Hoopstad: Piet Burger en Hentie Park	Bemesting-invoere en vermenging	Hannes Bester
	Hartswater: Danie Mathewson	Terugsterwing op bome	Hannes Bester
25 Nov 16	Johannesburg: CRI BOD meeting	Agenda	Hannes Bester Vaughan Hattingh Tim Grout Jon Pinker

28 Nov 16	Humansdorp: Gert Barnard	Aanplantings-beplanning	Hannes Bester
	Houers Depot, Marble Hall	Nuwe ontwerp hand-vou oop kartonne sonder endstukke	Dawid Groenewald
30 Nov 16	Stellenbosch: De Witt Le Grange	Plastiese palette	Hannes Bester Dawid Groenewald
	Exporters Technical Panel meeting	Agenda	Hannes Bester Dawid Groenewald MC Pretorius Liezl vd Linde
	Hortgro Science: Hugh Cambell	Navorsing en voorligting	Hannes Bester
1 Des 16	Stellenbosch: Kosie Human – Rovic Leers	Tegniese implimente Borgskap	Hannes Bester
	Petrus Du Toit - Byotrol	Saniteermiddels	Hannes Bester
	Andrew Paterson – M'Pact	Borgskap Akkreditasie: Karton-vervaardigers Sitruspakhuise	Hannes Bester
2 Des 16	Nelspruit - CRI	Na-oes vergadering met Wenkem en Citrosol	Keith Lesar Wilma du Plooy Schalk Visser Benito Orihuel Carmen Jimenez
5 Des 16	Loerie: Hennie Moolman	Aanplantings-beplanning	Hannes Bester
6 Des 16	Hectorspruit	Produsent besoeke GFC – Marinus Neethling en Karlien Grobler	MC PRetorius
7 Des 16	Nelspruit	Onderhoude vir AEM: North	Hannes Bester Tim Grout Jon Pinker
	Sappi Technology Centre	Bepaling van basiese massa van papier	Dawid Groenewald
12 Des 16	Irene Sakesentrum	Herwinning van papier by Neopak Recycling.	Dawid Groenewald

Opsomming van aktiwiteite deur Hannes Bester, MC Pretorius, Wayne Mommsen, Dawid Groenewald en Keith Lesar vir die periode Januarie - Maart 2017

Datum	**Studiegroep/Aktiwiteit**	**Onderwerpe/Aksies**	**Betrokkenes / Sprekers**
13 Jan 17	Innovation Hub, Pretoria	Chep beplan om Standaard Sitruspalette te vervaardig	Dawid Groenewald Gerhard Stander
19-20 Jan 17	Houers, Letsitele. Sappi Personeel	Vervaardiging van 150UF+ Eksperimentele A15C kartonne.	Dawid Groenewald. Wimpie Mostert Frikkie van Wyk Brian Percival
23-25 Jan 17	Nelspruit	Werkswinkel beplanning	Hannes Bester MC Pretorius Keith Lesar Liezl vd Linde
23 Jan 17	STC, Pretoria.	Toets van A15C 150UF+ kartonne en ontvang eerste plaaslik vervaardigde pulp sheets	Dawid Groenewald
26 Jan 17	Malelane: Esselen Kwekery	Kultivars Kwekery besoek	Hannes Bester
31 Jan - 1 Feb 17	Limpopo 1 CRI Na-oes werkswinkel	Agenda	Hannes Bester Dawid Groenewald Keith Lesar MC Pretorius Wayne Mommsen Liezl vd Linde

			Wilma Du Plooy Catherine Savage Sean Moore Paul Cronje
2-3 Feb 2017	Limpopo 2 CRI Na-oes werkswinkel	Agenda	Hannes Bester Dawid Groenewald Keith Lesar MC Pretorius Wayne Mommsen Liezl vd Linde Wilma Du Plooy Catherine Savage Sean Moore Paul Cronje
6-8 Feb 17	CRI Bestuurs-vergadering: Letsitele	Agenda	Hannes Bester MC Pretorius
8 Feb 17	Houers, Koöp	Kantoorruimte en samewerking	Hannes Bester Wayne Mommsen
7 & 10 Feb 17	STC Pretoria	Vergadering met Sappi senior personeel - 2017 strategie en water absorpsie toetse op plaaslike pulp.	Dawid Groenewald Craig Zorab Donald Nonyane
9 Feb 17	Tzaneen	Wayne Mommsen	Hannes Bester Wayne Mommsen
10 Feb 17	Polokwane	Marlene Calitz	Hannes Bester
14-15 Feb 17	Mpumalanga CRI Na-oes werkswinkels	Agenda	Hannes Bester Dawid Groenewald Keith Lesar MC Pretorius Wayne Mommsen Liezl vd Linde Wilma Du Plooy Catherine Savage Sean Moore
15 Feb 17	Johannesburg	CFB Bestuurder onderhoude	Hannes Bester
16-17 Feb 17	KZN & Swaziland CRI Na-oes werkswinkels	Agenda	Dawid Groenewald Keith Lesar MC Pretorius Liezl vd Linde Wilma Du Plooy Catherine Savage Sean Moore Chanel Steyn
21 Feb 17	Limpopo DAFF /PPECB Roadshow	Uitvoer Regulasies 2017	Wayne Mommsen
21-22 Feb 17	Oos-Kaap CRI Na-oes werkswinkel	Agenda	Hannes Bester Dawid Groenewald Keith Lesar MC Pretorius Liezl vd Linde Wilma Du Plooy Catherine Savage Sean Moore Karin Nel
23-24 Feb 17	Wes-Kaap CRI Na-oes werkswinkel	Agenda	Hannes Bester Dawid Groenewald Keith Lesar MC Pretorius Wayne Mommsen Liezl vd Linde Wilma Du Plooy

			Catherine Savage Sean Moore Paul Cronje Karin Nel
27 – 29 Feb 17	Letsitele studiegroep	Vrugtevlieg beheer Onderstamme Pakhuis werkswinkel terugvoer	MC Pretorius Aruna Manrakhan Johan Joubert Wayne Momsen
2 Mrt 17	Innovation Hub, Pretoria	NNZ Nederland. Ingevoerde toedraaipapier, (Fruit Wrappers)	Dawid Groenewald Jacques Coetzee
2 Mrt 17	Letsitele Pakhuisforum vergadering	Dompelbad,Chemiese bad en Waks behandeling	Wayne Mommsen
7 Mrt 17	Pallet Warehouse, Hartebeespoort	Vervaardiging van palette by sitruspakhuise	Dawid Groenewald Ferdi van Jaarsveld.
6-7 Mrt 17	Sondagsriviervallei	CGA Pre-summit tour	Hannes Bester MC Pretorius Wayne Mommsen Wayne Kirkman Johan Joubert Werner Swiegers
8-9 Mrt 17	CGA Citrus Summit	Agenda	Hannes Bester MC Pretorius Wayne Mommsen Wayne Kirkman Johan Joubert Werner Swiegers
11 Mrt 17	Kakamas	Jannie Spangenberg	Hannes Bester
13-14 Mrt 17	Windhoek	AMTA	Hannes Bester Hoppie Nel
15 Mrt 17	Alicedale Est, Tshipise JP Packers, Ohrigstad	A15C proef met 150UF+ kartonne. Probleme met Robot Palettiseerder	Dawid Groenewald Peter Nicholson John Wilkenson Wimpie Mostert Frikkie van Wyk Kobus Beetge
15 Mrt 17	Outjo	Produsente-vergadering	Hoppie Nel Hannes Bester
16 Mrt 17	Tsumeb	Produsente-vergadering	Hoppie Nel Hannes Bester
16 Mrt 17	Universiteit van Pretoria	Prof Nico Labuschagne Barend Strydom – Lugfoto- grafie MC Studies bespreking	MC Pretorius Prof Nico Labuschagne
17 Mrt 17	Outjo	Roelie van Wyk	Hannes Bester
20 Mrt 17	Walvisbaai	M'pact: Liina Muatunga & Frank Scholtz Wimpie Martin: Hawe	Hannes Bester
22 Mrt 17	Kakamas: Karsten Bdy Renosterkop Bdy Swartbooisberg Bdy	Vrugval Produksiepraktyke	Hannes Bester
23 Mrt 17	SRV en Patensie	Pakhuis Forum Vergaderings	Keith Lesar Wilma du Plooy Catherine Savage
23 Mrt 17	SRV en Patensie	Pakhuis Forum Vergaderings	Keith Lesar Wilma du Plooy Catherine Savage
23 Mrt 17	Innovation Hub, Pretoria,	Standaarde vir Hoekstukke Pride Pak en Corruseal.	Dawid Groenewald.
23 Mrt 17	Alzu	NSSA symposium vergadering met Bayer	MC Pretorius Dr. Mieke Daneel

24 Mrt 17	Karino	Boordbesoeke – Midknight boorde > 100t/ha	MC Pretorius James Warrington
24 Mrt 17	Kirkwood	Pakhuis besoeke/konsultasie	Keith Lesar Wilma du Plooy Catherine Savage
27 Mrt 17	Port Elizabeth	Water Versuiwering Vergadering	Keith Lesar Wilma du Plooy Catherine Savage
29 Mrt 17	Nelspruit	Produksiewerkswinkel beplanning	MC Pretorius Wayne Momsen Liezl vd Linde Johan Joubert Chris Kellerman James Warrington
30 Mrt 17	Ohrigstad studiegroep	Produsent en boord besoeke CRI – insette: Produksiewerkswinkels Pakhuis forum beplanning Kultivars Spuit tegnologie	MC Pretorius Johan Joubert Charl Kotze Johnny Roberts
	Hoedspruit studiegroep	Produsent en boord besoeke CRI insette; Produksiewerkswinkels Kultivars en onderstamme Spuit tegnologie FCM beheer CGACC kultivar bespreking	MC Pretorius Wayne Momsen Johan Joubert Charl Kotze Johnny Roberts

7.2 OTHER MEANS OF TECHNOLOGY TRANSFER

7.2.1 **SA Fruit Journal** by Tim G Grout (CRI)

Table 7.2.1.1. S A Fruit Journal articles in 2016-17 besides Extension Briefs.

Issue	Pages	Title	Author/s
April/May 15(2)	36	Research for citrus exports: A sector-specific Innovation fund from DST	T.G. Grout
	45	Uitstekende deelname aan 2016 se CRI Na-oes Werkwinkels	H. Bester
	52	Report back on a workshop to discuss CRI's horticultural research strategy for citrus	J. North, T. Vahrmeijer, O.P.J Stander& P. Cronje
Jun/Jul 15(3)	56	Drought management in citrus	T. Vahrmeijer
Aug/Sep 15(4)	12	New crop of entomologists	S.D. Moore
	52	Does molasses, applied with granulovirus against FCM, increase citrus thrips infestation and damage?	S. Thackeray, S. D. Moore, M. Parkinson & M. Hill
	94	New manager for National Extension at the CRI	L. Brodie
Oct/Nov 15(5)	64	Important considerations for citrus production under shade nets	O.P.J. Stander & P. Cronje
Dec/Jan 15(6)	43	Die Ballie Wahl-meriete toekenning vir die beste Hortologie student in Sitrusproduksie	P. Cronje
	84	Verspreiding van vrug kwaliteit op aan-jaar 'hardorcott' mandaryn bome	N. Jordaan & O.P.J. Stander
	88	Hennie le Roux orbituary	
Feb/Mar 16(1)	52	The European pepper moth as a citrus nursery pest in SA	S.D. Moore & F. Joubert
	54	Male Annihilation Technique (MAT) for the Oriental Fruit Fly	A. Manrakhan, J. Daneel & R. Beck
	62	Ninth Citrus Research Symposium	L. van der Linde

7.2.2 **CRI website** by Tim G Grout

Table 7.2.2.1. Visits and page requests on www.cri.co.za since April 2016.

Month	Unique visitors	Number of visits	Pages	Hits	Bandwidth
Total 2015/6	33 023	58 772	202 555	572 867	10.72 GB
Apr 2016	2418	4051	12834	45091	1.01 GB
May 2016	2188	3324	11251	42629	993.29 MB
Jun 2016	2124	3367	11200	44089	814.20 MB
Jul 2016	2166	3857	13535	48020	892.20 MB
Aug 2016	2475	4013	12735	49034	947.19 MB
Sep 2016	2611	4557	13791	47958	1.07 GB
Oct 2016	2793	5474	18731	64019	909.16 MB
Nov 2016	3228	5894	17199	53615	1.07 GB
Dec 2016	2277	5190	12867	35407	671.35 MB
Jan 2017	2887	5694	16589	56832	916.65 MB
Feb 2017	2855	5260	17773	59493	1.27 GB
Mar 2017	2957	5476	16863	56137	1.17 GB
Total 2016/7	30 979	56 157	175 368	602 324	11.20 GB

7.2.3 **CRInet** by Tim G Grout

CRInet provides a good opportunity for growers to share opinions or ask questions on any technical citrus topic but it is mostly being used for dissemination of information from CRI or CGA. The 45 messages sent during the 2016 calendar year are fewer than the average of 51 per annum for the last 9 years but this deviation is probably no cause for concern (Table 7.2.3.1). There are currently 467 CRInet members.

Table 7.2.3.1. Numbers of messages circulated per month on CRInet.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
2017	0	0	4										4
2016	6	3	0	3	1	8	10	3	1	6	3	6	50
2015	5	2	3	3	2	3	12	4	4	1	3	1	43
2014	4	3	4	1	12	6	13	1	0	1	3	1	49
2013	1	15	0	7	3	0	2	4	6	13	1	6	58
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

7.2.4. **Cutting Edge** by Tim G Grout (CRI)

Some growers consider the Cutting Edge 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 2016/7 are given in Table 7.2.4.1.

Table 7.2.4.1. Cutting Edge issues during 2016-17.

No.	Title	Issue	Author
212	Japan Carton Declarations in 2016	Apr	P. Hardman
213	CRI-Diagnostic Centre – Services and Price List	Apr	J. van Niekerk & E. Basson
214	Fruit Export Industry Guidelines on Implementation of IMO, SOLAS Chapter VI, Part A, Regulation 2: Shippers Mandatory Verification of the Gross Mass of a Packed Container (VGM Guideline Part 1 of 3: 21st April 2016)	Apr	M. Brooke

215	Consumer Assurance and MRL Update	May	P. Hardman
216	Fruit Export Industry Guidelines on Implementation of IMO, SOLAS Chapter VI, Part A, Regulation 2: Shippers Mandatory Verification of the Gross Mass of a Packed Container (VGM Guideline Part 2 of 3: 27th May 2016; Revision 1: 14th June 2016 – inclusion of section 4 and update to section 3, 8 & 10)	Jun	M. Brooke & A. Connell
217	Control recommendations in areas with high fruit fly population pressure	Jun	A. Manrakhan, M. Gilbert & S.D. Moore
218	Warning of possible phosphonate phytotoxicity on late mandarin fruit	Jul	J. van Niekerk, P. Cronje, C. Kotze & MC Pretorius
219	Fosetyl-Al, Phosphorous acid and Phosphonates MRLs	Jul	P. Hardman
220	Postharvest recommendations for 2016 – Guidelines according to the factsheets issued by CRI	Jul	W. du Plooy, J.J. Bester & K. Lesar
221	Chimeras in late Valencia cultivars	Aug	P. Fourie, J. Joubert & MC Pretorius
222	Dichlorprop-P use considerations for 2016/2017	Sep	P. Hardman
223	Fruit fly protocol for citrus production regions under high fruit fly populations (Western Cape and Eastern Cape)	Oct	A. Manrakhan, M. Gilbert & S.D. Moore
224	Introducing CRI-PhytRisk for improved Citrus Black Spot management	Oct	M. Kelleman & P. Fourie
225	Recommended fruit thinning strategies for 2017 season	Nov	P. Cronje & J. Stander
226	Status of the Benomyl and Mancozeb maximum residue levels in the USA for citrus	Nov	P. Hardman
227	Changes to the MRL status for Fenpyroximate on citrus in the EU and Canada	Nov	P. Hardman
228	Export recommendations for Satsumas to the EU in 2017	Mar	S.D. Moore, P. Cronje & V. Hattingh

8 PUBLICATIONS IN 2016

8.1 REFEREED PUBLICATIONS (OR ISI RANKED JOURNALS)

Chartier, F., V.C., M.P. Hill, S.D. Moore & J.F. Dames. 2016. Screening of entomopathogenic fungi against citrus mealybug, *Plannococcus citri* (Hemiptera: Pseudococcidae). African Entomology 24(2): 343–351.

Cook, G., S.P. van Vuuren, J.H.J. Breytenbach, J.T. Burger and H.J. Maree. 2016. Expanded Strain-Specific RT-PCR Assay for Differential Detection of Currently Known Citrus Tristeza Virus Strains: a Useful Screening Tool. J. Phytopathol. 164: 847-851.

Cook, G., S.P. van Vuuren, J.H.J. Breytenbach, C. Steyn, J.T. Burger and H.J. Maree. 2016. Characterization of Citrus tristeza virus Single-Variant Sources in Grapefruit in Greenhouse and Field Trials. Plant Dis. 100: 2251-2256.

Coombes, C.A., M.P. Hill, S.D. Moore, J.F. Dames. 2016. Entomopathogenic fungi as control agents of *Thaumatotibia leucotreta* in citrus orchards: field efficacy and persistence. BioControl 61: 729-739.

Defraeye, T., Nicolai, B., Kirkman, W., Moore, S., van Niekerk, S., Verbovena, P., Cronjé, P. 2016. Integral performance evaluation of the fresh-produce cold chain: A case study for ambient loading of citrus in refrigerated containers. Postharvest Biology and Technology 112: 1-13.

De Villiers, M., Hattingh, V., Kriticos, D.J., Brunel, S., Vayssières, J.-F., Sinzogan, A., Billah, M.K., Mohamed, S.A., Mwatawala, M., Abdelgader, H., Salah, F.E.E., De Meyer, M. 2016. The potential distribution of *Bactrocera dorsalis*: considering phenology and irrigation patterns. Bull. Entomol. Res. 106: 19-33.

Grout, T.G. & K.C. Stoltz. 2016. Eliminating *Macchiademus diplopterus* (Hemiptera: Lygaeidae) and *Siculobata sicula* (Acari: Oribatulidae) From Export Fruit Using Ethyl Formate. J. Econ. Entomol. 109: 2329-2333.

Hofmeyr, J.H., V. Hattingh, M. Hofmeyr & J.P. Slabbert. 2016. Postharvest phytosanitary disinfestation of *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) in citrus fruit: validation of an ionising radiation and cold combination treatment. Afr. Entomol. 24(1): 217-224

Hofmeyr, J.H., M. Hofmeyr, V. Hattingh & J.P. Slabbert. 2016. Postharvest phytosanitary disinfestation of *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) in citrus fruit: determination of ionising radiation and cold treatment conditions for inclusion in a combination treatment. Afr. Entomol. 24(1): 208-216.

Hofmeyr, H., Vaughan Hattingh, Marsheille Hofmeyr and Kobus Slabbert. 2016. Postharvest phytosanitary disinfestation of *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) in citrus fruit: Validation of an ionizing radiation treatment. Florida Entomologist 99 (Special Issue 2): 54-58.

Kellerman, M., J. Joubert, A. Erasmus, P.H. Fourie. 2016. The effect of temperature, exposure time and pH on imazalil residue loading and green mould control on citrus through dip application. Postharvest Biol. Tech. 121: 159-164.

Malan, A.P. & S.D. Moore. 2016. Evaluation of local entomopathogenic nematodes for the control of false codling moth, *Thaumatotibia leucotreta* (Meyrick, 1913), in a citrus orchard in South Africa. African Entomology 24(2): 489–501.

Manrakhan, A., J.H. Venter, V. Hattingh. 2015. The progressive invasion of *Bactrocera dorsalis* (Diptera: Tephritidae) in South Africa. Biol. Invasions 17: 2803-2809.

Moore, S.D., Wayne Kirkman, Peter R. Stephen, Sonnica Albertyn, Claire N. Love, Tim G. Grout, Vaughan Hattingh. 2017. Development of an improved postharvest cold treatment for *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae). Postharvest Biol. Technol. 125: 188-195.

Moore, S.D., W. Kirkman and V. Hattingh. 2016. Verification of Inspection Standards and Efficacy of a Systems Approach for *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) for Export Citrus From South Africa. J. Econ. Entomol. 109(4): 1564-1570.

Moore, S.D., W. Kirkman, S. Albertyn and V. Hattingh. 2016. Comparing the Use of Laboratory-Reared and Field-Collected *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) Larvae for Demonstrating Efficacy of Postharvest Cold Treatments in Citrus Fruit. J. Econ. Entomol. 109(4): 1571-1577.

Moore, S.D., W. Kirkman, S. Albertyn, C. N. Love, J. A. Coetzee and V. Hattingh. 2016. Partial Cold Treatment of Citrus Fruit for Export Risk Mitigation for *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) as Part of a Systems Approach. J. Econ. Entomol. 109(4): 1578-1585.

Opoku-Debrah, J.K., M.P. Hill, C. Knox, S.D. Moore. 2016. Heterogeneity in virulence relationships between *Cryptophlebia leucotreta* granulovirus isolates and geographically distinct host populations: lessons from codling moth resistance to CpGV-M. BioControl 61: 449-459.

Stander, O.P.J. and Cronje, P.J.R. 2016. Reviewing the Commercial Potential of Hand Thinning in Citrus with a Cost-benefit Analysis of Summer Hand Thinning of 'Nadorcott' Mandarin. Hort Technology 26(2): 206-212.

van Dael, M., S. Lebotsa, E. Herremans, P. Verboven, J. Sijbers, U.L. Opara, P.J. Cronje, B.M. Nicolaï. 2016. A segmentation and classification algorithm for online detection of internal disorders in citrus using X-ray radiographs. Postharvest Biology and Technology 112: 205-214.

Zimba, K., U. Heshula, S.D. Moore & M.P. Hill. 2016. Host searching and oviposition behaviour of *Agathis bishopi* (Hymenoptera: Braconidae), a larval parasitoid of false codling moth, *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae): a potential proxy indicator for fruit infestation. African Entomology 24(2): 524–529.

Zimba, K., S.D. Moore, U. Heshula & M.P. Hill. 2016. *Agathis bishopi*, a larval parasitoid of false codling moth *Thaumatotibia leucotreta*: laboratory rearing and effect of adult food on parasitism and longevity. Afr. Entomol. 24(1): 153-161.

8.2 SEMI-SCIENTIFIC PUBLICATIONS (Other than SA Fruit Journal)

None.

9 PRESENTATIONS AT SOCIETAL AND INTERNATIONAL CONGRESSES

Breytenbach, J.H.J, S.P. van Vuuren, G. Cook and C. Steyn. 2016. Elimination of graft transmissible diseases of citrus in southern Africa through shoot tip grafting. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.

Carstens, E., C.C. Linde, R. Slabbert, A. Miles, N. Donovan, H. Li, M.M. Dewdney, C. Glienke, G.C. Schutte, P.H. Fourie and A. McLeod. 2016. Population structure of the citrus black spot pathogen *Phyllosticta citricarpa* from five continets. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.

Carstens, E., C.C. Linde, R. Slabbert, A. Miles, N. Donovan, H. Li, M.M. Dewdney, C. Glienke, G.C. Schutte, P.H. Fourie and A. McLeod. 2016. Population structure of the citrus black spot pathogen *Phyllosticta citricarpa* from five continets. 13th International Citrus Congress, Foz Do Iguaçu, Brazil.

Cuellar, F., P.J.R. Cronje, G. Barry, M.J Rodrigo and L. Zacarias. 2016 Characterization of New Lycopene Accumulating Mutants of Sweet Oranges. 13th International Citrus Congress, Foz Do Iguaçu, Brazil.

Christie, C., C. Savage, C.L. Lennox, A. Erasmus, W. du Plooy and P.H. Fourie. 2016. Postharvest disease prevention begins with sanitation. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.

Cook, G, J.H.J. Breytenbach and S.P. van Vuuren. 2016. Field performance of various *Citrus tristeza* virus cross-protection sources trialled in grapefruit in different climatic regions. 20th IOCV Conference, Chongqing, China.

Cook, G., S.P. van Vuuren, J.H.J. Breytenbach, C. Steyn, J.T. Burger and H.J. Maree. 2016. *Citrus tristeza* virus: A journey of discovery. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.

Daneel, J., A. Manrakhan and M. de Meyer. 2016. Insights into the biology and ecology of two morphotypes and genotypes of the Natal fruit fly (Diptera, Tephritidae: *Ceratitis rosa*). TEAM (Tephritid workers group) fruit fly symposium, Stellenbosch.

Daneel, J., A. Manrakhan and M. de Meyer. 2016. Split of the Natal fruit fly into two species: implications and current investigations. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.

du Plooy, W., K. Lesar, A. Erasmus and P.H. Fourie. 2016. Postharvest disease management at the CRI: Past, present and future. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.

Ehlers J, K.I. Theron, F Alférez and P.J.R. Cronje. 2016. The use of pre-harvest foliar applied plant growth regulators to reduce rind pitting on 'Benny' Valencia. 13th International Citrus Congress, Foz Do Iguaçu, Brazil.

Ehlers J, K.I. Theron, F Alférez and P.J.R. Cronje. 2016. Non-chilling post-harvest pitting of Valencia orange. Combined Congress, Bloemfontein

Erusmas, A., C. Christie, C.L. Lennox, C. Savage and P.H. Fourie. 2016. Sanitisation of fungicide drench solutions and effects on green mould and sour rot control. 13th International Citrus Congress, Foz Do Iguaçu, Brazil.

Fourie, P.H., T. du Toit, M. le Roux and L. Olivier. 2016. The Southern African Citrus Improvement Scheme: meeting a dynamic industry's demands. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.

Fourie, P.H., M. Kellerman, E. Carstens, G.C. Schutte, V. Hattingh, M.M. Dewdney, A. Miles, R. Magarey and T. Gottwald. 2016. Citrus Black Spot epidemiology: what have we learnt in the past 4 years? 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.

Fourie, P.H., M. Kellerman, E. Carstens, G.C. Schutte, V. Hattingh, M.M. Dewdney, A. Miles, R. Magarey and T. Gottwald. 2016. Citrus Black Spot epidemiology: what have we learnt in the past 4 years? 13th International Citrus Congress, Foz Do Iguaçu, Brazil.

Gilbert, M. 2016. Monitoring of fruit flies (Tephritidae) on multi-crop farms in the Western Cape Province of South Africa. TEAM (Tephritid workers group) fruit fly symposium, Stellenbosch

Gilbert, M. 2016. Population fluctuations of Medfly & False Codling Moth on Western Cape multi-crop farms. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg

Gilbert, M. 2016. The autecology of fruit flies (Tephritidae) & false codling moth (Tortricidae) on multi-crop farms in the Western Cape region of South Africa. International Congress of Entomology, Florida, USA.

Gilbert, M. 2016. Monitoring of False Codling Moth in orchards of stone & pome fruit, citrus and vineyards. Hortgro FCM seminar, Stellenbosch.

Kellerman, M., G.J. van Zyl and P.H. Fourie. 2016. CRI-PhytRisk: improved Citrus Black Spot management through web-based information systems. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.

Kellerman, M., M.M. Dewdney, A. McLeod and P. H. Fourie. 2016. Germination parameters of pycnidiospores of *Phyllosticta citricarpa*. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.

Kellerman, M., M.M. Dewdney, A. McLeod and P. H. Fourie. 2016. Germination parameters of pycnidiospores of *Phyllosticta citricarpa*. 13th International Citrus Congress, Foz Do Iguaçu, Brazil.

Kellerman, M., G.J. van Zyl and P.H. Fourie. 2017. CRI-PhytRisk: improved Citrus Black Spot management through web-based information systems. 50th Congress of the Southern African Society for Plant Pathology (SASPP), Champagne Sports Resort, Drakensburg.

Kotze, C., M.C. Pretorius and J.M. van Niekerk. 2016. Trunk and dry root rot of citrus observed in some Southern African citrus orchards. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg

Love, C., M. Hill, S.D. Moore, M. Goddard and C. Daniel. 2016. Improving the cold tolerance of false codling moth through diet manipulation for improved performance in a sterile insect release programme. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.

Mamba, L.C., J.C. Meitz-Hopkins, M. Rosmarin, C. Stevens, M. Webber, W. du Plooy, P.H. Fourie, A. Erasmus and C.L. Lennox. 2016. Propiconazole drench application for citrus sour rot control. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.

Mamba, L.C., J.C. Meitz-Hopkins, M. Rosmarin, C. Stevens, M. Webber, W. du Plooy, P.H. Fourie, A. Erasmus and C.L. Lennox. 2017. Propiconazole drench application for citrus sour rot control. 50th Congress of the Southern African Society for Plant Pathology (SASPP), Champagne Sports Resort, Drakensburg.

Mamba, L.C., C.L. Lennox, P.H. Fourie, W. du Plooy, A. Erasmus and J.C. Meitz-Hopkins. 2017. Propiconazole baseline sensitivity of Penicillium digitatum isolates from a Western Cape orchard. 50th Congress of the Southern African Society for Plant Pathology (SASPP), Champagne Sports Resort, Drakensburg.

Meyer, J.B., P.H. Fourie, N.W. McLaren, G.C. Schutte. 2016. Determining ascospore release of *Phyllosticta* spp. in different citrus production areas of South Africa. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.

Nel, H., P.H. Fourie, J. Edmonds and P. Hardman. 2016. Projected future South African Citrus volumes: a comparison of projection models and an assessment of opportunities and risks related to global trade trends. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.

North, J.J., P.J.R Cronje. 2017. Post-harvest factors affecting the incidence of blossom-end clearing in grapefruit. Combined Congress, Klein Kariba.

Savage, C., C.L. Lennox, P.H. Fourie, W. du Plooy and A. Erasmus. 2016. Effect of imazalil solution temperature on citrus green mould development and survival of Rhizopus stolonifera. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.

Savage, C. 2016. Effect of imazalil solution temperature on citrus green mould development and survival of *Rhizopus stolonifer*. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.

Savage, C. 2017. Drench: Principles; Design; Products. Citrus Research International Postharvest Technical Forum, Six production regions, South Africa.

Savage, C. 2017. High pressure sprayers, Fungicide bath & Flooder: Products; Temperature, pH, time; Top-up. Citrus Research International Postharvest Technical Forum, Six production regions, South Africa.

Savage, C., C.L. Lennox, P.H. Fourie, A. Erasmus, W. du Plooy. 2017. Hot or cold fungicide application? The effect of temperature on citrus green mould control and survival of *Rhizopus stolonifer*. 50th Congress of the Southern African Society for Plant Pathology (SASPP), Champagne Sports Resort, Drakensburg.

Schreuder, W., A. Erasmus, G.C. Schutte, W. du Plooy, C.L. Lennox and P.H. Fourie. 2016. Effect of postharvest treatments on development of latent CBS infections. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.

Stander, O.P.J., Barry, G.H. and Cronjé, P.J.R. 2016. An investigation of the role of carbohydrates in alternate bearing of mandarins. International society of citriculture, Brazil.

Stander, O.P.J. 2017. Chemical fruit thinning strategies for late mandarins (*Citrus reticulata*). SASHS, Klein-Kariba, Bela-Bela.

Stevens, C., M. Rosmarin, L.C. Mamba, C.L. Lennox, A. Erasmus, W. du Plooy, P.H. Fourie, J.C. Meitz-Hopkins. 2017. Molecular identification and propiconazole baseline sensitivity of citrus sour rot. 50th Congress of the Southern African Society for Plant Pathology (SASPP), Champagne Sports Resort, Drakensberg.

Steyn, C., J.H.J. Breytenbach, S.P. van Vuuren and G. Cook. 2016. Citrus viroids: back with a bang. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.

Steyn, C., J.H.J. Breytenbach, S.P. van Vuuren and G. Cook. 2017. Citrus viroids: A re-emerging problem. 50th Congress of the Southern African Society for Plant Pathology (SASPP), Champagne Sports Resort, Drakensberg.

van Niekerk, J.M., C. Kotze and M.C. Pretorius. 2016. Integrated approach to preventative management of soilborne pathogens in citrus nurseries. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.

van Niekerk, J.M., E. Basson and A. McLeod. 2016. Identification of *Pythium* spp. occurring in South African citrus nurseries. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.

van Niekerk, J.M., C. Olivier, E. Basson and A. McLeod. 2016. Variation in mefenoxam sensitivity among *Phytophthora* spp. isolates from South African citrus nurseries. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.

van Niekerk, J.M., E. Basson, C. Olivier and A. McLeod. 2016. Mefenoxam sensitivity of *Phytophthora citrophthora* and *P. nicotianae* isolates in South African citrus nurseries. 13th International Citrus Congress, Foz Do Iguaçu, Brazil.

van Niekerk, J.M., E. Basson, C. Olivier and A. McLeod. 2017. Mefenoxam and Chlorine sensitivity of *Phytophthora citrophthora* and *P. nicotianae* isolates in South African citrus nurseries. 50th congress of the Southern African Society of Plant Pathology (SASPP), Champagne Sports Resort, Drakensberg.

van Niekerk, J.M., C. Kotze, H. Marais, J. North and P. Cronje. 2017. Effect on rind quality of phosphonate applications on “Nadorcott” mandarin fruit for the control of *Phytophthora* brown rot. 50th congress of the Southern African Society of Plant Pathology (SASPP), Champagne Sports Resort, Drakensberg.

van Niekerk, J.M., C. Kotze, H. Marais, J. North and P. Cronje. 2017. Understanding citrus replant disease in South Africa with the aim of developing a Methyl Bromide free management strategy. 50th congress of the Southern African Society of Plant Pathology (SASPP), Champagne Sports Resort, Drakensberg.

van Zyl, G., G.C. Schutte, M. Gilbert and P.H. Fourie. 2016. Evaluating reduced volume fungicide and pesticide spray application in South African citrus orchards: Towards more efficient spray application practices. 9th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.

Visser, M., G. Cook, J.T. Burger and H.J. Maree. 2017. An in-depth analysis of pathogen-associated gene regulation in grapefruit. 50th Congress of the Southern African Society for Plant Pathology (SASPP), Champagne Sports Resort, Drakensberg.