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CRI Group Annual Research Report for April 2018 to March 2019
Citrus Research International, Nelspruit

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1 MARKET ACCESS TECHNICAL COORDINATION

By Vaughan Hattingh and Elma Carstens (CRI)

1.1 SUMMARY

The FCM Management System (FMS) that CRI developed as an alternative to postharvest disinfestation (cold treatment) was implemented for the first time in 2018 for citrus exports to EU, with great success. Only nine FCM interceptions on citrus from South Africa were reported by the EU in 2018, from approximately 53 million cartons exported to EU. Based on experience gained during the 2018 season, CRI and DAFF agreed on further improvements to the FMS in December 2018, applicable to the 2019 season. Only two notifications of CBS interceptions on citrus from South Africa were notified by the EU in 2018 which is a major improvement on the 23 interceptions in the previous year. Continued access to the Japan market was secured when Japan-MAFF accepted the new scientific data generated by CRI, confirming that *B. dorsalis* is more cold sensitive than Medfly (*C. capitata*) and that Medfly cold treatments can be used for disinfestation of *B. dorsalis*. Market access to Japan was further facilitated by the approval of inspection, pre-cooling and loading of citrus fruit at two inland facilities. CRI contributed to the development and acceptance by USDA-APHIS of a Standard Operating Procedure (SOP) to enable continuation of bulk shipping to the USA, considering that the programme was under threat due to unavailability of the Fresh Produce Terminals (FPT) at Cape Town Port for cold treatment preparation and inspections as of 2019. Finalisation of the rule to enable export of citrus from South African production areas outside of the CBS-free areas was scheduled for September 2018, according to the USDA-APHIS Federal Register Semi-Annual Regulatory Agenda that was published in June 2018. However, this has not as yet occurred, despite assurance that all technical issues have been addressed. CRI supported the eSwatini National Plant Protection Organisation with technical inputs on the Pest Risk Analysis (PRA) conducted for access to the USA market. CRI and PPECB provided DAFF with sustained inputs during bilateral negotiations that in November 2018 culminated in China and South Africa agreeing to a protocol for bulk shipping of citrus to China. This enables co-loading of citrus destined for China and Japan and is expected to relieve some logistical pressure in the Durban Port. CRI supported the Zimbabwean citrus growers with inputs on the China PRA conducted for export of citrus from Zimbabwe. On request from CGA, CRI and PPECB revised guidelines for citrus shipments to Indonesia, to provide for handling temperatures that should reduce the incidence of chilling injury on citrus exports to Indonesia. CRI continued to support DAFF with scientific and technical information required in bilateral negotiations with Philippine authorities aimed at opening this market for South African citrus in the future.

OPSOMMING

Die VKM Bestuurstelsel (FMS) wat CRI as 'n alternatief vir na-oes disinfestasië (koue-behandeling) ontwikkel het, is vir die eerste keer in 2018 vir sitrusuitvoere na die EU geïmplementeer, met groot sukses. Slechs nege VKM onderskeppings op sitrus vanaf Suid-Afrika, is in 2018 deur die EU aangemeld, van ongeveer 53 miljoen kartonne wat na die EU uitgevoer is. Gebaseer op die ondervindinge van die 2018-seisoen, het CRI en DAFF in Desember 2018 op verdere verbeterings aan die FMS ooreengekom, vir toepassing in die 2019-seisoen. Slechs twee kennisgewings van SSV onderskeppings op sitrus vanaf Suid-Afrika is in 2018 deur die EU aangemeld, wat 'n groot verbetering is op die 23 onderskeppings in die vorige jaar. Voortgesette toegang tot die Japan-mark is verseker toe Japan-MAFF die nuwe wetenskaplike data wat deur CRI gegenereer is, aanvaar het, wat bevestig dat *B. dorsalis* meer koue-sensitief as Medvlieg (*C. capitata*) is en dat Medvlieg koue-handelings vir die disinfestasië van *B. dorsalis* gebruik kan word. Marktoegang tot Japan is verder verbeter deur die goedkeuring van inspeksie, voor-verkoeling en laai van sitrusvrugte by twee binnelandse fasiliteite. CRI het bygedra tot die ontwikkeling en aanvaarding van 'n Standaard-bedryfsprosedure (SOP) deur USDA-APHIS, om die voortsetting van grootmaat verskeping na die VSA moontlik te maak. Die program was onder druk omrede die Fresh Produce Terminals (FPT) in Kaapstad-Hawe nie meer vir voorbereiding van koue-behandelings en inspeksies vanaf 2019 beskikbaar sal wees nie. Finalisering van die wetgewing om die uitvoer van sitrus vanaf Suid-Afrikaanse produksiegebiede buite die SSV vrye areas moontlik te maak, was vir September 2019 geskeduleer volgens die USDA-APHIS Federal Register Semi-Annual Regulatory Agenda wat in Junie 2018 gepubliseer is. Dit het egter

nog nie gebeur nie, ondanks die versekering dat alle tegniese probleme aangespreek is. CRI het die eSwatini Nasionale Plantbeskermingsorganisasie ondersteun deur tegniese insette te lewer op 'n Pes Risiko Analise (PRA) wat vir toegang tot die VSA mark uitgevoer is. CRI het ook die Zimbabwiese sitrusprodusente ondersteun met insette op die China PRA wat gedoen is vir die uitvoer van sitrus vanaf Zimbabwe. CRI en PPECB het DAFF van insette tydens bilaterale onderhandelinge voorsien, wat in November 2018 tot gevolg gehad het dat China en Suid-Afrika tot 'n protokol vir grootmaat verskeping van sitrus na China ooreenstem. Dit maak die saamlaai van sitrus wat vir China en Japan bestem is moontlik, en daar word verwag dat dit van die logistieke druk in die Durbanse hawe kan verlig. Op versoek van die CGA, het CRI en PPECB die riglyne vir sitrusverskeping na Indonesië hersien, om voorsiening te maak vir temperature wat die voorkoms van koue-skade by sitrusuitvoere na Indonesië sal verminder. CRI het voortgegaan om DAFF te ondersteun met wetenskaplike en tegniese inligting vir bilaterale onderhandelinge met die Filippynse owerhede wat daarop gemik is om die mark vir Suid-Afrikaanse sitrus in die toekoms te open.

1.2 EUROPE (EU)

FCM

In April 2018 further meetings about the FMS and PhytClean were held with industry role players to ensure that all systems are in place for exports. The FMS and PhytClean were again presented at SA-DAFF and PPECB workshops in April 2018. An FMS Advisory Committee was established to guide implementation of the FMS in 2018. CRI produced a guideline for a recommended procedure when conducting on-arrival inspection to determine viability status of “live” FCM. Further meetings were held with shipping lines to include regime codes for conventional shipping to the EU.

The FCM Management System (FMS) that CRI developed as an alternative to postharvest disinfestation (cold treatment) was implemented for the first time in 2018 for citrus exports to EU, with great success. Only nine FCM interceptions on citrus from South Africa were reported by the EU in 2018, from approximately 53 million cartons exported to EU. A FCM Rapid Response Team was established to conduct assessments of FCM interceptions in the EU. Investigation reports on the 9 FCM interceptions were submitted to SA-DAFF and in all cases no anomalies or deviations from the FMS were detected/identified.

At the Citrus Coordinating Meeting on 24 October 2018 it was agreed that the FMS working group (industry role players) will meet to amend the current SA-DAFF approved FMS (dated 3 October 2017) to improve compliance in 2018 as officially requested by the EU. The workshop took place on 8 November 2018. CRI discussed the amendments with SA-DAFF on 22 November 2018 and a final revised version, authorised by SA-DAFF for implementation on 18 December 2018, was released on 20 December 2018. All the changes made were communicated to all role players in a Cutting Edge (No 261). The changes were also presented at CRI's Post Harvest Workshops during February 2019. Meetings between CRI, CGA, PPECB and shipping lines also took place to discuss and finalize the regime codes for break bulk shipping as well as several meetings of the FMS Advisory Committee to guide implementation of the amended FMS and to evaluate new requests from Industry to amend the FMS.

On 21 March 2019, the EU Commission Implementing Directive (EU) 2019/523 was published that amends Annexes I to V to Council Directive 2000/29/EC. The amended regulation stipulates that for 16.6 (d), fruit (except lemons and *C. aurantifolia*): “have been subjected to an effective cold treatment to ensure freedom from *Thaumatotibia leucotreta* (Meyrick) or another effective treatment to ensure freedom from *Thaumatotibia leucotreta* (Meyrick) and the treatment data should be indicated on the certificates referred to in Article 13(1)(ii), provided that the treatment method together with documentary evidence of its effectiveness has been communicated in advance in writing by the national plant protection organisation of the third country concerned to the Commission”. At the workshop of 7 May 2019, the decision was taken that SA-DAFF will include the available scientific publications that demonstrate the efficacy of the FCM Systems Approach in their communication to the EU to indicate

compliance with the amended wording of the regulation. CRI submitted the publications to SA-DAFF on 20 May 2019.

CBS

In the 2017 citrus export season, 23 CBS interceptions from South Africa on citrus fruit were notified by the EU. As most of the interceptions were on Valencias that were exported in September and October 2017, the CBS-RMS was amended by the CBS-RMS working group to improve compliance in 2018. The draft CBS-RMS were submitted to SA-DAFF for adoption and implementation in the 2018 export season. However, on 28 March 2018, SA-DAFF released an update of the CBS-RMS, including some changes that had not been agreed upon at the 2017 CBS-RMS Working Group meeting. These changes included validity periods for Valencia orchard inspection reports as of August, a requirement for repeated orchard inspections for lemons, and requirements for orchard inspections for degreened fruit. Objections were raised and on 9 April 2018 SA-DAFF released a revised CBS-RMS. Objections were again raised by members of the CBS-RMS Working Group. A teleconference took place on 02 May 2018 and CRI compiled a report on the CBS risk associated with degreened fruit and presented this with recommendations on further CBS-RMS changes to SA-DAFF on 18 May 2018. SA-DAFF released an updated version of the CBS-RMS on 23 July 2018.

Two notifications of CBS interceptions from South Africa on citrus fruit were notified by the EU in 2018. At the Citrus Coordinating Meeting on 24 October 2018 it was agreed that the CBS working group (industry role players) will meet to amend the current CBS-RMS dated 23 July 2018. The workshop took place on 8 November 2018 and only one issue was identified - to allow co-loading of fruit from CBS free areas with fruit from CBS areas. CRI discussed the amendments with SA-DAFF on 22 November and DAFF indicated that before the request can be approved, PhytClean must provide the assurance that the pre-verification report should indicate that the fruit is from CBS free or non CBS free areas. PhytClean was updated and SA-DAFF released the CBS RMS for the 2019 citrus export season on 6 May 2019.

FRUIT FLIES

The EU Commission Implementing Directive (EU) 2019/523 of 21 March 2019 that amends Annexes I to V to Council Directive 2000/29/EC was published and non EU trading partners must comply with the regulation from 1 September 2019. The new set of rules stipulates compliance measures for fruit flies (Tephritidae (non-European)). NPPOs of non EU trading partners must communicate to the Commission before 1 September 2019, notifying which compliance measure will be used for fruit flies. The regulation states that citrus fruit from Africa, amongst other crops, must either be sourced from a fruit fly free area or must be subjected to an effective treatment to ensure freedom from fruit flies. A meeting between the implicated industries, PPECB, FPEF and SA-DAFF to discuss this new development and to agree on the effective treatment took place on 7 May 2019. The decision was taken that a basic Citrus Fruit Fly System Approach would be the best option to comply with the new regulation. CRI compiled the Citrus Fruit Fly Management System (Citrus FF-MS) and submitted it to SA-DAFF on 20 May 2019.

OTHER

The EU has published a new Plant Health Regime that will replace Council Directive 2000/29/EC (the framework plant health regulation). Implementation of the new Regime will come into force in late 2019. Industries need to assess how these changes will affect export operations. On 7 May 2019 CRI, representatives from other implicated industries, PPECB and SA-DAFF met to discuss Regulation 2016/2031. Possible concerns were highlighted and discussed.

1.3 JAPAN

The outstanding issues pertaining to this market remain the three requests: to allow access for all mandarins (except Satsumas), under the current protocol for Clementines (pending from November 2009), to revise the current cold treatment conditions for the export of all eligible citrus types to Japan by the inclusion a cold treatment of 1.4°C or lower for 16 consecutive days (pending from November 2009) and to include all navel orange cultivars as the protocol currently only specifies Washington and Cara Cara Navel cultivars (pending from September 2016). Despite many industry meetings and following communication made by SA-DAFF, the Japan-MAFF response is that they deal with one market access request from a country at a time and currently Japan-MAFF is working on the protocol for importing South African avocados.

In November 2017 Japan-MAFF indicated that they are concerned that the current cold treatment would not be adequate for *Bactrocera invadens*. In March 2018, CRI supplied SA-DAFF with the scientific information to confirm that Medfly (*C. capitata*) cold treatments can be used for disinfestation of *B. dorsalis*. SA-DAFF submitted the reports to Japan-MAFF in April 2018. On 27 March 2019 Japan-MAFF responded that they had evaluated the scientific data and that they concluded that Medfly is more cold tolerant than *B. dorsalis* and that they agreed with South Africa that Medfly cold treatments can be used for disinfestation of *B. dorsalis*. Japan-MAFF also indicated that South Africa will be listed as a country where *B. dorsalis* is present and that the import conditions will be updated accordingly.

In October 2018, CRI received a request from citrus producers and exporters for inland inspection, pre-cooling and loading of citrus fruit destined for Japan as the current bilateral protocol only allows for the following: "*Point 2 (1) A: Cold treatment facilities for the fresh fruits shall be located at the point of pier areas, where it is not necessary to transport the sterilized fresh fruits on land for loading*". The industry submitted a proposal to SA-DAFF on 16 November 2018 to request Japan-MAFF to amend the current bilateral protocol to include inland operations. In a meeting between CRI, CGA and SA-DAFF on 18 February 2019, SA-DAFF indicated that the request to inspect, pre-cool and load citrus fruit from inland facilities will be discussed with the Japanese Inspector, expected to arrive on 1 April 2019. A meeting took place between SA-DAFF and the Japan-MAFF inspector on 2 April 2019 and on 12 April 2019 two facilities (PE Cold Storage and Cape Fruit Coolers) were approved by Japan-MAFF for inland inspection, pre-cooling and loading of citrus fruit.

1.4 USA

On 31 May 2018 a Digital Video Conference (DVC) took place between USDA-APHIS and SA-DAFF and all the outstanding and new issues were discussed.

The new issue for discussion was that the Fresh Produce Terminals (FPT) at Cape Town Port will no longer be available in 2019 as an inspection point and a cold treatment facility for the Citrus Export Programme to the United States of America (USA) due to business related reasons. As this programme makes use of both specialized refrigerated vessels (conventional vessels) and containers, an alternative approach for inspection and pre-cooling of citrus fruit destined for this market is required. A Standard Operating Procedure (SOP) was developed by PPECB, SA-DAFF, CRI and Summer Citrus for: i) pre-clearance inspection and pre-cooling of citrus fruit (according to the Work plan) at approved outside cold treatment facilities; ii) transporting of inspected/approved and pre-cooled fruit to Cape Town Port; iii) and for loading of the fruit on the quay side at Cape Town Port into approved specialized refrigerated vessels (conventional vessels). SA-DAFF submitted the SOP document on 14 May 2018 to USDA-APHIS with a request to allow three trial shipments during the 2018 export season. USDA-APHIS requested further information and SA-DAFF provided the information to USDA-APHIS on 18 May 2018 after consultation with CRI and PPECB. The approved and signed SOP document was received from USDA-APHIS on 20 July 2018 and the first trial operation took place on Monday, 30 July 2018. The second and third trial operation took place on 15 August 2018 and 5 September 2018. All three trial operations were successful and PPECB and CRI drafted a report on the outcome of the SOP and submitted it to SA-DAFF in December 2018 for submission to USDA-APHIS. SA-DAFF informed USDA-APHIS on 25 February 2019 that the three trials were a success and that the new operational procedure proved to be feasible. The procedure will enable the South

African citrus industry to continue with bulk shipping of fresh citrus fruit to the USA. No temperature breaks were identified in the cold chain during the loading of the pallets into the RRMTs, transporting of the pallets and offloading and loading of pallets from the RRMTs into the vessels. USDA-APHIS acknowledged and approved that the South African citrus industry can continue with bulk shipping of fresh citrus fruit to the USA. Fruit will be inspected and pre-cooled at approved outside depots and transported in refrigerated road motor transport to be loaded into specialized refrigerated vessels.

One of the long outstanding issues for this market remained the equivalence between USA domestic CBS regulations and USA import regulations (access to USA for all SA citrus production areas). On 11 June 2018, USDA-APHIS published the Federal Register Semi-Annual Regulatory Agenda. This Agenda indicated a projection of when the final rule will be published. According to this Agenda, the rule was expected to be published in September 2018. However, despite follow up communication by SA-DAFF and Industry the publishing of the rule is still pending.

The other long outstanding issues: expansion of CBS pest free areas to include the whole of the W Cape in the work plan, adoption of CBS pest free places of production in the area of low pest prevalence (Far Northern Limpopo) and the revised work plan and pest list, are also pending despite the Digital Video Conference (DVC) that took place and the follow up communications by SA-DAFF and Industry.

1.5 CHINA

Only one issue remained pending for this market by the end of this reporting period – a request submitted to AQSIQ to exempt lemons from the current cold treatment requirement (24-days) by recognising the non-host status of lemons for fruit flies.

In August 2017, SA-DAFF received feedback from AQSIQ requesting further scientific evidence that lemons are not a host of fruit flies. They requested that experimental work be conducted in accordance with the guidelines of the relevant ISPM (ISPM 37 - Determination of host status of fruit to fruit flies (Tephritidae)). CRI submitted a draft response to SA-DAFF on 14 November 2017 which included the scientific publication demonstrating that lemons are not a host for fruit flies. The research was conducted according to the IPPC guidelines as included in ISPM 37 and as requested by AQSIQ. After further meetings, SA-DAFF submitted the information to AQSIQ on 28 February 2018. On 5 June 2018, SA-DAFF received feedback from GACC, but they still declined to accept that lemons are not a host for fruit flies. GACC again requested research to be conducted according to ISPM 37 and to be submitted to the IPPC as a draft for inclusion in an ISPM. GACC referred to ISPM 28 – PT 26 (Cold treatment for *Ceratitis capitata* on *Citrus limon*) which stipulated a fruit fly cold treatment for lemons, as justification for their ongoing refusal to recognise non-host status of lemon for fruit flies. On 1 October 2018 CRI provided a response to SA-DAFF. In the response the reasons were highlighted why GACC cannot refer to ISPM 28 - PT 26 for not accepting the non-host status of lemons for fruit flies. Further discussions took place between CRI and SA-DAFF on 25 October 2018 and SA-DAFF submitted the response to GACC on 9 November 2018.

In September 2017, SA-DAFF received feedback from AQSIQ, indicating that they remain concerned about the number of temperature sensors for bulk shipping. A response, drafted by CRI and PPECB, was submitted to GACC (AQSIQ) on 23 February 2018. In the response, a detailed explanation was given about the number of sensors per m³ within a vessel. The detail of a USDA approved vessel was used (South Africa only uses USDA approved vessels for bulk shipments of citrus fruit to trading partners requesting a cold treatment disinfestation for quarantine pests), a plan of the deck was included (detailing the number of fruit sensors per independent deck and/or common cooling space), together with a table indicating the minimum number of sensors required per deck or cooling space. The information indicated that the vessels to be used for shipments to China would be equipped with as many or more temperature sensors than the USDA specification (these specifications are used internationally as the benchmark for temperature monitoring in fresh produce shipping). On 23 October 2018 SA-DAFF received a response from GACC indicating that they will accept bulk shipments of fresh citrus fruit from

South Africa. CRI, FPEF and PPECB discussed the protocol and identified that one of the requirements cannot be met. The requirement entails that each pallet must be covered with a mesh after the DAFF phytosanitary inspection to prevent contamination with phytosanitary pests. A telecom took place on 9 November 2018 between CRI and SA-DAFF to discuss and agree upon a solution to include in the feedback to GACC. On 16 November a bilateral meeting took place between South Africa and China. South Africa discussed the problem identified in the protocol for bulk shipments of Citrus. The Chinese delegation agreed to remove the requirement that each pallet must be covered with mesh. The two Agriculture Ministers signed the protocol to allow bulk shipments of fresh citrus fruit from South Africa to the People's Republic of China on 16 November 2018.

1.6 INDIA

Two trial shipments of Citrus were sent in April 2018. The first included grapefruit and lemons and the second Novas (soft citrus). The container with grapefruit and lemons was cleared, but not the container with soft citrus. No further trial shipments have been sent. The Indian Authorities did not release the container due to the claim that *Elsinoë australis* was detected on the fruit. The container was returned to South Africa and the fruit was submitted to an independent laboratory for analysis. A negative result for this pathogen was received from the laboratory. The pathogen *Elsinoë australis* is not known to be present in South Africa. SA-DAFF did communicate to the Indian Authorities, requesting details about the laboratory methods and also indicated that this pathogen is not present in South Africa. A scientific article was published confirming that *P. citricarpa*, the causative organism of CBS, is present in India. SA-DAFF did communicate to India the concerns about the current import conditions for India that stipulated consignments must be inspected and found to be free from CBS. To date, SA-DAFF is still awaiting reports from India on trial shipments sent in 2017 and 2018, feedback on the erroneous report of *Elsinoë australis* interception and the presence of *Phyllosticta citricarpa* in India.

1.7 VIETNAM

On 14 March 2018 SA-DAFF submitted a response to the Plant Protection Department of the Ministry of Agriculture and Rural Development of Vietnam (PPD) indicating that the listing of *A. nerii* as a quarantine pest with a low risk rating will be accepted, pending a scientific survey to provide further support that the pest is not present in commercial citrus orchards. The latest scientific articles by Moore *et al.* on an improved cold treatment for FCM and information on the standard industry sorting and inspection procedures as used for exports to other trading partners were provided. Further scientific evidence to support the removal of *P. syringae* *py syringae* as a quarantine pest was included. Despite follow up communication by SA-DAFF and industry meetings, by the end of this reporting period feedback was still pending from the PPD. No feedback was received from the PPD on the Pest Information Packages for lemons, mandarins and grapefruit that had been submitted in 2013.

1.8 THE PHILIPPINES

Although SA-DAFF submitted the Pest Information Package (PIP) to the Philippine authorities (BPI) in 2009 to allow for importation of fresh Citrus fruit from South Africa, the import protocol was not yet finalised by the end of this reporting period. The BPI sent the first draft PRA in February 2015. SA-DAFF and CRI agreed that considerable revision of the draft PRA was needed. Although the BPI agreed to remove most of the pests that are not associated with citrus fruit and/or are not recorded pests of Citrus and /or Citrus in South Africa, they still declined to remove *Ceratitis quinaria* from the list despite all the technical/scientific information submitted in 2015, 2016 and 2017 as proof that Citrus is not a host for this fruit fly. In May 2018, the BPI again responded, maintaining their position on *C. quinaria* and requesting further scientific information on studies conducted in South Africa to support the non-host status of *Citrus* spp for this pest. The BPI again referred to old reports to support their position. CRI provided the data pack on 08 October 2018. The data package, submitted to the BPI in 18 October 2018, included expert opinion letters from several fruit fly experts stating that citrus fruit is not a host for *C. quinaria*, as well as a report on the results of field surveys conducted in South Africa that confirm that citrus fruit is not a host for *C. quinaria*. The BPI acknowledged receipt. On 26 November 2018 the Philippines responded that *C. quinaria*

will be retained as a quarantine pest until such time that SA-DAFF can provide published scientific data or official data following the outlines of ISPM 37 to demonstrate that citrus is not a host to this fruit fly. CRI and CGA met on 7 December 2018 to discuss the detail of the response to the BPI and in January 2019, SA-DAFF submitted the response to the BPI. On 31 January 2019, the BPI acknowledged SA-DAFF's decision to provisionally keep *C. quinaria* listed as a quarantine pest. On 10 April SA-DAFF provided CRI with the draft phytosanitary requirements as received from the BPI for comments and inputs.

1.9 INDONESIA

In response to queries raised by CGA about shipping conditions to Indonesia, PPECB and CRI reviewed the PPECB guidelines for citrus shipments to Indonesia. The objective was to identify the shipping condition options, with the least risk of chilling injury, that remain compliant with the Indonesian import requirements. Changes were accordingly made to the PPECB guidelines applicable to citrus shipments to Indonesia. It is anticipated that these adjustments will be valuable in reducing the incidence of chilling injury and generally improving fruit quality of citrus exports to Indonesia.

2 BIOSECURITY

By Solomon Gebeyehu, Wayne Kirkman, Elma Carstens and Vaughan Hattingh (CRI)

2.1 SUMMARY

The writing of the citrus industry's Biosecurity Master Plan is underway; with first draft expected by end of May, and final draft before September 2019. Engagement with APHIS attaché in Pretoria was initiated for funding support to hold a regional training workshop on citrus greening at ICIPE, Kenya. The main objective is to use the week long workshop to raise awareness about the threat of HLB and ACP to citrus industries in Africa. SG participated in the 21st IOCV & 6th IRCHLB conference in Riverside, California 10-17 March 2019, and gave an invited presentation on the HLB and ACP situation in Africa and used the opportunity for networking and information exchange.

WK and Hannes Bester attended a meeting with key members of the Namibian Ministry of Agriculture, Water and Forestry (MAWF) in Windhoek on 20 March 2019. Biosecurity issues and threats to the Namibian and South African citrus industries were presented, and the importance of obtaining citrus material only from the Citrus Foundation Block and certified nurseries in South Africa was emphasized. WK has compiled, maintained and updated a file containing facts and correspondence on major pests and diseases that have biosecurity threats to the citrus industry.

The Biosecurity Division has continued providing inputs as the draft HLB and ACP Action Plan has been undergoing several revisions. Engagements with contacts in East Africa have continued to undertake surveys for detection and tracking distribution of ACP in Tanzania, as well as HLB in Ethiopia, with efforts being made to initiate surveys in Mozambique in light of new information about detection of ACP in southern Tanzania close to the Mozambique border. Two 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 however remained the same – the pest is considered to be present in specified regions, actionable and under official control in South Africa.

The discovery of Citrus Leprosis (CL-N), known to occur in South and Central American countries, on three farms in the Addo area of the Eastern Cape Province in May 2018 was reported to the Department of Agriculture, Forestry and Fisheries (DAFF) as is required, and the regulatory status of the pest is pending. CRI formed a Citrus Leprosis Advisory Committee and developed the Citrus Leprosis Response Plan (CLRP) to control and contain the disease. The affected growers were made aware of actions required, focussing on removal of CL-N inoculum (symptomatic material), and the containment and control of mites, and these actions were implemented according to the CLRP.

CRI continued its contribution to the Phyto Risk Forum, and in this reporting period two meetings took place. At both meetings feedback was provided on surveys conducted related to citrus.

Two African Greening surveys were conducted under the auspices of DAFF in 2018 in the Western Cape. When results became available in February 2019, SA-DAFF issued orders in March 2019 to three implicated home garden owners to remove the trees. A monitoring survey conducted in November 2018 under the auspice of SA-DAFF in a transect of the 5 km citrus free zone outside the CFB discovered four places with several citrus trees following which SA-DAFF issued orders to all the implicated owners. In a follow up survey in February 2019, in another transect, citrus trees were found on five (5) more places. A monitoring survey to cover the 5 km radius is scheduled for April 2019.

OPSOMMING

Die skryf van die sitrusbedryf se Meesterplan vir Biosekuriteit is in proses, met die eerste konsep wat teen einde Mei verag word en die finale konsep voor September 2019. Daar was skakeling met APHIS se attaché in Pretoria vir befondsingsondersteuning om 'n streeksopleidingswerkswinkel oor sitrusvergroening by ICIPE, Kenia te hou, met die hoofdoel om die week lange werkswinkel te gebruik om bewustheid oor die bedreiging van HLB en ACP vir sitrusbedrywe in Afrika te verskerp. SG het aan die 21ste IOCV & 6 de IRCHLB konferensie in Riverside, Kalifornië 10-17 Maart 2019 deelgeneem, waar hy uitgenooi is om 'n aanbieding oor die HLB- en ACP-situasie in Afrika te lewer asook om die geleentheid vir netwerk- en inligtinguitruiling te gebruik.

WK en Hannes Bester het op 20 Maart 2019 'n vergadering met belangrike beamptes van die Namibiese Ministerie van Landbou, Water en Bosbou (MAWF) in Windhoek bygewoon waar biosekuriteitskwessies en bedreigings vir die Namibiese en Suid-Afrikaanse sitrusbedrywe bespreek is. Die belangrikheid van die verkryging van sitrusmateriaal slegs van die Sitrus Grondvesblok en gesertifiseerde kwekerie in Suid-Afrika, is ook beklemtoon. WK het 'n stelsel ontwikkel om inligting en korrespondensie oor belangrike plae en siektes wat bedreigings vir die biosekuriteit van die sitrusbedryf inhou, te versamel en op te dateer.

Die Biosekuriteitsafdeling het voortgegaan om insette te lewer soos die konsep HLB- en ACP aksieplan verskeie hersienings ondergaan het. Skakeling met kontakte in Oos-Afrika het voortgegaan om opnames te doen vir die opsporing en die volging van die verspreiding van ACP in Tanzanië, asook HLB in Ethiopië. Daar word gepoog om met opnames in Mosambiek te begin in die lig van nuwe inligting oor die opsporing van ACP in die suide van Tanzanië naby aan Mosambiek se grens. Twee nuwe pesverslae is aan die IPPC gestuur oor die ontdekking van *B. dorsalis* (voorheen *B. invadens*) in Suid-Afrika. Die status van die vrugtevlug het egter dieselfde gebly - die pes word beskou as teenwoordig in gespesifieke streke, "actionable" en onder amptelike beheer in Suid-Afrika.

Die ontdekking van Sitrus Leprose (CL-N), wat bekend is om in lande in Suid- en Sentraal-Amerika voor te kom, op drie plase in die Addo-omgewing van die Oos-Kaap provinsie in Mei 2018, is soos vereis aan die Departement van Landbou, Bosbou en Visserye (DAFF) gerapporteer. Die regulatoriese status van die pes is steeds hangende. CRI het 'n Sitrus Leprosis Advieskomitee gevorm en die Sitrus Leprosis-responsplan (CLRP) ontwikkel om die siekte te beheer en te beperk. Die betrokke produsente is oor die nodige aksies ingelig, met die fokus op die verwydering van CL-N-inokulum (simptomaties materiaal), die beperking en beheer van myte, en hierdie aksies is volgens die CLRP geïmplementeer.

CRI het bydraes tot die Phyto Risk Forum voortgesit, en in hierdie verslagtydperk het twee vergaderings plaasgevind. Op albei vergaderings is terugvoering oor opnames wat met sitrus verband hou, gegee.

Twee Afrika vergroeningsopnames is onder die vaandel van DAFF in 2018 in die Wes-Kaap uitgevoer. Resultate is in Februarie 2019 beskikbaar gestel, en SA-DAFF het in Maart 2019 lasgewings aan die drie betrokke eienaars uitgereik om die bome te verwyder. Tydens 'n moniteringsopname in November 2018 wat onder die vaandel van

SA-DAFF in 'n gedeelte van die 5 km sitrusvrye sone rondom die CFB uitgevoer is, is vier plekke met verskeie sitrusbome ontdek. SA-DAFF het lasgewings aan al die betrokkenes uitgereik. In 'n opvolg opname in Februarie 2019, in 'n ander gedeelte, is sitrusbome op nog vyf plekke gevind. 'n Moniteringsopname om die totale 5 km radius te dek, is vir April 2019 geskeduleer.

2.2 Develop and maintain a comprehensive Citrus industry biosecurity plan - to ensure overall mitigation of the Southern African Citrus industry's biosecurity risks

2.2.1 Project 1: Develop a Southern Africa Citrus industry biosecurity master plan

In a bid to develop a comprehensive citrus industry biosecurity master plan that would serve as a road map to guide industry in addressing biosecurity threats in the context of increasing threats from invasive pests and diseases, biosecurity strategic plans and published articles written by similar industries around the world have been reviewed. Outline of a draft master plan has been developed, and write up of the full master plan is underway. The first draft is scheduled for completion by end of May 2019, which will be distributed to various stakeholders for comments with the expectation that the final version will be completed before September 2019.

2.3 Biosecurity portfolio: Design, develop and oversee the implementation and operation of appropriate biosecurity structures, engagements, procedures, co-operations, resources, projects and other appropriate actions

2.3.1 Project 2: Identify, assess and initiate engagement with international funding providers, for future support of Southern African biosecurity projects

SG held a meeting with APHIS attaché in SA Mr Jeromy McKim in Pretoria in November 2018, and proposed to APHIS to provide funding for a regional training workshop on citrus greening diseases and associated vectors at ICIPE, Kenya, and secured support for the proposal. APHIS agreed in principle, and wanted to receive a concept note and budget required to implement the training workshop. SG wrote the concept note, developed a comprehensive budget in consultation with ICIPE, and submitted these to Mr McKim. SG subsequently has made follow ups in early 2019, and the feedback received was that the proposal has been presented to the regional director of APHIS, but has not received the green light for funding at this stage due to competing priorities relative to available funding. Engagement with APHIS will continue, should the funding situation change in coming months.

2.4 Networking and awareness: Obtain supportive participation of relevant stakeholders and interested parties

SG participated in the 21st IOCV & 6th IRCHLB conference in Riverside/California March 10-17, 2019, and gave an invited presentation entitled "Current state of Liberibacter complex and associated vectors threatening citrus production on the African continent." Networking and one-to-one discussions held with several experts from around the world on HLB/ACP surveillance and management, and obtained the latest version of HLB and ACP Action Plan for California from contacts at the California Department of Food and Agriculture. This latest version is being used as a reference as CRI continues to develop SA's HLB and ACP Action Plan.

WK and Hannes Bester attended a meeting with Eddie Hasheela and other key members of the Namibian Ministry of Agriculture, Water and Forestry (MAWF) in Windhoek on 20 March 2019. Biosecurity issues and threats to the Namibian and South African citrus industries were presented to MAWF and discussed. The importance of obtaining citrus material only from the Citrus Foundation Block and certified nurseries in South Africa was emphasized, and the use of the South African quarantine facilities was offered to MAWF, as they have none. MAWF agreed to coordinate the monitoring for *D. citri* by distributing ACP traps to growers in Outjo, Tsumeb and the northern areas of Namibia, and sending them back to CRI for evaluation after collection. CRI agreed to supply

traps and finance the transportation of the traps. Forty ACP traps were provided to MAWF. The outcome of the meeting was positive, and MAWF indicated that they were willing to collaborate with CRI on all biosecurity industry matters, but did indicate that their funding was limited.

WK and Hannes Bester visited the farm of Dr Roelie van Wyk outside Outjo on 21 March 2019. A meeting was held with growers and industry representatives at the farm on 22 March 2019. The structure and operations of the CGA and CRI were presented to them in order to assist them with the formation of biosecurity and industry structures. Biosecurity threats to the Namibian and SA citrus industries were discussed, and they agreed to monitor for ACP in their citrus orchards. ACP traps were provided to them, with the agreement that they would replace them every two weeks, photograph the used traps and send the images to CRI. If anything suspicious was noted on the images by CRI, the traps would be sent to SA for evaluation. To date several images have been received by CRI, but no signs of ACP were recorded.

WK and EC attended the CGA Citrus Summit in Port Elizabeth on 13-14 March 2019.

2.5 Ensure successful implementation of processes, procedures and interactions to ensure the timely identification and assessment of biosecurity threats facing the Southern African Citrus industry

2.5.1 Project 3: Develop and oversee implementation of Southern African Citrus industry pest- specific action plans for priority biosecurity pests

2.6 Ensure that there is a comprehensive and up to date list of Citrus pests and diseases perceived to hold a biosecurity risk for the industry

CRI and SA-DAFF compiled a comprehensive list of global citrus pests which included mites, insects, bacteria, fungi, viruses, nematodes and snails. The list also includes the following information: hosts other than citrus, plant parts associated with and presence/absence in South Africa. The list of mites (140) has been evaluated and a shorter list for further risk profiling was compiled.

2.7 Ensure effective implementation of processes, procedures, interactions to advance actions required to successfully mitigate the risks and consequences of biosecurity incursions

WK has, as per instruction, compiled, maintained and updated a file containing facts and correspondence on all the other major Biosecurity threats, including, Fall Army Worm, PSHB (Ambrosia beetle), Citrus Canker, Citrus Variegated Chlorosis (Sharpshooters), Sudden Death (CTV virus), Post-Bloom Fruit Drop (virus), *Pseudocercospora angolensis* (fungus), Malseco (lemons), Cotton Mealybug (Zimbabwe, Letsitele and SRV) and spotted lantern fly. WK subscribed to the mailing list of several relevant websites, such as APHIS and Australian Biosecurity alerts. Several meetings were held with Californian and Australian Biosecurity personnel between sessions at the Citrus Research Symposium in 2018. WK represented Biosecurity and IPM at the Citrus Foundation Block evaluations and the Citrus Improvement Scheme Advisory Committee (CISAC) meeting on 19 July 2018, as well as the IPM and DM project proposal meetings on 22 and 23 October 2018 at City Lodge, OR Tambo.

2.7.1 Project 4: Develop and oversee implementation of a Southern African Citrus industry HLB action plan and safe tree production system

This activity is being coordinated by PF in the division of CIS. WK attended a meeting with the Biosecurity Advisory Committee in Nelspruit on 20 June 2018, where priorities and biosecurity actions were communicated and discussed. WK, EC and SG participated in biosecurity meetings and ACP and HLB Action Plan and Safe Tree Production System workshops on 27 September and 1 November 2018. The Biosecurity Division has continued

providing inputs as the draft Action Plan has been undergoing several revisions. SG and WK attended an HLB/SACNA/SANA workshop along with PF and VH on March 5, 2019 to workshop the Action Plan with stakeholders from nurseries. SG gave a presentation at this workshop sharing an overview of the state of affairs of ACP and HLB distribution in East Africa and ongoing research activities by ICIPE and other stakeholders where CRI is involved as a technical partner aimed at raising awareness and developing management strategies to combat the threat of ACP and HLB in citrus value chains in Africa.

2.7.2 Project 5: Ensure that HLB and ACP surveillance is undertaken in Eastern Africa

Aruna Manrakhan and Claire Love conducted a survey of the Asian Citrus Psyllid (ACP), *Diaphorina citri* in collaboration with Tanzanian partners along the Morogoro-Songea transect in southern Tanzania from November 17 to 23, 2018 using the Biosecurity budget. The survey of ACP was carried out by trapping (use of double sided lime-green sticky cards) and visual sampling. Morogoro was one of the southernmost detection points of ACP in Tanzania in the surveys conducted in the country in 2016. In this survey, ACP was found to have spread south west of Morogoro possibly to a distance of up to 240 km. The identity of the single ACP specimen found furthest from Morogoro could however not be confirmed by molecular analysis. The unconfirmed ACP specimen also contained an unknown *Liberibacter* species. No Las was detected in any of the psyllid samples.

In subsequent follow ups on the aforementioned results from Tanzania, an unofficial report from a credible contact working as entomologist in agricultural research in Tanzania (Chris Materu) in February 2019 indicated that ACP has been detected further in southern Tanzania about 30 km north of the border with Mozambique. This has led us to focus on Mozambique and initiate engagement with Dr. Domingos Cugala of Eduardo Mondlane University of Mozambique and NPPO of Mozambique to start detection surveys of ACP and HLB starting with southern Mozambique. The purpose of this survey is determine the southern frontier of ACP in Africa. Willingness from Mozambique to undertake ACP surveys was obtained, and tentative dates for an initial trip by CRI biosecurity team with our contacts in Mozambique is planned for the 2nd week of May.

SG gave two presentations on behalf of Dr. Tim Grout at a workshop organized by ICIPE in Mombasa, Kenya on December 2-6, 2018 to review progress of research in East Africa on citrus greening and associated vectors in the past 3 years, with a focus on HLB and ACP. Also during this meeting, SG participated in a strategic planning and proposal writing session with ICIPE and a few donors for applying for funding with various agencies to continue citrus greening research and surveys across Africa. SG made useful contribution to the strategy and proposal development while following up on subsequent steps by ICIPE as CRI will continue as technical partner going forward. While in Kenya SG discussed a proposal for holding a regional training workshop on citrus greening at ICIPE, provided SG secures funding from APHIS. ICIPE agreed with the proposal, and subsequently developed a draft budget for training 20 participants for one week. The main objective of this training workshop is to use it as a platform for raising awareness about citrus greening and the invasion by HLB and ACP, and build a network of collaboration across African NPPOs, research organisations, and universities.

2.7.3 Project 6: Facilitate initiation of an HLB eradication plan in Ethiopia

SG held meetings in Ethiopia in December 2018 to advance collaboration with key potential partners. SG met with the country head of ICIPE, Ethiopia, Dr. Tadele Tefera to explore opportunities to collaborate in the management of citrus pests and diseases, focusing on HLB and ACP surveys. Goodwill and support for collaboration was obtained including an offer to use existing ICIPE networks and logistics for future survey activities. SG also met with a group of researchers - horticulturists, entomologists and pathologists in a research centre that serves as a satellite station of Ethiopia's National Agriculture Research Organization (equivalent of ARC in SA), and is responsible for research in the central rift valley region where some of the country's large commercial citrus orchards are located in the outskirts of the city of Nazareth (120 km south-east of Addis) to explore avenues for collaboration. SG was briefed on ongoing research activities on citrus pests and diseases, and then gave a brief presentation on HLB and ACP, left some literature and sticky traps for possible use. This is also the area Hennie

le Roux had visited back in 2014 to offer technical consultancy to a private company growing passion fruit for production of juice. Traps that were left behind have subsequently been deployed to detect ACP in back yard and commercial citrus fields in the central rift valley region. Engagement with contacts has continued to follow up on survey results, and ensure sustained collaboration and information exchange.

2.7.4 Project 7: Ensure, in close collaboration with relevant government officials, that regulations of relevance to biosecurity risk mitigation are appropriately updated and compliance effectively implemented

In this reporting period two 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 however remained the same – the pest is considered to be present in specified regions, actionable and under official control in South Africa.

In 2010 and 2013 a “scab-like” disease was observed on fruit of pomegranates in orchards in the Eastern Cape and Western Cape provinces. Detection surveys were conducted under the auspices of DAFF and an *Elsinoë* species was isolated that could pose a serious threat to the citrus industry. Different reference strains (*Elsinoë* species) were imported and pathogenicity tests were conducted in an official quarantine environment on pomegranate plants and a range of citrus types grown in South Africa. It was demonstrated and confirmed that although *Elsinoë punicae* is responsible for a new disease on pomegranates in South Africa, it is not a pathogen of citrus. An article on these results has been published in Australasian Plant Pathology: Elma Carstens, Shaun D. Langenhoven, Romain Pierron, Wilhelm Laubscher, Jakobus J. Serfontein, Carolien M. Bezuidenhout, Elrita Venter, Paul H. Fourie, Vaughan Hattingh & Lizel Mostert. 2018. *Elsinoë punicae* causing scab of pomegranates in South Africa does not cause disease on citrus. Australasian Plant Pathology, 47:405 – 411.

2.7.5 Project 8: Monitoring and control of Leprosis

Citrus Leprosis (CL), one of the oldest citrus diseases, is known to occur in South and Central American countries, but has not previously been reported on citrus in South Africa. CL is caused by several RNA viruses, commonly referred to as either the cytoplasmic type (CL-C), or the nuclear type (CL-N). Symptoms similar to that of CL were observed on three farms in the Addo area of the Eastern Cape Province. Molecular diagnostics were conducted by CRI Nelspruit, with initial results reported on 07, 14 and 24 May 2018. Duplicate testing was conducted by ARC Tropical and Subtropical Crops, reported on 12 May 2018. The presence of a single virus belonging to the *Dichorhavirus* genus was detected in samples from all three orchards. Limited sequence data indicated that the associated virus is a strain of *Orchid fleck virus* (OFV) with closest sequence identity to a strain previously characterised on cymbidium orchids (*Cymbidium* spp.), but not previously reported on citrus. *Brevipalpus californicus* mites, which are known vectors of some leprosis-causing viruses, were observed in all three orchards, and their identity was confirmed. This resulted in activation of precautionary actions by CRI, while further clarification of virus identity and confirmation of causative association was being sought. Clarification of identity was required to determine the official regulatory status of the virus on citrus in South Africa (e.g. quarantine pest or non-quarantine regulated pest). The discovery was reported to the Department of Agriculture, Forestry and Fisheries (DAFF) as is required, and the regulatory status of the pest is pending.

The virus persists around mite feeding sites, and mites become infected when feeding on symptomatic tissue, and transmit the virus by subsequent feeding. The virus cannot spread systemically, which increases the chances of containment and eradication of this disease.

Subsequently the disease was identified on a fourth farm in the Addo area, and another farm in the Gamtoos River Valley. Several other suspicious sites were inspected but samples tested negative for leprosis.

CRI responded by forming a Citrus Leprosis Advisory Panel. This panel studied all applicable literature and through much workshopping developed the Citrus Leprosis Response Plan (CLRP) to control and contain the disease. The affected growers were made aware of actions required, focussing on removal of CL-N inoculum (symptomatic

material), and the containment and control of mites, and these actions were implemented according to the CLRP. Growers were monitored and assisted with implementation of the CLRP by CRI and SRCC.

A delimitation survey procedure and survey methods were developed. These surveys were initiated to determine the spread of the disease and infected mites. New findings came to light as a result of the surveys and were confirmed by molecular diagnostics. This information was communicated to the affected growers, and the response plan was implemented. Owners of neighbouring orchards were also informed of their responsibilities according to the response plan. Surveys were continually coordinated, conducted and communicate by CRI and SRCC. All suspicious samples were collected by CRI, inspected for the presence of flat mites, dipped in an acaricide and sent to Nelspruit for molecular diagnosis. Mites were collected in 99% ethanol and sent to Nelspruit to determine if they were infected. Infected orchards were classified as Red1, and all orchards with an edge within 50 metres of Red1 orchards were classified as Yellow according to the response plan. Survey found a total 27 CL infected orchards on five farms. Four infected farms were in close proximity whereas one farm was identified in a different area, 96 km from the initial find site. Infected orchards were either Navel or Valencia orange and mites collected at the find sites were identified as *B. californicus*. No symptoms were found in adjacent lemon and mandarin orchards despite the presence of *B. californicus* in some of these orchards.

The affected farms in the Sundays River Valley, Kleinplaas, Halaron and Bellevue were audited for compliance to the CLRP in 2018, and Elim East in March 2019. The audits focussed on pruning, spray programmes, weed control, controlled movement of people and record keeping.

There were no major non-compliances on Halaron, Bellevue and Elim East. Bellevue was subsequently sold to SRCC, and the Delta and Midnight Valencia orchards where leprosis was first discovered were removed and destroyed. The only remaining affected orchards are young Cara Cara navels, which were not as severely infected. The major finding at Kleinplaas was that the weeds and ground cover (Wandering Jew) had not been removed. The grower was given three months to rectify this by CRI and SRCC. The deadline was not met, but sufficient attempts and progress were made, with the plant being extremely hardy and difficult to destroy. The farms will be audited again in June 2019.

Random orchards on the affected farms were surveyed in 2019 for the presence of symptoms and mites. To date none were found. All affected farms will be officially audited for compliance to the CLRP one year after initial diagnosis. These audits will be conducted mostly in June and July 2019, and the outcome will determine whether the status of all orchards on the farms will change.

The affected orchard in the Gamtoos River valley was sprayed immediately after diagnosis. It was harvested a week later, and then the orchard was removed. The material was then burned in accordance with the CLRP.

Several communications, informal and formal meetings were held and presentations were given to create and increase awareness about CL in the SA citrus industry. WK arranged and attended informal meetings per opportunity with most of the biggest growers in the Eastern Cape. Presentations were made to them on symptoms, actions and the importance of early detection. They were provided with material and encouraged to train their managers and staff. These meetings included SRCC agronomists, Unifrutti/Dunbrody Estates (Deon Joubert, Steve Lloyd, Carla Marais and packhouse quality department), Sunriver Citrus (Willem Bouwer and Waldo Gerber), San Miguel (JP Robin, Louis de Bruin and managers), Habata (Hannes Joubert, Gary Webb and managers) and Cape Citrus packhouse quality department. Presentations were made to service providers and agrochemical company agents including Agriwide (Marius Ferreira and staff), Nexus (Morne Gouws and staff), Inteligro, and Xsit. Greg Jones, CEO of the Sundays River Forum, was contacted to disseminate a leprosis Cutting Edge to all producers' packhouses in the Eastern Cape. The Forum, SRCC and CRI decided to disclose the names of the affected farms to all packhouses to reduce the risk of leprosis spread by uncontrolled transport of fruit.

WK convened, coordinated and chaired a meeting of the Eastern Cape Technical Association (ECTA) meeting on 26 June 2018. This association comprises of all researchers, technical consultants and agrochemical company

representatives who make recommendations to growers. A presentation was given on leprosis symptoms and management, awareness material was distributed and discussions were held on the importance of awareness and training of staff. WK and Patensie Citrus (PSB) arranged a meeting with PSB staff, growers, consultants and agrochemical company representatives on 6 November 2018. A presentation was given on leprosis symptoms and management, awareness material was distributed and discussions were held on the importance of awareness and training of staff.

Presentations: CRI Citrus Research Symposium – Oral Presentation by WK – “Response to the first case of Citrus Leprosis-N in South Africa”. IPM Roadshow workshops. Two presentations by WK at 5 workshops titled “Biosecurity” and “Control of Flat Mite” on 4-5 Sep (Letsitele), 6-7 September (Groblersdal), 10-11 September (Nelspruit), 11-12 September (Addo) and 18-19 September (Simondium).

Awareness material: Cutting Edge no 248 on leprosis was compiled and distributed. An article on leprosis was compiled and published in the Landbouweekblad. WK and EC are co-authors on a peer-reviewed short communication titled “Orchid fleck virus associated with the first case of Citrus Leprosis-N in South Africa” which has been submitted for publication. Leprosis awareness fliers were compiled and distributed at all the IPM workshops and per opportunity at other meetings.

Due to the hypothesis that OFV was transmitted from orchids to citrus, WK and Sean Moore attended the National Orchid show in Port Elizabeth on 26 September 2018, to engage with delegates and discuss the transmission of OFV and the control of flat mite in orchids. Delegates and presenters were adamant that OFV was systemic in orchids and were unaware of spread by vectors.

WK visited Christo Botes at his collection in Feb 2019 in an attempt to collect infected mites, but none were found. Infected material was collected and sent to the molecular laboratory in Nelspruit, where they tested positive for OFV. Further collections of seemingly infected orchids were arranged from nurseries in Brits and Tzaneen and Nelspruit. Samples from Brits also tested positive for OFV.

2.7.6 Project 9: Phytosanitary Risk Forum

In this reporting period two meetings took place. At both meetings feedback was provided on surveys conducted related to citrus. Inputs were provided on various import protocols related to other crops and pests of biosecurity concern to other industries.

2.7.7 Project 10: Greening surveys (African greening - *candidatus liberibacter Africanus* & Asiatic greening - *candidatus liberibacter asiaticus*)

Two African Greening surveys were conducted under the auspices of DAFF in 2018. A detection survey had been conducted in the greening free Tulbagh and Wolseley magisterial districts of the Western Cape Province and five (5) samples were drawn and submitted to DAFF’s Laboratory in Stellenbosch. All the samples tested negative for African Greening and Asiatic Greening. A delimiting survey had been conducted in November 2018 in the town of Knysna in the official greening free buffer zone in the Western Cape Province after an African Greening positive tree was found during the 2017 detection survey. Twenty-two (22) samples were drawn and duplicate samples were submitted to DAFFs Laboratory in Stellenbosch and CRI’s Laboratory in Nelspruit. Four of the 22 samples tested positive in both laboratories for African greening. All the samples tested negative for Asiatic Greening. The positive samples were collected from home gardens neighbouring the garden in which the positive tree had been found in 2017. As the results became available in February 2019, SA-DAFF issued orders in March 2019 to the three implicated owners to remove the trees.

A planning meeting between CRI and SA-DAFF took place in February 2019 to discuss and scheduled the African and Asiatic greening surveys for 2019. African greening surveys will be conducted in June (delimiting in Knysna)

and July (detection in Eastern Cape buffer zone). Asiatic greening survey will be conducted in August (detection in KZN).

2.7.8 Project 11: Citrus Free Zone (5 km) outside the Citrus Foundation Block (CFB) in the Eastern Cape Province in the magisterial district of Uitenhage

In January 2011 legislation was published which prohibited the keeping, cultivation and planting of specific plants, including Citrus species, in the area of 5 km radius outside the citrus foundation block (CFB) in the Eastern Cape province in the magisterial district of Uitenhage. A survey had been conducted by SA-DAFF prior to 2011 and farms were identified with citrus trees and orders were given to all the implicated owners. Most of the trees were removed but one of the owners refused to remove the trees despite an order issued and follow up visits by SA-DAFF. In November 2018 SA-DAFF reported the case to SAPS in Uitenhage. In January 2019 the SAPS indicated that the case was referred to the Senior Prosecutor for a decision. By the end of the reporting period no outcome on the reported case was available.

A monitoring survey was conducted in November 2018 under the auspice of SA-DAFF in a transect of the 5 km citrus free zone outside the CFB. Four places with several citrus trees were found and the decision was taken that the monitoring survey should be repeated to cover the 5 km radius. SA-DAFF issued orders to all the implicated owners. In a follow up survey in February 2019, in another transect, citrus trees were found at five (5) more places. A monitoring survey to cover the 5 km radius is scheduled for April 2019.

3 **PORTFOLIO: INTEGRATED PEST MANAGEMENT**

3.1 **PORTFOLIO SUMMARY**

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

The Integrated Pest Management (IPM) Portfolio has been simplified from the five research programmes of last year into only three research programmes this year. The False Codling Moth (FCM) and Fruit Fly Programmes remain as is. However, the remaining three programmes (Mealybug and other phytosanitary pests, Non-Phytosanitary Key Pests, and Minor Pests and Mites) have been merged into one new programme, namely Other Pests. The backdrop to the IPM Research Portfolio during the last year was once again dominated by market access forces. This saw us entering the second year of production and export under the False codling moth risk management system (the FMS) for citrus exports to the EU. The performance of the FMS and the industry during the first year of export was considering overwhelmingly successful, with only 9 interceptions of live FCM in South African citrus from around 800 000 tons exported to Europe. However, despite this, the message from the market was that further improvement was required, leading to changes towards greater stringency in the FMS requirements.

A new market access force came in the form of an amendment to the phytosanitary measures required by the EU for mitigating fruit fly risk. This required the rapid development of a Fruit fly management system (FFMS) for implementation by the EU as of 1 September 2019.

A third and very significant market access pressure comes in the form of ever increasing requirements for reduced numbers and levels of chemical residues on fruit, mainly coming from European retailers. This is nothing new, but requirements are escalating and the effect within the IPM Portfolio is an intensification of research on biological alternatives for pest management.

FCM remained as the top entomological and market access research priority during the last research cycle. Within the FCM programme, 15 different projects were conducted, of which 11 addressed pre-harvest issues and four were postharvest projects. Research within this programme focussed on monitoring of FCM using female

attractants, microbial control of FCM, management of FCM using semiochemicals, improvement of the sterile insect technique (SIT) and the effect of nets on FCM management. The postharvest studies focussed on disinfestation of fruit and detection of FCM-infested fruit. Execution of field trials proved to be particularly and uncharacteristically difficult. It became clear that in the current environment of lower FCM levels in the field, our trial methodology needs to be revised, something that we are already working on. Nonetheless, two of the virus trials have produced exciting results, promising for improved control in the field. These entailed the successful selection of a UV-resistant virus (CrleGV) and the establishment of synergism between a yeast and CrleGV, improving the insecticidal effect of the latter. Two different technologies led to improvement in SIT efficacy i.e. the use of a cryoprotectant in the larval diet, and release of sterile moths under nets. Disinfestation of fruit from FCM was determined for a series of cold treatments and with a combination of CO₂ fumigation and a short cold treatment. Finally, detection of infested fruit, relative to healthy fruit, is being achieved through detection of specific volatiles, using a couple of volatile detection technologies.

The market access importance of fruit flies increased during the last year, with the impending regulation of fruit flies by the EU. There were nine projects carried out under the fruit fly programme. Four of these projects were on baits used for pre-harvest control of fruit flies and three addressed post-harvest cold treatments. The efficacy of different bait stations was compared and it was demonstrated that starting fruit fly baiting in October, reduced fruit fly pressure in February. Additionally, bait and copper phytotoxicity was found to be reduced when baits were only applied in April. In postharvest studies, Medfly was found to be more cold tolerant than the other fruit fly pests on citrus, potentially allowing future cold treatment trials to only be conducted with Medfly.

The Other Pests programme covered research on mealybugs, thrips, psylla (African and Asian), woolly whitefly, leafhoppers, mites and various pests occurring under nets. This included 12 projects, with some highlights as follows. Research conducted over five years resulted in an emergency registration for green citrus leafhopper. A range of soil drenches were found to be effective for the suppression or control of the Asian citrus psyllid (ACP), either applied against ACP in Mauritius or an indicator species in South Africa. In the quest for efficacious entomopathogenic fungal (EPF) treatments, EPF were found to be extremely susceptible to UV radiation, showing the importance of good formulation. In a preventative programme, EPF based products showed no significant efficacy against red scale, mealybug or thrips. However, a low degree of control was recorded as corrective treatments against mealybug. In a couple of projects investigating pest management under nets, trap counts of fruit fly were higher in open orchards, but FCM trap counts were higher under net, as was infestation of mites and red scale.

During the past year, CRI research entomologists were also very active in transferring technology to growers, in the form of the biennial Citrus Research Symposium, the IPM and Disease Management spring workshops, the pre-packing season Postharvest workshops and several other smaller forums. This was extremely important, particularly in communicating critical messages, such as the FMS, Leprosis management and the threat of ACP and HLB. Several Cutting Edges on important topics were also released to growers, as were articles in the SA Fruit Journal. Additionally, CRI research entomologists participated in certain international scientific meetings, such as the International Invertebrate Pathology and Microbial Control Congress, the International Chemical Ecology Congress and the International Symposium on Fruit Flies of Economic Importance. Additionally, several key papers were published in scientific peer-reviewed journals.

PORTEFEULJE OPSOMMING

Die Geïntegreerde Plaagbeheer (IPM) Portefeulje is vereenvoudig vanaf die vyf navorsingsprogramme laas jaar, na slegs drie navorsingsprogramme hierdie jaar. Die Valskodlingmot (VKM) en Vrugtevlieg programme bly dieselfde. Die oorblywende drie programme (Witluis en ander fitosanitêre plae, Nie-fitosanitêre Sleutel Plae, en Geringe Plae en Myte) is in een nuwe program saamgevoeg, naamlik Ander Plae. Die agtergrond van die IPM Portefeulje is gedurende die afgelope jaar weereens oorheers deur marktoegangsmagte. Dit het gelei tot die betreding van die tweede jaar van produksie en uitvoer onder die valskodlingmot risiko bestuur stelsel (die FMS)

vir sitrus uitvoere na die EU. Die prestasie van die FMS en die bedryf gedurende die eerste jaar van uitvoer was oorweldigend suksesvol, met slegs nege onderskeppings van lewendige VKM in Suid-Afrikaanse sitrus vanuit ongeveer 800 000 ton uitgevoer na Europa. Ten spyte van dit was die boodskap vanaf die mark dat verder verbetering nodig is, wat gelei het tot veranderinge na groter strengheid in die FMS vereistes.

'n Nuwe marktoegangsmag het in die vorm van 'n wysiging tot die fitosanitêre maatreëls benodig deur die EU vir versagende vrugtevlug risiko. Dit het gelei tot die ontwikkeling van 'n vrugtevlug bestuur stelsel (FFMS) vir implementering deur die EU vanaf 1 September 2019.

'n Derde en baie belangrike druk op marktoegang kom in die vorm van die alewige toenemende vereiste vir 'n vermindering aan die aantal en vlakke van chemiese residue op vrugte, hoofsaaklik afkomstig vanaf Europese supermarkte. Dit is niks nuut nie, maar die vereistes neem toe en die gevolg binne die IPM Portefeulje is 'n intensivering van navorsing op biologiese alternatiewe vir plaagbeheer.

VKM het as die hoogste entomologiese en marknavorsingsprioriteit gebly gedurende die laaste navorsingsiklus. Binne die VKM program is 15 verskillende projekte uitgevoer, waarvan 11 die voor-oes aspekte toespreek en vier na-oes projekte was. Navorsing binne hierdie programme fokus op die monitering van VKM deur die gebruik van wyfie lokmiddels, mikrobiële beheer van VKM, beheer van VKM deur semiochemikalieë, verbetering van die steriele insek tegniek (SIT) en die effek van nette op VKM beheer. Die na-oes studies fokus op die ontsmetting van vrugte en die opsporing van VKM-besmette vrugte. Uitvoering van veldproewe was besonders onoverwags uitdagend gewees. Dit het duidelik geword dat in die huidige omgewing van laer VKM vlakke in die veld, dat ons proef metodes aangepas moet word, iets waaraan ons alreeds werk. Nietemin het twee van die virus proewe opwindende resultate gelewer, belowend vir verbeterde beheer in die veld. Dit het die suksesvolle seleksie van 'n UV-bestande virus (CrleGV) en die vestiging van sinergisme tussen 'n gis en CrleGV behels, wat die insekdodende effek van laasgenoemde verbeter het. Twee verskillende tegnologieë het gelei tot 'n verbetering in SIT effektiwiteit, d.w.s. die gebruik van 'n kouebeskermer in die larvale dieet, en die loslating van steriele motte onder nette. Ontsmetting van vrugte van VKM is vasgestel vir 'n reeks koue behandelings en met 'n kombinasie van CO₂ beroking en 'n kort koue behandeling. Laastens word besmette vrugte van gesonde vrugte onderskei deur die opsporing van spesifieke vlugtige stowwe, deur die gebruik van 'n paar vlugtige opsporingstegnologieë.

Die belang van marktoegang vir vrugtevlugte het gedurende die laaste jaar toegeneem, met die dreigende regulering van vrugtevlugte deur die EU. Daar is nege projekte onder die vrugtevlugte program uitgevoer. Vier van hierdie projekte was op die gebruik van lokase vir voor-oes beheer van vrugtevlugte en drie het op na-oes koue behandelings gefokus. Die effektiwiteit van verskillende lokaas stasies is vergelyk en dit is ook gewys dat inisiasie vrugtevlugte bestryding in Oktober het gelei na verlaagde vrugtevlugte druk in Februarie. Daar is ook getoon dat lokaas en koper fitotoksisiteit verminder word wanneer lokase in April toegedien word. In na-oes studies is gevind dat Medvlugte meer koue bestand is as die ander vrugtevlugte plaeg op sitrus, wat dit moontlik maak dat toekomstige koue behandelings proewe dalk op slegs Medvlugte gedoen kan word.

Die Ander Plaeg programme het navorsing gedoen op witluis, blaaspootjies, bladvlooie (Afrika en Asiaties), wollerige witvlugte, bladspringers, myte en verskeie ander plaeg wat onder nette voorkom. Dit het 12 projekte ingesluit, met 'n paar hoogtepunte soos volg. Navorsing gedoen oor vyf jaar het gelei tot 'n nood-registrasie vir groensitrusbladspringer. 'n Reeks grond toedienings is gevind om effektief te wees vir die onderdrukking of beheer van die Asiatiese sitrusbladvlooi (ACP), of toegedien teen ACP in Mauritius of teen 'n indikator spesie in Suid-Afrika. In die soektog na effektiewe entomopatogeniese swam (EPS) behandelings is daar gevind dat EPS uiters vatbaar is tot UV-bestraling, wat die belangrikheid van 'n goeie formulering toon. In 'n voorkomende program het EPS gebaseerde produkte geen beduidende effektiwiteit getoon teen rooidopluis, witluis of blaaspootjie nie. Daar is egter 'n lae mate van beheer aangeteken as 'n regstellende behandeling teen witluis. In 'n paar projekte wat plaagbeheer onder nette ondersoek het, was lokval vangste vir vrugtevlugte hoër in oop boorde, maar VKM lokval tellings, sowel as myte en rooidopluis besmetting was hoër onder die nette.

Gedurende die laaste jaar was CRI navorsingsentomoloë ook baie aktief in die oordrag van tegnologie aan produsente, in die vorm van die tweejaarlikse Sitrus Simposium, die IPM en Siektebestuur lente werksinkels, die voor-verpakking seisoen Na-oes werksinkels en verskeie ander kleiner forums. Dit was uiters belangrik, veral in die kommunikasie van kritiese boodskappe, soos die FMS, Leprose bestuur en die bedreiging van ACP en HLB. Verskeie Snykante oor belangrike onderwerpe is ook aan produsente vrygestel, so ook artikels in die SA Vruchtejoernaal. Boonop het CRI-navorsingsentomoloë aan sekere internasionale wetenskaplike kongresse deelgeneem, soos die International Invertebrate Pathology and Microbial Control Congress, die International Chemical Ecology Congress en die International Symposium on Fruit Flies of Economic Importance. Daarbenewens is verskeie sleutel artikels in internasionale wetenskaplike joernale gepubliseer

3.2 **PROGRAMME: FALSE CODLING MOTH**

Programme coordinator: Sean D Moore (CRI)

3.2.1 **Programme summary**

FCM remained as the top entomological and market access research priority during the last research cycle. The 2017/18 citrus season was the first in which the majority of citrus produced in the country was exported to markets that regulate FCM as a phytosanitary pest. This occurred with the implementation of the new EU regulation on 1 January 2018. The FCM Risk Management System (including a systems approach), which emanated from research conducted in this programme, was very successful, but did not eliminate FCM from export fruit. Within the FCM programme, 15 different projects were conducted, of which 11 addressed pre-harvest issues and four were postharvest projects.

One of the pre-harvest projects focussed on developing an improved monitoring system for FCM, by identifying a female moth attractant (3.2.9). Field-cage no-choice trials have not yet been able to identify a volatile or combination of volatiles that is sufficiently attractive.

Four of the pre-harvest projects researched some aspect of microbial control of FCM with baculovirus. Firstly, a series of field trials were conducted to measure the efficacy of the novel baculovirus, CrpeNPV, and other treatments, some of which were also novel, against FCM (3.2.3). Results were generally inconclusive, as there was a high degree of variability, partly due to greatly improved FCM control and thus generally lower levels of FCM. Further field trials against FCM will be conducted next season under a new project. However, it is clear that in this environment of lower FCM levels in the field, our trial methodology may need to be revised. Another virus project selected for UV-resistant CrleGV (3.2.8), as UV irradiation is the biggest environmental impediment to the efficacy and persistence of insect viruses. After five UV-exposure and repassaging cycles, there was more than 1000-fold improvement in virulence of CrleGV on exposure to UV light relative to after the first cycle, indicating selection of UV-resistant virus. A third virus project has the objective of selecting for improved virulence to FCM or resistance by FCM using a homologous and a heterologous baculovirus, CrleGV and CrpeNPV respectively (3.2.13). This is still a new project and no meaningful results are available yet. The final virus project, which has been running for 3 years now, is investigating yeast-virus synergism for improved insecticidal efficacy against FCM (3.2.15). To date, this study has successfully demonstrated synergism in a laboratory environment. In the last year, three new promising yeast species were identified, the volatiles of these yeasts were tested for their attractiveness to FCM females, and yeast-virus bioassays were refined.

Three further pre-harvest projects were dedicated to investigating semiochemical management of FCM. One of them examined the use of very high application rates of the conventional male pheromone-based products (3.2.17). When mating disruption was applied in a sterile insect technique (SIT) site, FCM control was improved. However, increasing mating disruption dosage application, did not appear to improve control, even though trap catches were reduced. The second project investigated the use of a novel compound, 7-vinyl decyl acetate (7-VDA), for inhibiting, rather than disrupting mating (3.2.2). After several seasons of preparatory trials in the laboratory and in field cages, a large-scale field trial was conducted, in which greater trap shutdown was achieved

with a combination of 7-VDA (10%) and FCM pheromone. However, this did not translate into a reduction in fruit infestation. The last of these three projects is studying sexual attraction and mating compatibility between FCM populations and the potential impact that any differences might have on management practices, such as monitoring with pheromone traps, mating disruption and SIT. Currently, composition of sex pheromones in females from different regions is being compared, as is attraction of males to females from regionally distinct populations.

Remaining on the topic of SIT, two projects are focussing on assessing and improving the application of this technology. The first of these covered both the improvement in quality of moths for SIT and the improvement of quality control tests for these moths (3.2.10). Firstly, cold temperature performance of moths is being improved by the addition of the cryoprotectant, trehalose, to the larval diet. Secondly, it has been determined that the measurement of spermatophore transfer from male to female moths in the laboratory, is a good gauge of their mating competitiveness. Finally, AFLP tests are being developed as a means to differentiate between wild larvae and Xsit larvae, in order to determine whether any larvae infesting fruit in the field are F1 steriles. The second SIT project is an offshoot of the first (3.2.16). In the year in question, this project has mainly focussed on the use of anoxia, as an alternative to cold for immobilisation of irradiated moths during transportation. Unfortunately, anoxia negatively affected flight ability and can therefore not be used.

The final pre-harvest project addressed the question of whether netting over citrus orchards could facilitate localised and temporary eradication of FCM (3.2.12). On the contrary, FCM infestation was higher under nets (erected over mature trees at the start of the season). However, so too were sterile moth recaptures and sterile to wild ratios, providing confidence that superior control of FCM under nets might be achievable in a second season.

Of the projects that focussed on postharvest technologies, two addressed disinfestation of fruit and two addressed detection of infested fruit. The first disinfestation project continued to investigate cold treatments for FCM, particularly incomplete cold treatments that can be used in a systems approach (3.2.6). The efficacy of each of 3°C, 4.5°C and 5°C for periods ranging from 16 to 26 days was established. Studies on the behaviour and survival/development of larvae surviving cold treatments were also conducted, but there was no absolute relationship between movement and survival to adulthood. The second disinfestation project is expanding on a study investigating the combination of CO₂ fumigation and a short cold treatment, as an improved disinfestation measure to a standalone cold treatment (3.2.5). Post-fumigation cold treatments against fifth instars, of 12 and 14 days at 2°C, provided 99.20% and 99.92% mortality respectively, which increased to 99.84% and 99.99% if one also considered post-treatment mortality.

The first postharvest detection project analysed volatiles emitted by FCM infested fruit as a means to identify infested fruit (3.2.7). In the trials conducted in 2018 with Navels and Valencias, D-limonene levels decreased significantly and naphthalene levels increased with time after infestation, and the ratio between the two compounds was significantly lower than with healthy fruit. In Clementines there was a significant increase in beta-Ocimene. Infested Midknights showed significantly lower levels of caryophyllene. Lastly, Selected Ion Flow Tube Mass Spectrometry (Sift-MS) demonstrated great potential to rapidly differentiate between healthy and injured fruit. The final postharvest detection trial aimed to develop a remote vapour detection system, using a sniffer dog (3.2.11), previously shown to detect very accurately. Vapours from infested or healthy fruit were suctioned into filters, which were then presented to the dog. The dog found detection of odours more challenging when doing so from a remote filter than from the fruit itself.

Programopsomming

VKM het aangebly as die top entomologiese en marktoegang navorsingsprioriteit gedurende die laaste navorsingsiklus. Die 2017/18 sitrus seisoen was die eerste een waar die meerderheid sitrus wat in die land geproduseer was, uitgevoer is na markte wat VKM as 'n fitosanitêre plaag reguleer. Hierdie het gebeur met die

implementering van die nuwe EU regulasie op 1 Januarie 2018. Die VKM Risiko Bestuur Stelsel (insluitend 'n stelselsbenadering), wat sy oorsprong het uit navorsing wat in hierdie program gedoen is, was baie suksesvol, maar het VKM nie in uitvoer vrugte uitgewis nie. Binne die VKM program is 15 verskillende projekte uitgevoer, waarvan 11 voor oes kwessies toegesprek het en vier was na-oes projekte.

Een van die voor oes projekte het gefokus op die ontwikkeling van 'n verbeterde moniterings stelsel vir VKM, deur 'n wyfie lokmiddel te identifiseer (3.2.9). Veld-hok nie-keuse proewe kon nog nie 'n vlugtige stof of kombinasie van middels identifiseer wat genoegsaam aantreklik is nie.

Vier van die voor oes projekte het 'n aspek van mikrobiese beheer van VKM met bakulovirus ondersoek. Eerstens, is 'n reeks veldproewe uitgevoer om die werking van 'n nuwe bakulovirus, CrpeNPV, en ander behandelings, sommige wat ook oorspronklik was, teen VKM te toets (3.2.3). Resultate is oor die algemeen onduidelik omrede daar baie variasie was, gedeeltelik as gevolg van baie verbeterde VKM beheer en dus algemene laer vlakke van VKM. Verdere veldproewe teen VKM sal volgende seisoen onder 'n nuwe projek uitgevoer word. Dit is egter duidelik dat in hierdie omgewing van verlaagde VKM druk in die veld, ons proef metodiek waarskynlik hersien sal moet word. Nog 'n virus projek het vir UV-bestande CrleGV geselekteer (3.2.8), omrede UV bestraling die belangrikste omgewings struikelblok vir doeltreffendheid en nawerking van insek virusse is. Na vyf UV-blootstellings en hervoedings siklusse, was daar meer as 1000-voudige verbetering in virulensie van CrleGV na blootstelling aan UV-bestraling, in vergelyking met na die eerste siklus, wat suksesvolle seleksie van UV-bestande virus aangedui het. 'n Derde projek se doel is om te selekteer vir verbeterde virulensie teen VKM of bestandheid deur VKM met gebruik van 'n homoloë en heteroloë bakulovirus, onderskeidelik CrleGV en CrpeNPV (3.2.13). Hierdie is nogsteeds 'n nuwe projek en daar is dus nog geen beduidende resultate beskikbaar nie. Die finale virus projek, wat nou 3 jaar aan die gang is, ondersoek gis-virus sinergisme vir verbeterde insekdodende werking teen VKM (3.2.15). Tot hede het hierdie studie sinergisme in 'n laboratorium omgewing suksesvol gedemonstreer. In die laaste jaar is drie nuwe en belowende gis spesies geïdentifiseer, die vlugtige stowwe van hierdie gisse is getoets vir hulle aantreklikheid vir VKM wyfies, en gis-virus bio-toetse is verfyn.

Drie verdere voor oes projekte is tot die ondersoek van semiochemiese bestuur van VKM toegewy. Een van hulle het die gebruik van baie hoë toedienings dosisse van konvensionele mannetjie feromoon-gebaseerde produkte ondersoek (3.2.17). Wanneer paringsontwrigting in 'n steriele insek tegniek (SIT) perseel toegedien is, was daar 'n verbetering in VKM beheer. 'n Verhoging in die paringsontwrigting dosis wat toegedien is het blykbaar egter nie VKM beheer verbeter nie, al is daar 'n vermindering in lokvalvangste. Die tweede projek het die gebruik van 'n nuwe verbinding, 7-vinil desiel asetaat (7-VDA), wat verhoeding eerder as ontwrigting van paring ondersoek (3.2.2). Na verskeie seisoene van voorbereidende proewe in die laboratorium en in veldhokke, is 'n grootskaalse veldproef uitgevoer. 'n Groter mate van lokval afsluiting is met 'n kombinasie van 7-VDA (10%) en VKM feromoon bereik, maar hierdie het nie tot 'n vermindering in vrugbesmetting gelei nie. Die laaste van hierdie drie projekte bestudeer seksuele aantrekking en parings verenigbaarheid tussen VKM populasies en die potensiële impak wat enige verskille mag hê op bestuurspraktyke soos monitering met feromoon lokvalle, paringsontwrigting en die steriele insek tegniek (SIT). Tans word die samestelling van die seksferomoon in wyfies van verskillende streke vergelyk, so ook aantrekking van mannetjies tot wyfies van verskillende streke.

Nog steeds op die onderwerp van SIT, fokus twee projekte op die ontleding en verbetering van hierdie tegnologie. Die eerste van hierdie het beide die verbetering in motgehalte vir SIT en die verbetering in gehalte beheer toetse vir hierdie motte gedek (3.2.10). Eerstens, word koue temperatuur prestasie van motte verbeter deur byvoeging van die kouebeskermer, trehalose, tot die larwale dieet. Tweedens, is dit vasgestel dat bepaling van spermatofoor oordrag van mannetjie tot wyfie motte in die laboratorium, 'n goeie maatstaf van hulle parings mededingendheid is. Laastens, word AFLP toetse ontwikkel om tussen wilde en Xsit larwes te onderskei, om te bepaal of enige larwes wat vrugte besmet in die veld, F1 steriele is. Die tweede SIT projek is van die eerste een afkomstig (3.2.16). In die laaste jaar het hierdie projek hoofsaaklik gefokus op anoksie as 'n alternatief vir koue immobilisasie van bestraalde motte gedurende vervoer. Ongelukkig het anoksie vlugvermoë benadeel en kan daarom nie gebruik word nie.

Die finale voor oes projek het die moontlikheid van plaaslike en tydelike vernietiging van VKM onder nette oor sitrusboorde aangespreek (3.2.12). Inteendeel was VKM besmetting eintlik hoër onder nette (opgerig oor volwasse bome aan die begin van die seisoen). Steriele mot hervangste en steriele tot wilde mot verhoudings is egter ook hoër onder nette en dus sal verbeterde beheer gedurende 'n tweede agtereenvolgende seisoen moontlik bereikbaar wees.

Van die projekte wat op na-oes tegnologieë gefokus het, het twee ontsmetting van vrugte aangespreek en twee het opsporing van besmette vrugte aangespreek. Die eerste ontsmetting projek het voortgegaan met die ondersoek van koue behandelings vir VKM, veral onvolledige koue behandelings wat in 'n stelsels benadering gebruik kan word (3.2.6). Die doeltreffendheid van elk van 3°C, 4.5°C en 5°C vir tydperke van 16 tot 26 dae is vasgestel. Studies op die gedrag en oorlewing/ontwikkeling van larwes wat koue behandelings oorleef het is uitgevoer en daar was geen absolute verband tussen beweging en oorlewing tot volwassenheid nie. Die tweede ontsmettings projek brei uit op 'n studie wat 'n kombinasie van CO₂ beroking en 'n kort koue behandeling ondersoek het as 'n verbeterde ontsmettings behandeling in vergelyking met 'n alleenstaande koue behandeling (3.2.5). Koue behandelings na beroking, teen vyfde instars, van 12 en 14 dae teen 2°C, het onderskeidelik 99.20% en 99.92% mortaliteit veroorsaak. Hierdie het gestyg tot 99.84% en 99.99% wanneer mortaliteit na behandeling ook ingesluit is.

Die eerste na-oes opsporings projek het vlugtige stowwe wat deur VKM besmette vrugte vrygestel is ontleed as 'n metode om besmette vrugte te identifiseer (3.2.7). In die proewe wat in 2018 met Nawels en Valencias uitgevoer is, het D-limonien vlakke betekenisvol afgeneem en naftaleen vlakke het met tyd na besmetting vermeerder; die verhouding tussen die twee stowwe is ook betekenisvol laer as van gesonde vrugte. In Clementines is daar 'n betekenisvolle verhoging in beta-Osimeen. Besmette Midknights het betekenisvolle laer vlakke van kariofeleen gewys. Laastens, Geselekteerde loon Vloeibare Massaspektrometrie (Sift-MS) het groot potensiaal getoon om vinnig tussen gesonde en beskadigde vrugte te onderskei. Die finale na-oes opsporings projek se doel was om 'n afstand vlugtige stof opsporings stelsel te ontwikkel, met gebruik van 'n snuffelhond (3.2.11), wat voorheen gewys is om baie akkuraat besmette vrugte op te spoor. Vlugtige stowwe van besmette of gesonde vrugte is deur filters gesuig, wat toe aan die hond voorgelê is. Dit was duidelik dat dit vir die hond meer uitdagend was om reuke in filters op te spoor as van die vrugte self.

3.2.2 FINAL REPORT: Evaluation of 7-Vinyl-Decyl Acetate for mating inhibition in FCM

Project 1063 (April 2012 – June 2018) by Sean Moore, Wayne Kirkman (CRI), Mellissa Peyper, Tammy Marsberg, Sonnica Albertyn (RU) and Ben Burger (SU)

Summary

Several years ago it was discovered, almost accidentally, 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. Field cage and laboratory-based mating inhibition trials indicated that 7-VDA was not as effective as the female FCM pheromone at preventing mating. Consequently, laboratory mating inhibition trials were conducted to compare the ability of combinations of 7-VDA and FCM pheromone to reduce mating, measured by egg laying, relative to the two compounds on their own and to untreated moths. There was no significant difference in egg laying between the different treatments. However, egg laying was lowest for the 5% and 10% 7-VDA treatments. Variation in egg laying, measured by the standard error, was lower for the 10% 7-VDA and thus this mixture was selected for further trials. Egg laying for FCM pheromone, 7-VDA and 10% 7-VDA treated moths was significantly lower than for the untreated control moths. Egg laying was lowest for females exposed to the FCM pheromone. However, all eggs laid by 7-VDA and mixture exposed moths were non-viable and 8.3% (one) of the egg batches laid by moths exposed to the FCM pheromone was viable, compared to 68.7% of egg batches laid by untreated control moths. Consequently, release rate trials were conducted with this combination and with pure FCM

pheromone in novel polyethylene dispensers, recording a release rate of approximately 3 ug per day. In a large-scale replicated field trial, a total of 300 moths were caught in untreated control blocks, 17 in blocks treated with FCM pheromone dispensers, 10 in blocks treated with 7-VDA + FCM pheromone treatment and 20 in a Splat treatment. Splat reduced infestation by 50%, FCM pheromone by 23% and 7-VDA + FCM pheromone not at all. Therefore, infestation results do not concur with trapping results. This project has come to an end, but if sufficient 7-VDA can be synthesized, another such field trial will be conducted.

Opsomming

Jare gelede is dit ontdek, amper toevalig, dat 7-viniel-desielasetaat 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 'n oorspronklike paringsontwrigting – of liever paringsverhoeding – tegnologie te ontwikkel. Veldhok en laboratorium-gebaseerde paringsverhoedings proewe het aangedui dat 7-VDA nie so doeltreffend was soos die wyfie VKM feromoon om paring te verhoed nie. Gevolglik is verdere laboratorium paringsverhoedings proewe uitgevoer om die vermoë van kombinasies van 7-VDA en VKM feromoon om paring te verminder te vergelyk met die vermoë van die stowwe op hul eie. Hierdie is bepaal deur eierlegging. Daar is geen betekenisvolle verskil in eierlegging tussen verskillende behandelings, maar eierlegging was die laagste vir die 5% en 10% 7-VDA behandelings. Variasie in eierlegging was die laagste vir die 10% 7-VDA en dus is hierdie mengsel vir verdere proewe gekies. Eierlegging vir VKM feromoon, 7-VDA en 10% 7-VDA behandelde motte was betekenisvol laer as vir die onbehandelde kontrole motte. Eierlegging was laagste vir wyfies wat aan die VKM feromoon blootgestel was. Alle eiers gelê deur 7-VDA en mengsel blootgestelde motte is egter nie lewensvatbaar nie en 8.3% (een) van die eierbondels wat gelê is deur motte blootgestel aan VKM feromoon was lewensvatbaar, in vergelyking met 68.7% van eierbondels wat deur onbehandelde motte gelê is. Gevolglik is vrystellings tempo proewe met hierdie kombinasie en met suiwer VKM feromoon in oorspronklike poliëtileen vrystellers uitgevoer, en 'n vrystellings tempo van ongeveer 3 ug per dag is gemeet. In 'n grootskaalse herhaalde veldproef is 'n totaal van 300 motte in onbehandelde kontrol blokke gevang, 17 in blokke wat met VKM feromoon behandel is, 10 in 7-VDA + VKM feromoon blokke en 20 in 'n Splat behandeling. Splat het VKM besmetting met 50% verminder, VKM feromoon met 23% en 7-VDA + FCM feromoon het geen vermindering getoon nie. Daarom het besmettings resultate nie met lokval resultate ooreengestem nie. Hierdie projek het nou tot einde gekom maar as genoeg 7-VDA gesintetiseer kan word, sal nog so 'n veldproef uitgevoer word.

Introduction

Mating disruption has been used for management of FCM in citrus orchards for several years now with generally good success. The two main isomers found in the sex pheromone of the female FCM are trans-8-dodecenyl acetate [(E)-8-12:Ac] and cis-8-dodecenyl acetate [(Z)-8-12:Ac] (Persoons et al 1976 & 1977). Hence these are the two main isomers – and sometimes the only two isomers (although a third component, dodecyl acetate (12:Ac), was later discovered (Angelini *et al.*, 1981)) – used in the formulation of the mating disruption products (and lures for monitoring). However, in a study in which solventless introduction of the sex pheromone glands of the insect into the injector of a gas chromatograph (GC) was used, a wide variation in the proportion of the two acetates was found in populations from different geographical origins (Attygalle et al 1986). This has been proposed as a possible reason for erratic results experienced with commercial FCM pheromone lures in field trials and commercial usage in different parts of the country (Burger et al 1990). If this is so, then one can expect efficacy with mating disruption to also vary in different geographic regions.

In an evaluation of differently formulated synthetic false codling moth lures, formulations in which a certain batch of (E)-8-dodecenyl acetate was used, did not attract any male moths. On closer inspection it was found that, although the major constituents of this material had the correct structure, this batch of the ester contained an impurity in a concentration of approximately 9%. A typical formulation in which (E)- and (Z)-8-dodecenyl acetate were used in a ratio of 1:1 would therefore have contained the impurity in a concentration of 4.5%. The impurity

had a slightly shorter GC retention time on an apolar phase than (E)-8dodecenyl acetate or its Z-isomer. From a comparison of mass spectral dose obtained by GC/HR-MS analysis of the mixture, (E)-8-dodecenyl acetate and the impurity appeared to be structurally related, the only significant difference being between the mass spectra of the two compounds (Burger et al 1990). The impurity was isolated by preparative GC for NMR spectral analysis and conclusively identified as 7-vinyldecyl acetate 1. In the preparation of (E)-8-dodecenyl acetate 6 by a catalyst-induced coupling of (E)-2-hexenyl acetate 2 with the Grignard reagent 3, the branched-chain impurity 1 is formed by attack at the vinylogous position with a concurrent shift of the double bond (Attygalle et al 1986; Burger et al 1990). It is then isolated by careful fractionation and preparative GC.

The presence of the branched-chain acetate 1 strongly reduced the attractiveness of synthetic FCM lures. The strong inhibition of pheromonal response in FCM by this compound was illustrated in experiments in which its effect on the attractiveness of live virgin females for males of the species was determined. It was, for instance, found that whereas 370 males were caught over a period of 21 days in traps, each baited with a virgin female, not a single moth was caught in traps baited with female plus polyethylene cap dispensers containing 2 µl of the branched-chain acetate 1 (Burger et al 1990). In these experiments females were exposed in small wire gauze cages suspended centrally in tube traps and were replaced with fresh females every 3-4 days. Although further research is needed to determine the inhibitory threshold of this compound, its mode of action, the activity of analogues, etc., it is clear that 7-vinyldecyl acetate acts as a strong pheromonal inhibitor in FCM and might find application in controlling this insect pest by mating disruption.

It is unlikely that the same geographic variation by FCM populations in sensitivity to the formulated pheromone (whether as a lure or as a mating disruption product) will occur to this single acetate. It may therefore form the basis of a more effective and reliable mating disruption – or rather mating inhibitory – product than those currently available based on the FCM pheromone.

Objectives

- Determine the mating inhibitory ability of 7-Vinyl-Decyl Acetate in field situations – specifically:
- Determine the density required of the compound in order to prevent mating/infestation
- Determine the maximum distance between insects and the compound to prevent mating/pheromone trap location
- Ultimately determine the potential efficacy of 7-Vinyl-Decyl Acetate to control FCM populations and reduce citrus fruit damage in orchards

Materials and methods

Dispenser trials (2015/16)

Initially dispensers used were 0.75 ml Epindorff vials covered with a breathable membrane (once the compound was loaded), as these had previously proved effective in mating disruption trials with the FCM pheromone (Moore et al., 2014). Dispensers were loaded with FCM pheromone from the Lorelei or with 7 VDA. Additionally, Isomate dispensers (with FCM pheromone) were used. Six dispensers were used per treatment and 50 mg loaded into each. Dispensers were then placed into an incubator set at 27°C. Every seven days for four weeks, dispensers were removed and weighed to determine loss of compound.

A second dispenser trial was conducted, but this time Isomate dispensers were used. These were emptied of pheromone, dried in an incubator at 40-45°C. On a daily basis, dispensers were removed and weighed. Once there was no more than negligible loss of weight i.e. all pheromone had volatilised, dispensers were removed from the incubator. The same treatments and volumes as described above were used and again in six dispensers each. Dispensers were loaded and then both ends of the Isomate dispenser sealed. Dispensers were then placed

into an incubator set at 27°C and removed and weighed on a daily basis for 19 days. Loss of compound was calculated and compared between treatments.

A third dispenser trial was conducted, exactly as the previous one, except that 12 dispensers were used for each of the FCM pheromone treatments and 48 for 7 VDA; and evaluation was continued for only eight days.

(2018/19)

Dispensers were made from polyethylene (PE) tubing (inside diameter of 1.4 mm and outside diameter of 1.9 mm) and from two types of silicon tubing (inside diameter of 0.75 mm and outside diameter of 1.65 mm; and inside diameter of 0.76 mm and outside diameter of 0.86 mm). Dispensers were cut so that they would hold either 100 or 200 µl of FCM pheromone. Six dispensers of each were used: three were placed into an incubator with a constant temperature of 26°C and three were hung outside in the shade, but exposed to the elements. Daily, if possible, otherwise as frequently as possible, dispensers were weighed to determine weight loss. This was done over a 14-day period for PE dispensers and over a 7-day period for silicon dispensers.

Before field trials commenced, release rate trials were conducted to compare a solution of 10% 7-VDA and 90% FCM pheromone with pure FCM pheromone in PE dispensers to ensure that the required release rate of approximately 3 µg per day will be achieved. Six dispensers containing the FCM pheromone and 7 VDA mix and 7 dispensers with FCM pheromone only were kept at a constant temperature of 26°C for 4 days. Dispenser weights were recorded every 24 h.

Laboratory mating inhibition trials

2015/16

Six buckets with a 5 L capacity were used for the trial per treatment. The three treatments were control (empty dispensers), FCM pheromone (from the Lorelei) and 7 VDA. A volume of 5 mg was placed into each dispenser (which was a 0.75 ml capacity Epindorff vial) and one dispenser was placed into each bucket, suspended from the inside of the lid. Each lid had a window, covered with gauze for ventilation. After 24 h (in order to allow the volatiles from the compounds to saturate the bucket space), one virgin male and one virgin female adult moth were placed into each bucket. After seven days, buckets were opened and eggs laid in each bucket were counted. Additionally, dispensers were weighed to determine loss of compound.

A second trial was conducted in a similar manner, except that Isomate dispensers were used, 100 mg of compound was used per dispenser, and in addition to the three treatments used in the first trial, FCM pheromone from Isomate was also used. Additionally, two 7 VDA dispensers were used per bucket, whereas only one of each of the others were used. This was due to the inferior release rate of 7 VDA relative to FCM pheromone.

A third trial was conducted in a similar manner to the previous one, with the following differences. FCM pheromone from the Lorelei was not used; six 7 VDA dispensers were placed in each bucket, whereas two FCM pheromone (from Isomate) dispensers were placed into each bucket.

2016/17

Previously 6 buckets with a 5 L capacity were used for the trial per treatment. To reduce the chances that the male and female could encounter each other accidentally in such a small space, it was decided to conduct these trials in larger 80 L capacity plastic boxes. The three treatments, control, Isomate and 7 VDA were placed on 23 October 2014. After 24 h (in order to allow the volatiles from the compounds to saturate the box space), one virgin male and one virgin female adult moth were placed into each box. After seven days, boxes were opened and eggs laid in each box were counted. Additionally, dispensers were weighed to determine loss of compound.

A second trial was conducted in a similar manner, where the treatments were placed on 6 November 2014. Sponge was placed between the lids and the boxes to reduce the chance of moths escaping. Moths were placed in the boxes on 7 November, and removed on 14 November.

A third trial was conducted in a similar manner to the previous one, with treatments placed on 26 November 2014. Moths were placed in the containers on 27 November and removed on 4 December.

A fourth trial was conducted in a similar manner to the previous one, with the inclusion of an extra treatment – a combination of 7VDA and Isomate. Treatments placed on 16 February 2015. Moths were placed in the containers on 18 February and removed on 2 March.

A fifth trial was conducted in a similar manner to the previous one. This time 7VDA was compared with FCM pheromone, as well as combinations of 7VDA (2.5, 5 and 10%) and FCM pheromone, formulated in plastic dispensers. Treatments were placed in the boxes on 24 June 2015. Moths were placed in the boxes on 26 June and removed on 3 July.

2017/18

Six rectangular plastic containers with 80 L capacity were used for the trial per treatment. The five treatments were an untreated control, FCM pheromone, 7-VDA, 10% 7-VDA in FCM pheromone, 5% 7-VDA in FCM pheromone and 2.5% 7-VDA in FCM pheromone. Twenty-four hours after hanging treatments in containers (in order to allow the volatiles from the compounds to saturate the box space), one virgin male and one virgin female adult moth were placed into each box. After seven days, boxes were opened and eggs laid in each box were counted. Additionally, dispensers were weighed to determine loss of compound. Four replicates of the trial were conducted, being initiated on 24 June, 19 August, 21 September and 26 October 2015.

The laboratory trials were then modified in order to provide greater clarity on the differences in efficacy between the treatments. Additionally, only one mixture of FCM pheromone and 7VDA was used i.e. the combination which appeared to be the most effective (albeit not significantly more so than the other ratios). The trials entailed keeping unmated moth pairs in the large containers (6 x 80 L per treatment) for only 48 h. This was in order to reduce the possibility of an incidental meeting of the pair. After 48 h, females were individually transferred to petri dishes and egg laying and egg viability (hatch) were monitored on a daily basis. The trial was replicated three times, being initiated on 12 February, 19 March and 14 May 2016.

Field cage trials

2015/16

A Washington Navel orange orchard (on Swingle rootstock) on Arundel Farm in the Sundays River Valley was used for the trials. The orchard was planted in 2009 at a spacing of 5 m x 2 m (rows x trees) – therefore 1000 tree per ha – and was irrigated with microsprinklers. Twelve wooden-framed mosquito net-covered cages were erected over pairs of trees in the orchard. Nets had zip-doors so that a person could enter and cages were spacious enough to accommodate the presence of a person. Netted trees were laid out to maximise the distance between caged trees – a minimum of 36 m between cages.

In the first trial in the cages, six different treatments were used – two cages per treatment. These were a control, Isomate (two dispensers per cage) and 7 VDA (one, two, four and eight dispensers per cage). In this trial, the dispensers used for 7 VDA were 0.75 ml Epindorff dispensers loaded with 10 mg each. Dispensers were hung in the cages on 13 December 2013 at 11h30 and at 15h30 on the same day, 10 virgin male and 10 virgin female moths were released into each cage. Males and females were released as far apart from one another as possible. Weekly, from 2 January 2014, fallen fruit in cages were collected and dissected to determine FCM infestation. This was continued for five weeks.

In the second cage trial, Isomate dispensers were loaded with the relevant compound. Treatments used were: control, Isomate (two per cage) and 7 VDA (two, four and eight per cage). Dispensers were hung on 30 April 2014 and moths were released on 2 May. FCM infestation in fallen fruit was monitored weekly from 30 May to 26 June 2014. Thereafter, all remaining fruit on trees were stripped and evaluated for FCM infestation.

2016/17

A Lane Late Navel orange orchard on Bernol Farm in the Sundays River Valley was used for the trials. The orchard was planted in 2006 at a spacing of 6 m x 3 m (rows x trees) – therefore 555 trees per ha – and was irrigated with drippers. Twelve wooden-framed mosquito net-covered cages were erected over pairs of trees in the orchard. Nets had zip-doors so that a person could enter and cages were spacious enough to accommodate the presence of a person. Netted trees were laid out to maximise the distance between caged trees – a minimum of 36 m between cages.

In the first trial in the cages, four different treatments were used – three cages per treatment. These were a control, Isomate (one dispenser per cage) and 7 VDA (one and two dispensers per cage). The dispensers used for 7 VDA, were perimeter sealed polyethylene double sheets, containing 50 mg per dispenser. Dispensers were hung in the cages on 10 December 2014, and the following day 10 virgin male and 10 virgin female moths were released into each cage. Males and females were released as far apart from one another as possible. Egg counts on fruit were conducted on 18 and 23 December 2014, on 20 randomly selected fruit per cage. Weekly, from 23 December 2014 to 29 January 2015, fallen fruit in cages were collected and dissected to determine FCM infestation.

In the second cage trial, treatments used were: control, Isomate (one per cage), 7 VDA (one per cage) and a combination of Isomate and 7VDA (one each per cage). Dispensers were hung on 17 March 2015 and moths were released on the evening of 18 March. Egg counts on fruit were conducted on 1 April 2015, on 20 randomly selected fruit per cage. FCM infestation in fallen fruit was monitored weekly from 9 April to 20 May 2015.

Field trials

2018/19

A field trial was initiated in November 2017. Four treatments were used: untreated control, FCM pheromone, 7-VDA (10%) + FCM pheromone (90%) and Splat. Each treatment was replicated twice in a randomised block design, each to a 1 ha block of Navel orange trees on Boerboon Farm in the Sundays River Valley. Splat was applied by River Bioscience (in November and January) and FCM pheromone and 7-VDA + FCM pheromone were applied at 1 dispenser per tree i.e. 555 dispensers per hectare each (only in November). One FCM pheromone trap was simultaneously hung in the centre of each replicate and monitored weekly. The trial was monitored until the end of February (15 weeks).

Results and discussion

Dispenser trials

2015/16

It was clear from the first release rate trial conducted in an incubator that rate of release of both 7 VDA and FCM pheromone from the polyethylene Epindorff dispensers was way inferior to that of FCM pheromone from Isomate dispensers (Fig. 3.2.2.1) and hence probably inadequate. Release rate per day averaged 20.1 mg for Isomate, 0.3 mg per day for 7 VDA and 0.4 mg per day for Lorelei pheromone.

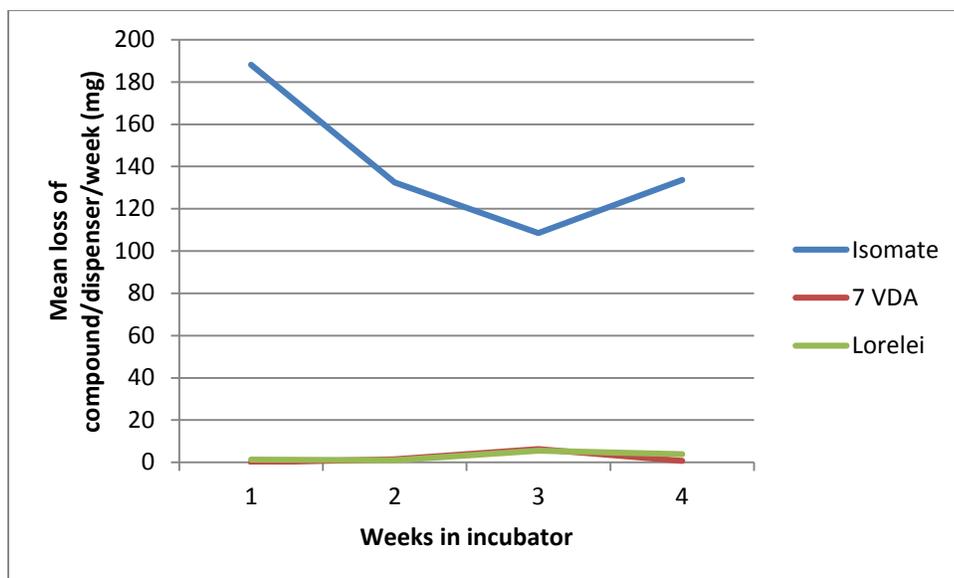


Figure 3.2.2.1. Average loss of compound per dispenser per week in an incubator set at 27°C. (Isomate FCM pheromone is Isomate dispensers; 7 VDA and Lorelei FCM pheromone in 0.75 ml Epindorff vials).

In the second release rate trial, the release of both 7 VDA and the Lorelei pheromone were clearly greater from the Isomate dispensers than they had been from the Epindorff vials in the previous trial (Fig. 3.2.2.2). However, release of the FCM pheromone (Isomate and Lorelei) was almost double that of the rate of 7 VDA release i.e. 58.4 and 68.0 mg per day respectively as opposed to 35.8 mg per day. This lower release rate of 7 VDA was due to it being a larger molecule (and a branched molecule) than the molecules in the FCM pheromone.

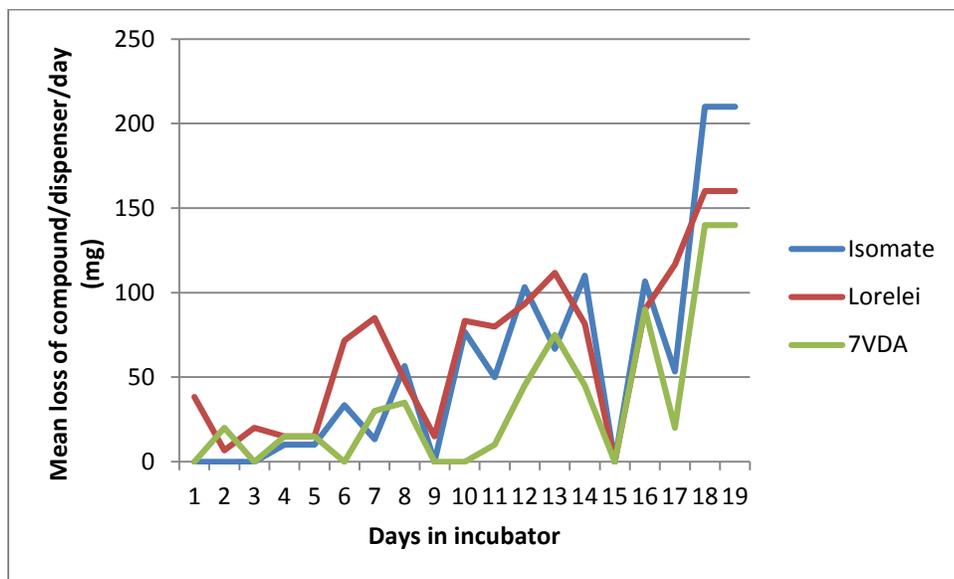


Figure 3.2.2.2. Average loss of compound per dispenser per day in an incubator set at 27°C. (Isomate dispensers used for all compounds).

The findings were confirmed in the third release rate trial, where the two FCM pheromones were released at 63.0 and 63.3 mg per day and 7 VDA was released at 34.4 mg per day (Fig. 3.2.2.3). In this and the previous trial, rate of release per day was not constant over time. There was apparently vacillation in temperature in the incubator.

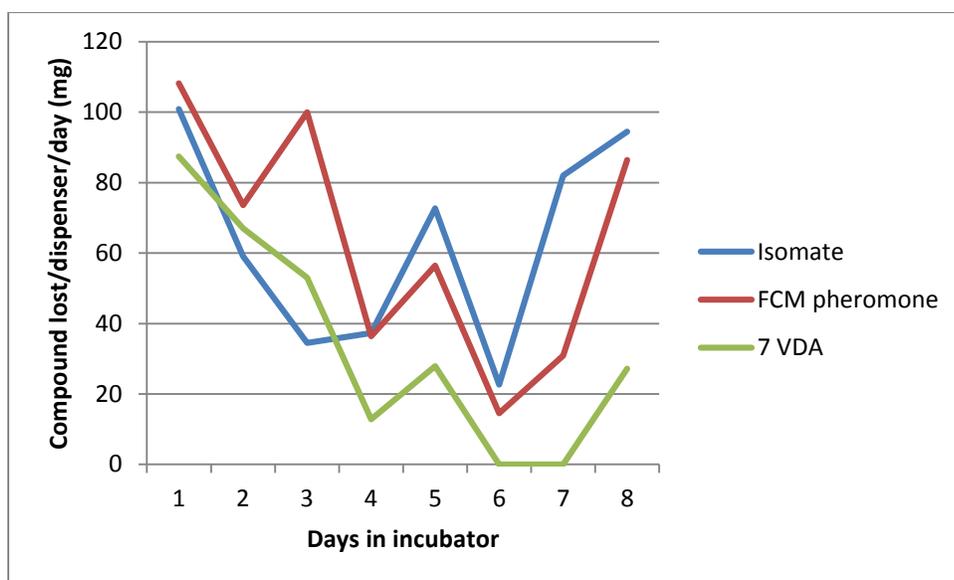


Figure 3.2.2.3. Average loss of compound per dispenser per day in an incubator set at 27°C. (Isomate dispensers used for all compounds).

It was clear from the incubator trials that a higher number of dispensers with 7 VDA would be required in field trials in order to achieve a comparable release of compound to that of the FCM pheromone.

2018/19

In order to achieve a release rate similar to Isomate, a release of approximately 2 mg per dispenser per day is required. PE therefore appears to be an extremely suitable compound for the dispensers, releasing around this rate with all of the PE dispenser specifications (Table 3.2.2.1). PE is a commonly used compound in dispensers used both for monitoring and for mating disruption. A concern though was that release rate in a dispenser made purely of PE might be too variable, particularly as the volume of compound in the dispenser reduced over time. This did not seem to be so in the very narrow tube dispensers used. On the other hand, silicon does not appear to be a suitable compound for the dispensers. The release rate was very variable and generally excessive (Table 3.2.2.1).

Table 3.2.2.1

Dispenser type	Dispenser volume	Incubator or Outside	Average daily loss of pheromone (mg)
Polyethylene	100 ul	Incubator	1.9
		Outside	1.7
	200 ul	Incubator	2.7
		Outside	2.3
Silicon 0.75 & 1.65 Ø	100 ul	Incubator	3.1
		Outside	8.1
	200 ul	Incubator	6.0
		Outside	13.3
Silicon 0.76 & 0.86 Ø	100 ul	Incubator	5.2
		Outside	9.5
	200 ul	Incubator	9.4
		Outside	36.9

Prior to conducting field trials an average release rate of 2.5 mg and 2.7 mg per day was recorded for the 7-VDA and FCM pheromone mix and pure FCM pheromone respectively.

Laboratory mating inhibition trials
2015/16

In the first trial FCM pheromone succeeded in reducing egg laying (as a result of reduced mating). However, 7 VDA made no difference to egg laying (Fig. 3.2.2.4). This was disappointing, as although it is understandable that insufficient 7 VDA may have been released to have an effect, it would have been expected that this would have been the same for the FCM pheromone, as both compounds were placed into Epindorff vials. Mean release rate of each per day was 0.4 and 0.6 mg, respectively.

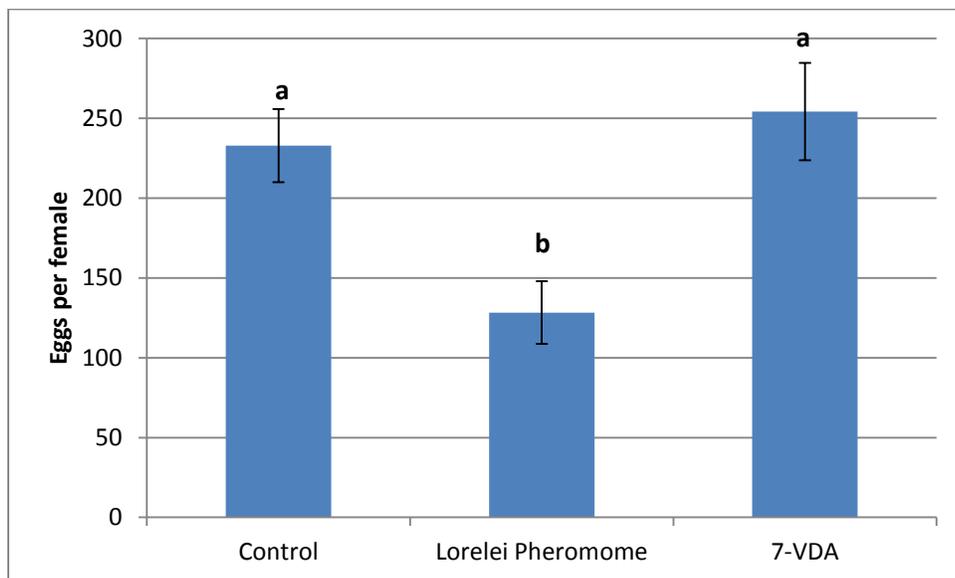


Figure 3.2.2.4. Mean eggs laid per female when exposed to different compounds in buckets in the laboratory

In the second trial, all treatments appeared to reduce egg laying, but these differences were not statistically significant (Fig. 3.2.2.5). Release rate per day for Isomate FCM pheromone was 50.5 mg and was 62.8 mg for the Lorelei FCM pheromone. However, release rate for 7 VDA was only 18.7 mg per dispenser per day and consequently 37.4 mg per bucket per day (two dispensers per bucket).

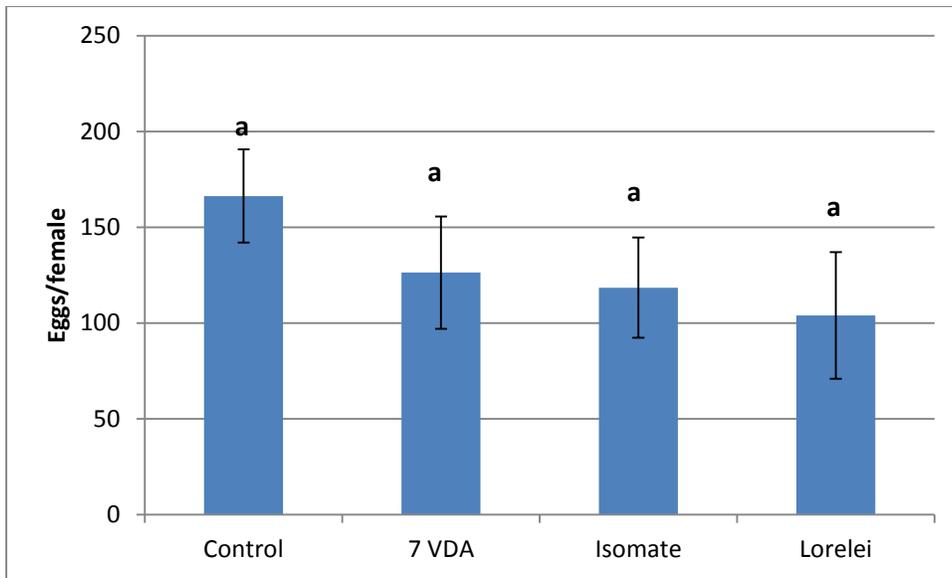


Figure 3.2.2.5. Mean eggs laid per female when exposed to different compounds in buckets in the laboratory (both Isomate and Lorelei were FCM pheromone)

In the third trial, both FCM pheromone (two dispensers per bucket) and 7 VDA (six dispensers per bucket) succeeded in significantly reducing egg laying (Fig. 3.2.2.6). Release rate per bucket per day for each was 122.7 and 210.0 mg, respectively.

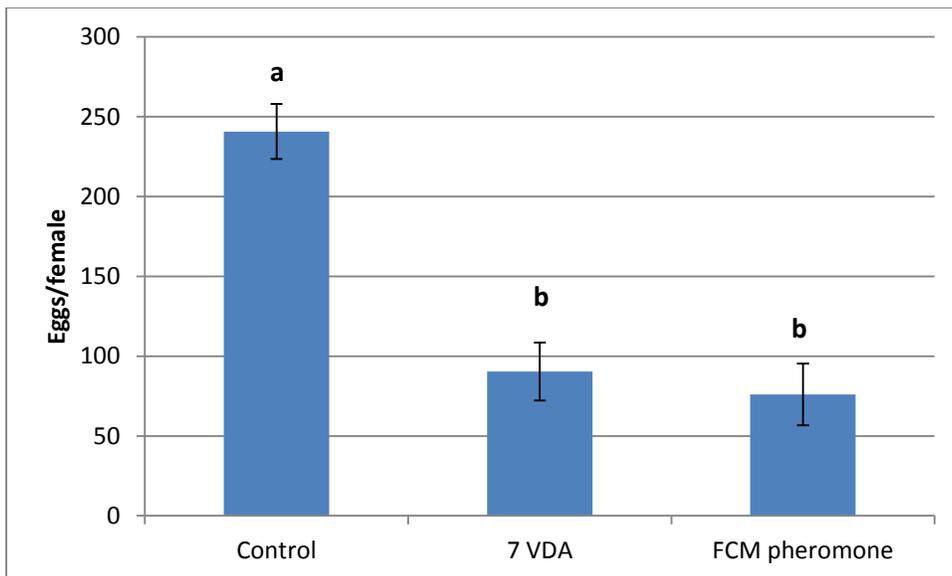


Figure 3.2.2.6. Mean eggs laid per female when exposed to different compounds in buckets in the laboratory (FCM pheromone was the Lorelei blend)

2016/17

In the first trial, mean release rate per day was 2.2 mg and 3.1 mg for 7VDA dispensers and Isomate respectively. Unfortunately, most of the moths escaped from the boxes through gaps between the lids and boxes. This trial was abandoned. In the second trial, 7VDA dispensers lost 1.2 mg per day, compared to 1.8 mg for Isomate. Egg laying was reduced (as a result of reduced mating) by 39% and 56% by 7VDA and Isomate respectively (Fig 3.2.2.7).

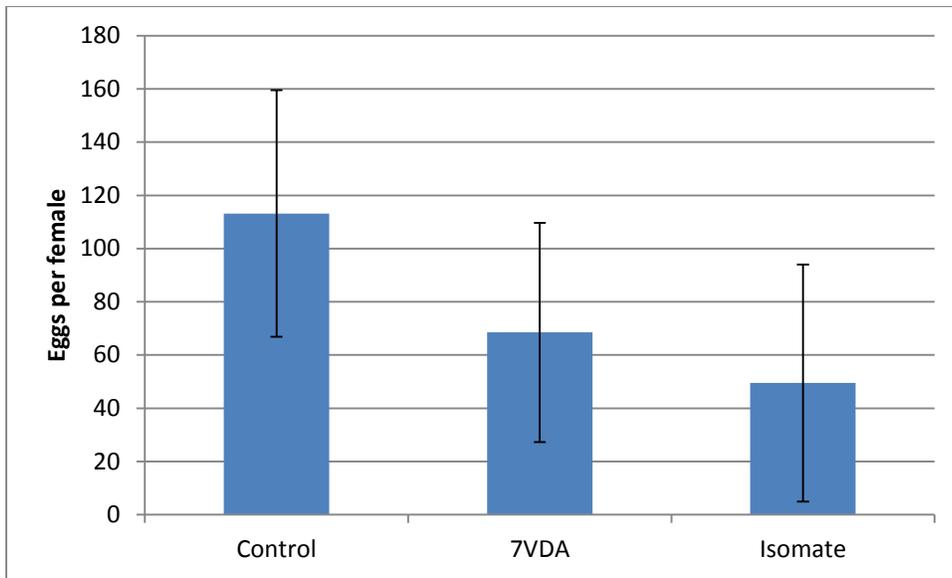


Figure 3.2.2.7. Mean eggs laid per female when exposed to different compounds in boxes in the laboratory (Trial 2).

In the third trial, mean release rate per day was 2.4 mg and 2.8 mg for 7VDA dispensers and Isomate respectively. Egg laying was reduced (as a result of reduced mating) by 49% and 77% by 7VDA and Isomate respectively (Fig 3.2.2.8).

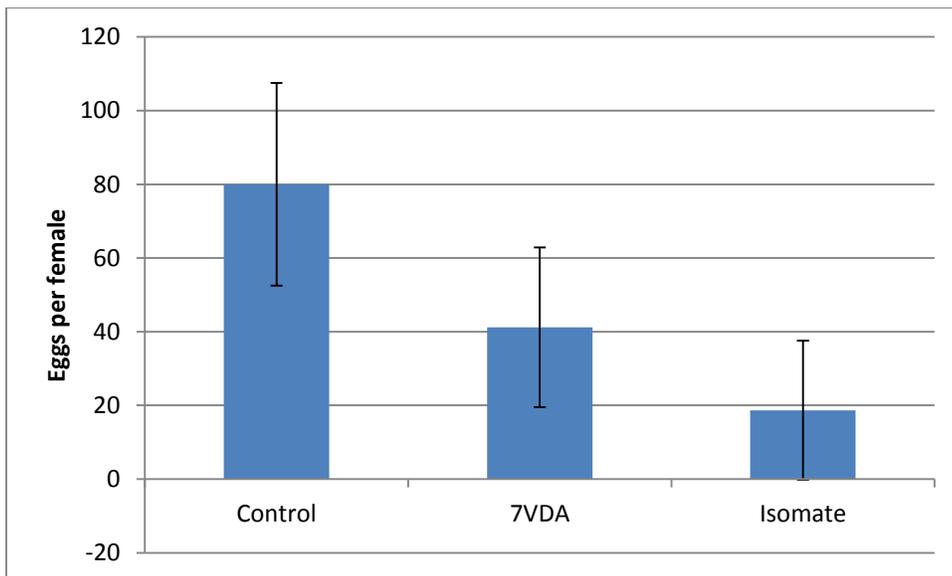


Figure 3.2.2.8. Mean eggs laid per female when exposed to different compounds in boxes in the laboratory (Trial 3).

As 7VDA did not reduce mating and hence egg laying to the same extent as did Isomate in the second and third laboratory trials, a combined hanging of separate 7VDA and Isomate dispensers was used as a treatment. It was postulated that this might be more effective, as the original discovery of 7VDA was as a contaminant in FCM pheromone dispensers, completely preventing catching of male moths in traps (Burger et al., 1990). The mean release rates per day for the fourth trial are given in Table 2. In this trial, unlike the previous trials, egg laying was reduced more by 7VDA (68%) than Isomate (42%) (Fig 3.2.2.9). The combination of 7VDA and Isomate reduced egg laying by 76%.

Table 3.2.2.2. The average daily release rate per dispenser for various treatments in laboratory trials for the disruption of FCM mating (Trial 4).

Treatment	Average release rate per day (mg)
7VDA	16
7VDA (used with Isomate)	22
Isomate	26
Isomate (used with 7VDA)	25

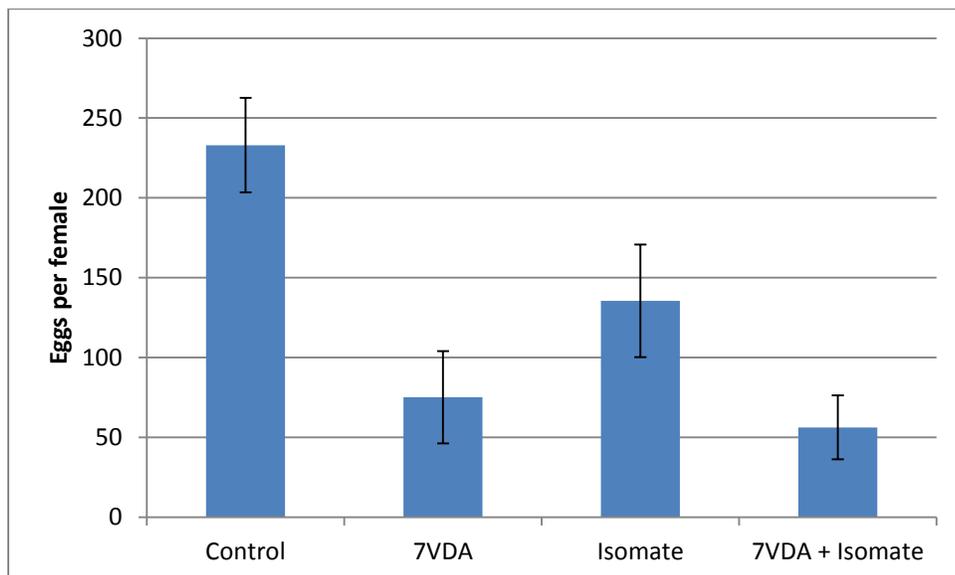


Figure 3.2.2.9. Mean eggs laid per female when exposed to different compounds in boxes in the laboratory (Trial 4).

Due to the positive result in trial four, 7VDA and FCM pheromone were combined into the same dispensers for three of the treatments in the fifth trial. The mean release rates per day for the fifth trial are given in Table 3.2.2.3. In this trial, egg laying was reduced significantly by FCM pheromone as well as combinations of FCM pheromone and 7VDA (Fig 3.2.2.10). The 10% 7VDA combination was the most effective. Surprisingly 7VDA alone did not reduce egg laying as much as in previous trials.

Table 3.2.2.3. The average daily release rate per dispenser for various treatments in laboratory trials for the disruption of FCM mating (Trial 5).

Treatment	Average release rate per day (mg)
7VDA	1.2
FCM pheromone	1.1
7VDA (10%) + FCM pheromone	1.2
7VDA (5%) + FCM pheromone	1.2
7VDA (2.5%) + FCM pheromone	1.2

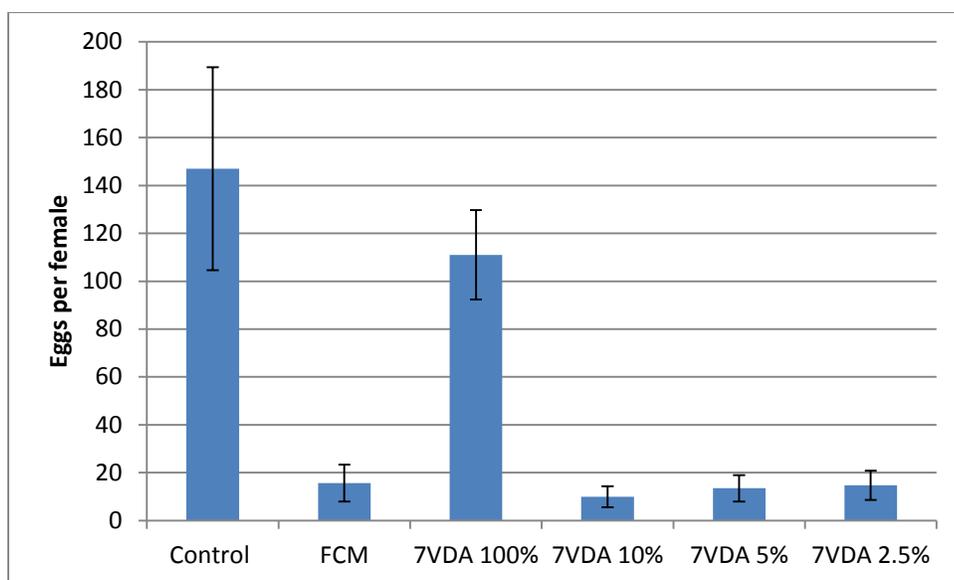


Figure 3.2.2.10. Mean eggs laid per female when exposed to different compounds in boxes in the laboratory (Trial 5).

2017/18

Release rates of the five treatments in the four replicates was relatively constant, usually ranging from 6.6 to 9.2 mg per day (Table 3.2.2.4). However, in two of the replicates (Rep 2 and 3) the pure 7-VDA did release at a markedly lower rate. It is not clear why. 7-VDA is known to be a larger branched molecule than the FCM pheromone. However, if this was the reason for the lower release rate, one would have expected this to also be so in the other two replicates (Reps 1 and 4), which was not the case.

Table 3.2.2.4. The average daily release rate per dispenser for various treatments in laboratory trials for the disruption of FCM mating for four replicates.

Treatment	Average release rate per day (mg)			
	Rep 1	Rep 2	Rep 3	Rep 4
FCM pheromone	6.6	8.3	7.6	8.3
7VDA	7.0	4.8	4.4	9.2
7VDA (10%) + FCM pheromone	7.3	9.3	8.4	8.6
7VDA (5%) + FCM pheromone	7.2	9.9	9.0	8.7
7VDA (2.5%) + FCM pheromone	7.0	6.9	6.3	6.2

Mean number of eggs laid per female for all of the treatments was lower than that for control (untreated moths) (Fig. 3.2.2.11). There was no significant difference in egg laying between the different treatments. However, egg laying was lowest for the 5% and 10% 7-VDA treatments. Variation in egg laying, measured by the standard error, was lower for the 10% 7-VDA and thus this mixture was selected for further trials.

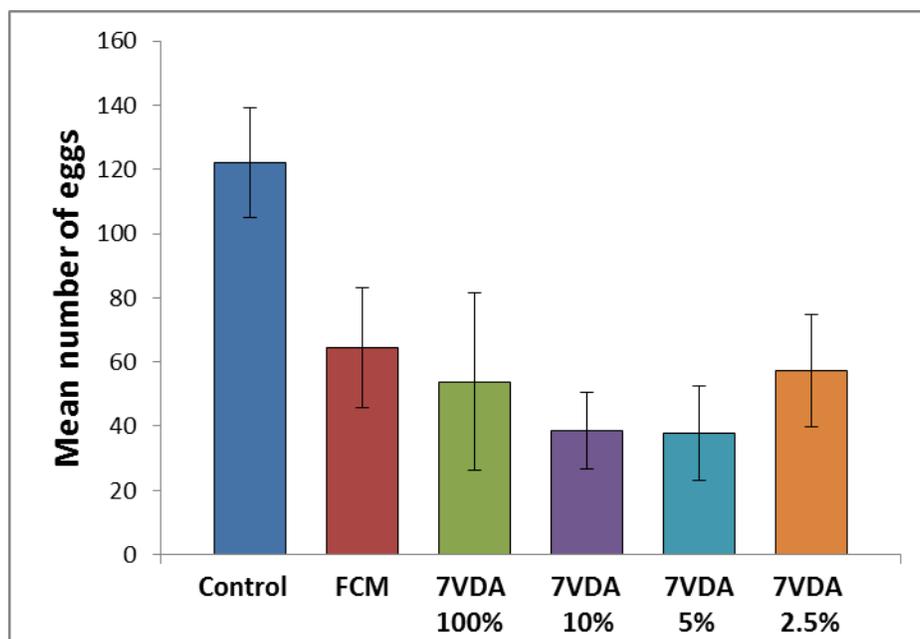


Figure 3.2.2.11. Mean eggs laid per female when exposed to different compounds in boxes in the laboratory, determined from four replicates.

In the improved trials, in each successive replicate, release rate was markedly lower than the previously one, but remained relatively consistent between treatments (Table 3.2.2.5). This was despite the trials being conducted at the same temperature on each occasion. This is a concern and something that must be monitored and if necessary, rectified, in the execution of field trials.

Table 3.2.2.5. The average daily release rate per dispenser for various treatments in laboratory trials for the disruption of FCM mating for three replicates.

Treatment	Average release rate per day (mg)		
	Rep 1	Rep 2	Rep 3
FCM pheromone	13.6	3.6	1.0
7VDA	16.6	4.5	1.1
7VDA (10%) + FCM pheromone	12.4	3.9	1.2

Egg laying for all three treatments was significantly lower than for the untreated control moths (Fig. 3.2.2.12). Egg laying was lowest for females exposed to the FCM pheromone, significantly lower than moths exposed to the mixture. However, in monitoring of egg viability, it became apparent that this was an important aspect to measure for determining treatment efficacy. All eggs laid by 7-VDA and mixture exposed moths were non-viable i.e. did not hatch, therefore not fertilised (Table 3.2.2.6). However, 8.3% (one) of the egg batches laid by moths exposed to the FCM pheromone was viable, compared to 68.7% of egg batches laid by untreated control moths.

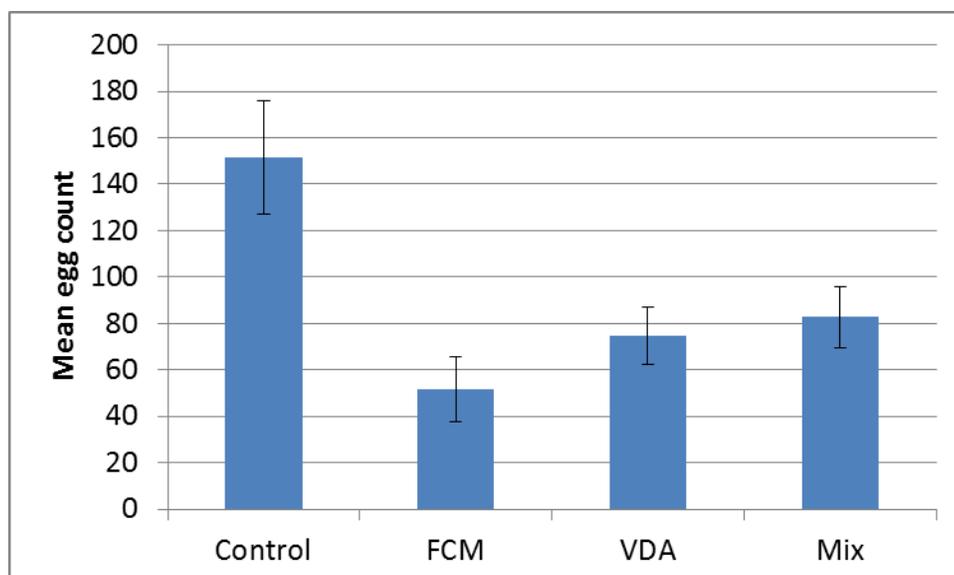


Figure 3.2.2.12. Mean eggs laid per female in petri dishes after being exposed to different compounds in 80 L containers for 48 h in the laboratory, determined from three replicates.

Of the 18 control moths, 16 laid eggs, of which 11 egg batches were viable i.e. fertilised and hatched. Of the 18 FCM pheromone treated moths, 12 laid eggs, only one batch being viable. Of the 18 7VDA treated moths and the 18 pheromone-VDA mixture treated moths, 16 of each laid eggs. However, none of these eggs were viable.

Table 3.2.2.6. Females out of a total of 18 per treatment, which laid eggs and numbers of these egg batches that were viable (i.e. fertilised and hatched) per treatment.

	Females laying and viable egg batches per treatment			
	Control	FCM pheromone	7-VDA	Mixture
Moths laying eggs (n = 18)	16	12	16	16
Viable egg batches	11	1	0	0

Field cage trials

2015/16

Results from the first field trial were not very helpful, as FCM infestation was generally very low and although no infested fruit were recorded in the Isomate cages, so too were there no infested fruit for one of the lower 7 VDA treatments (Fig. 3.2.2.13). Additionally, FCM infestation for two of the 7 VDA treatments (including the highest one) was higher than for the untreated control. It was concluded that the results were meaningless but that insufficient 7 VDA had been released and that improved dispensers to the polyethylene ones should be used.

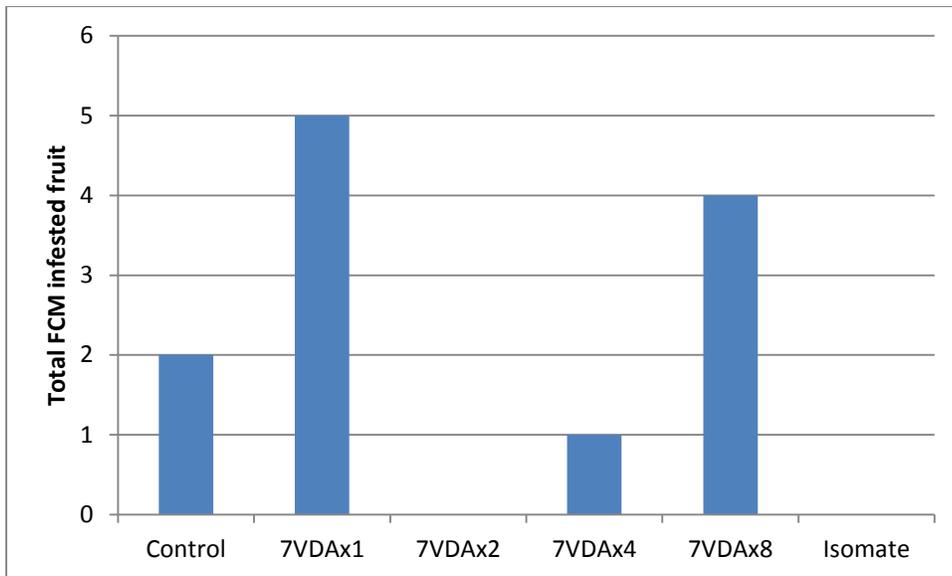


Figure 3.2.2.13. Total fruit infested with FCM from caged trees in which dispensers with either FCM pheromone or 7 VDA were hung (FCM pheromone in Isomate dispenser; 7 VDA in 0.75 ml Epindorff polyethylene dispenser), monitored weekly for five weeks from 2 January 2014.

Results from the second caged trial looked more promising than the first, with a reduction in infestation (relative to the control) for all treatments (Fig. 3.2.2.14). However, again, FCM infestation was generally low. Isomate was the only treatment for which no infestation was recorded. Average release rate per day of the FCM pheromone was 61.3 mg and of 7-VDA was 18.8 mg. Therefore, total release rate per tent for FCM pheromone was 122.6 mg and for 7-VDA was 37.6 mg, 75.2 mg and 150.4 mg.

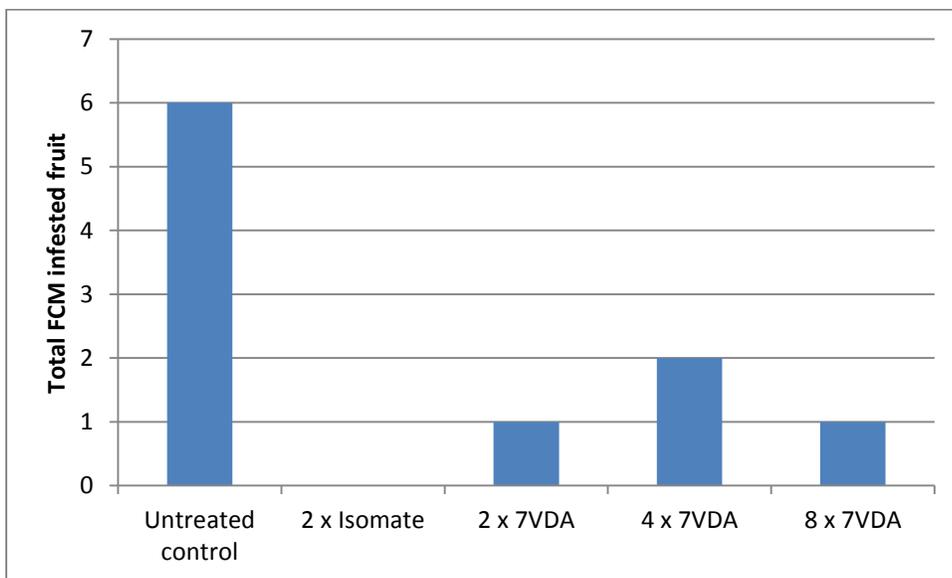


Figure 3.2.2.14. Total fruit infested with FCM from caged trees in which dispensers with either FCM pheromone or 7 VDA were hung (all in Isomate dispensers), monitored weekly for four weeks from 30 May 2014.

Once fruit was stripped from the trees and evaluated for FCM infestation, results became unclear, as they did not reflect the on-tree assessments and they did not show any particular trend (Fig. 3.2.2.15).

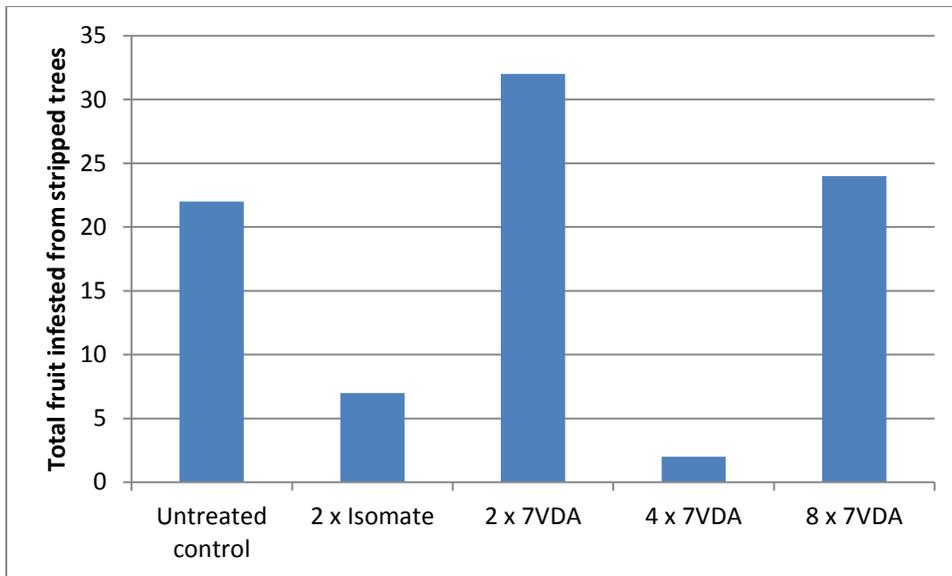


Figure 3.2.2.15. Total fruit infested with FCM stripped from caged trees in which dispensers with either FCM pheromone or 7 VDA were hung (all in Isomate dispensers).

2016/17

In the first trial, the average release rate per day was 6.3 mg and 3.4 mg for 7VDA and Isomate respectively. Egg counts were low and showed no trend, as more fruit had eggs laid on them in some treatments than in the untreated control (Fig 3.2.2.16 and 3.2.2.17). FCM infestation was generally very low, and as with the eggs, no trend was visible (Fig. 3.2.2.18). Additionally, FCM infestation for some treatments was again higher than for the untreated control. It was concluded that the results were meaningless.

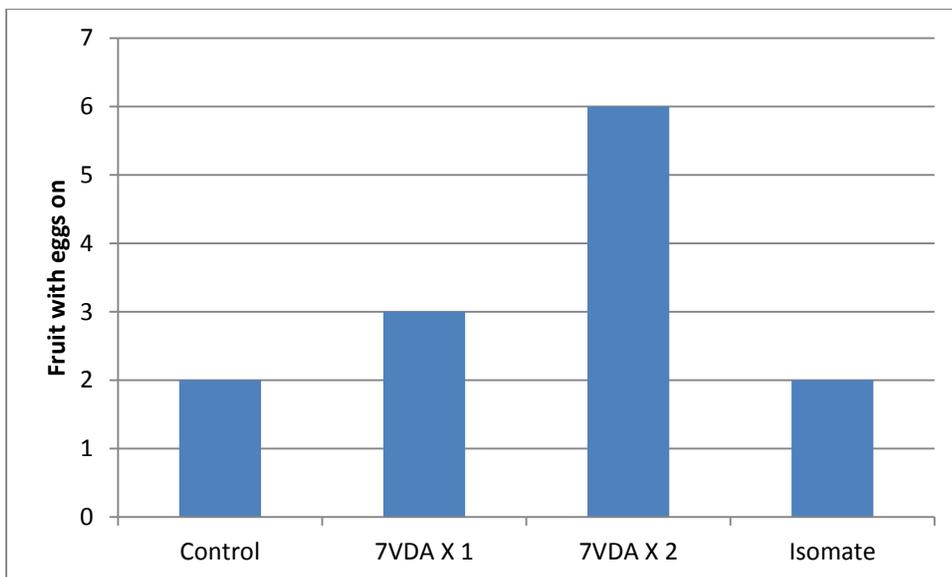


Figure 3.2.2.16. Total number of fruit (of 20) with eggs laid on, for various treatments, evaluated on 18 December 2014

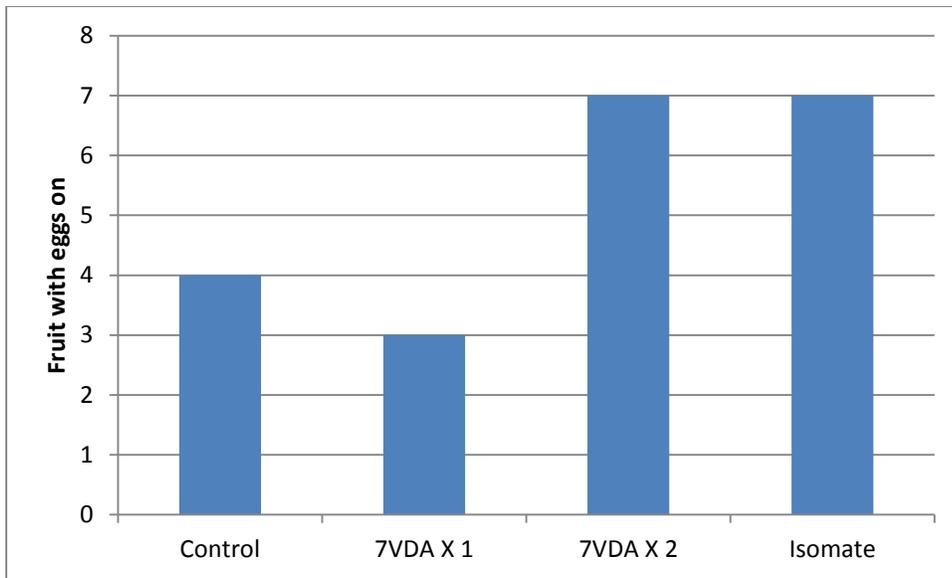


Figure 3.2.2.17. Total number of fruit (of 20) with eggs laid on, for various treatments, evaluated on 23 December 2014.

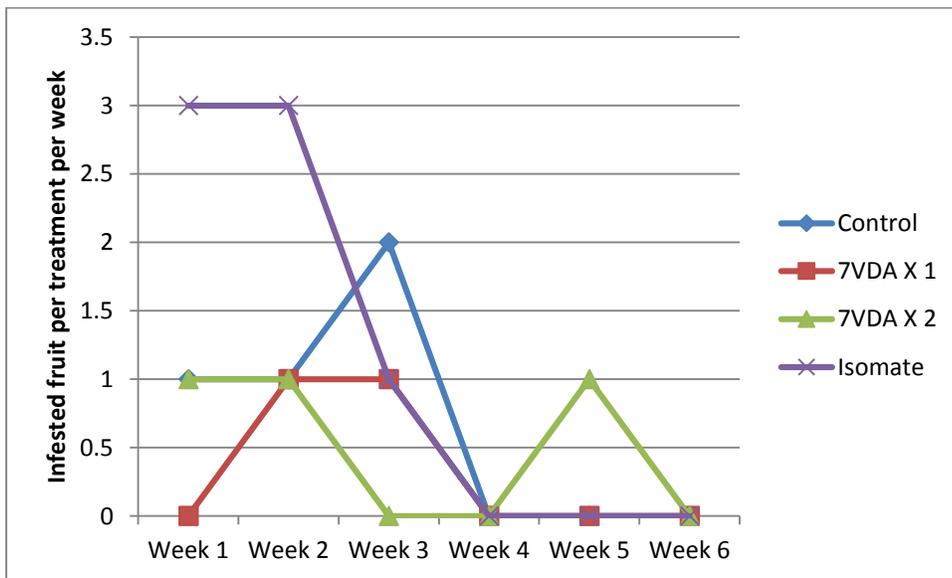


Figure 3.2.2.18. Fruit infested per week with FCM from caged trees for various treatments, monitored weekly for six weeks from 23 December 2014.

In the second trial the average release rate per day was 4.3 mg and 2.7 mg for 7-VDA and Isomate respectively. More eggs were laid, possibly due to the moths being released in the evening. However, there was no visible trend in egg laying or FCM infestation (Fig 3.2.2.19 and 3.2.2.20). Consequently, it was concluded that there was fault with this particular trial design and that changes must be made for field trials in the following season.

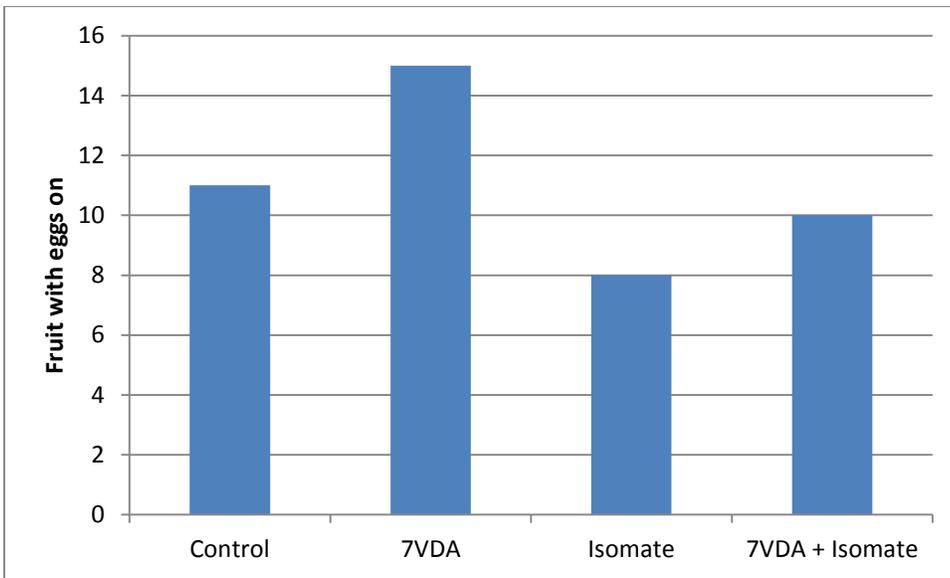


Figure 3.2.2.19. Total number of fruit (of 20) with eggs laid on, for various treatments, evaluated on 1 April 2015.

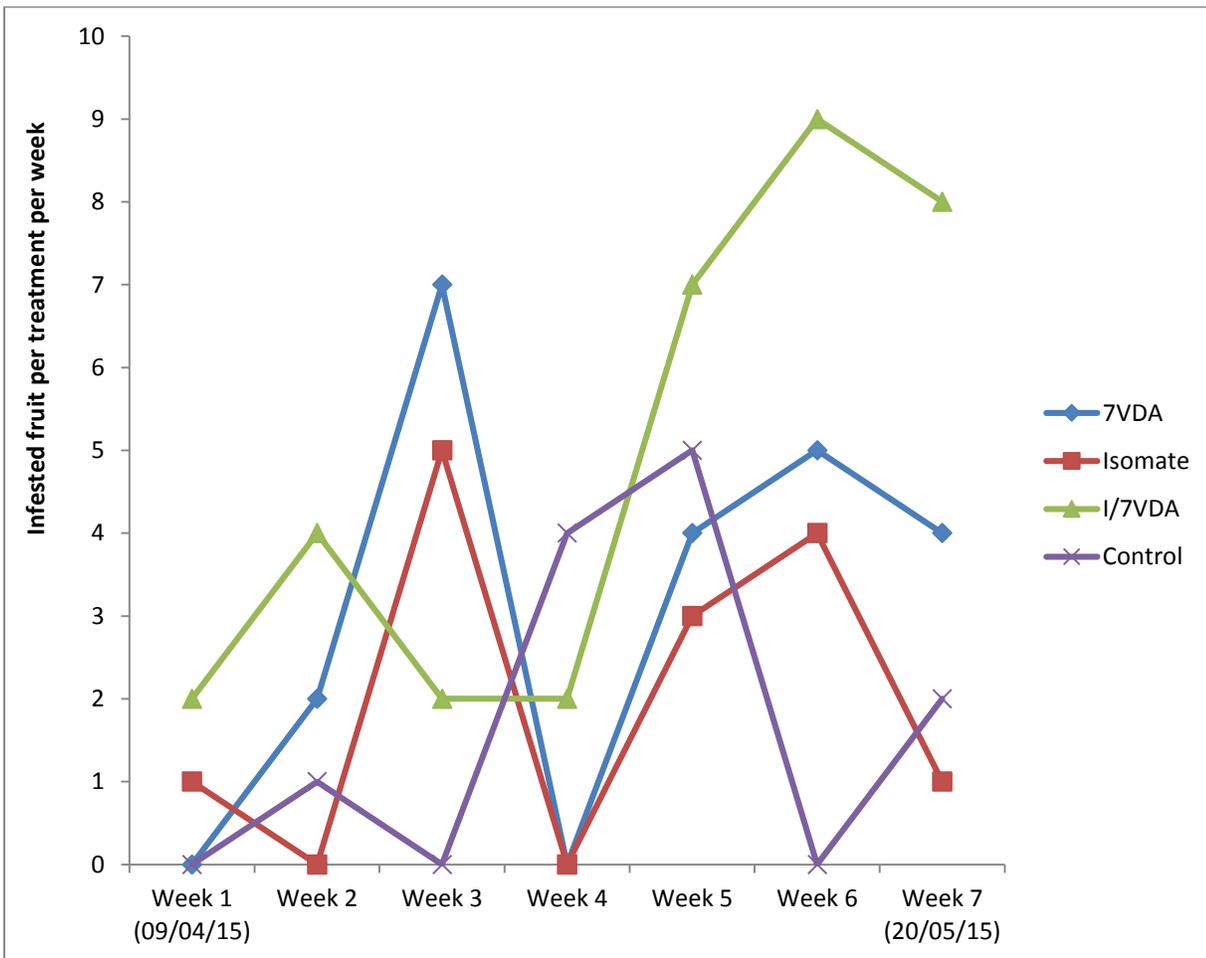


Figure 3.2.2.20. Fruit infested per week with FCM from caged trees for various treatments, monitored weekly for seven weeks from 9 April 2015.

Field trials
2018/19

A total of 300 moths were caught in the untreated control, 17 in the FCM pheromone treatment, 10 in the 7-VDA + FCM pheromone treatment and 20 in the Splat treatment. Infestation was monitored during January and February. Splat reduced infestation by 50%, FCM pheromone by 23% and 7-VDA + FCM pheromone not at all.

Conclusion

Laboratory and field cages trials indicated that mating disruption/inhibition was superior using FCM pheromone than 7-VDA. Consequently, combinations of 7-VDA and FCM pheromone were tested, demonstrating that 10% 7-VDA + 90% FCM pheromone was the most effective treatment at reducing mating, measured by fecundity and fertility of females. A suitable narrow-diameter polyethylene dispenser with the ability to release approximately 3 ug per dispenser per day, as required for field trials, was developed. A large-scale replicated field trial showed that the 7-VDA + FCM pheromone mixture was the most effective treatment at reducing trap catches of moths. However, fruit infestation results did not concur with trapping results.

Future Research

Although this project has now come to an end, if sufficient 7-VDA can be synthesized, the field trial will be repeated.

Technology Transfer

A poster was presented at the 2016 Citrus Research Symposium and at the International Chemical Ecology Society Congress in Budapest in 2018.

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3.2.3 FINAL REPORT: Potential of novel products, including a novel nucleopolyhedrovirus, for control of FCM

Project 1161 (April 2017 – March 2019) by Sean Moore, Wayne Kirkman, Martin Gilbert, Claire Love (CRI), Mellissa Peyper, Tammy Marsberg, Sonnica Albertyn and Michael Jukes (RU)

Summary

On 14 July 2017 FCM was declared a regulated pest by the EU. Consequently, it has become extremely important to develop and test a full suite of control options for FCM. Recently, a novel nucleopolyhedrovirus (NPV) was discovered infecting both litchi moth and FCM. As it appears that the litchi moth is the natural host, the virus has

been named the *Cryptophlebia peltastica* nucleopolyhedrovirus (CrpeNPV). Laboratory bioassays have indicated that the NPV has a relatively broad host range, being similarly virulent to FCM as to litchi moth. Consequently, nine field trials were conducted in total, eight of which included different concentrations of CrpeNPV. It was also applied in combination with the FCM granulovirus, CrleGV (Cryptogran), as laboratory bioassays indicated some synergism between these two viruses, particularly at a 3:1 ratio of CrpeNPV:CrleGV. Additionally, some experimental chemical and biological insecticides were included in trials on the request of the manufacturers or suppliers of the products. The novel virus, CrpeNPV, demonstrated potential for control of FCM in some field trials, as did Cryptogran "Dual Isolate". The experimental products were generally not as effective. However, at least one of them showed sufficient potential to warrant further testing. Some combinations of products showed promise in the field. Field conditions during one of the seasons were extremely challenging, with a high degree of fruit splitting, facilitating easier access into the fruit for FCM and thus elevating FCM levels in the orchards. Consequently, all of the trials were repeated the following season. Results generally remained inconclusive, as there was a high degree of variability, partly due to generally improved FCM control and thus generally lower levels of FCM. Further field trials against FCM will be conducted under a new project.

Opsomming

Op 14 Julie 2017 is VKM deur die EU as 'n gereguleerde plaag verklaar. Gevolglik het dit uiters belangrik geword om 'n volle reeks beheer opsies vir VKM te ontwikkel en te toets. Onlangs is 'n nuwe nukleopolihedrovirus (NPV) ontdek wat albei lietsjiemot en VKM besmet het. Omdat dit voorkom dat lietsjiemot die natuurlike gasheer is, is die virus die *Cryptophlebia peltastica* nukleopolihedrovirus (CrpeNPV) genoem. Laboratorium biotoetse het aangedui dat die NPV 'n relatiewe breë gasheer reeks het en sy virulensie teen VKM is omtrent gelyk aan die teen lietsjiemot. Gevolglik is in totaal nege veldproewe uitgevoer, waarvan agt verskillende konsentrasies van CrpeNPV ingesluit het. Dit is ook in kombinasie met die VKM granulovirus, CrleGV (Cryptogran), toegedien want laboratorium biotoetse het sinergisme tussen hierdie twee viruse aangedui, veral teen 'n verhouding van 3:1 van CrpeNPV:CrleGV. Daarbenewens is sekere eksperimentele chemiese en biologiese insekdoders in proewe ingesluit op versoek van die produk vervaardigers of verskaffers. Die nuwe virus, CrpeNPV, het goeie belofte vir beheer van VKM getoon in sekere veldproewe, en ook Cryptogran "Dubbele Isolaat". Die eksperimentele produkte is hoofsaaklik nie so doeltreffend nie, maar minstens een van hulle het genoegsame potensiaal getoon om verdere toetsing te regverdig. Sekere kombinasies van produkte het belowend gelyk. Omstandighede in die veld gedurende een van die seisoene was uiters uitdagend, met 'n hoë vlak van vrugbars wat maklike toegang vir VKM tot in die vrug fasiliteer het en dus VKM vlakke in die boorde verhoog het. As gevolg hiervan is al hierdie proewe gedurende die volgende seisoen herhaal. Resultate was oor die algemeen onoortuigend as gevolg van 'n hoë mate van variasie, gedeeltelik as gevolg van verbeterde VKM beheer en dus algemeen lae vlakke van VKM. In die toekoms sal veldproewe teen VKM onder 'n nuwe projek uitgevoer word.

Introduction

Recently, a novel nucleopolyhedrovirus (NPV) was discovered infecting both litchi moth, *Cryptophlebia peltastica* (Marsberg et al, 2016) and FCM (Jukes et al, 2016a). These discoveries emanated from an informal collaboration between CRI and the University of Gdansk and a separate litchi industry funded project. The NPV has now been fully genetically sequenced and named *Cryptophlebia peltastica* nucleopolyhedrovirus (CrpeNPV), as litchi moth is in all likelihood the homologous host. Despite this, laboratory bioassays have indicated that the NPV, unlike the FCM GV (CrleGV), has a relatively broad host range, being similarly virulent to FCM as to litchi moth (Marsberg et al., 2016). (Additionally, there is also significant virulence against codling moth). Preliminary field trials seem to support this observation (Chambers et al., 2016).

Consequently, CrpeNPV appears to have significant potential for use within an IPM programme against FCM. This could be of critical value for resistance management, as contrary to original expectations, the possibility of FCM developing resistance to CrleGV does exist. This is deduced from the extensive recording of resistance by codling moth to its homologous virus, CpGV, in European apple orchards (Fritsch et al. 2005; Jehle et al. 2006;

Asser-Kaiser et al. 2011). Additionally, due to CrpeNPV's apparently broader host range, it may even have some effect against other lepidopteran pests of citrus, such as carob moth, citrus flower moth and leafrollers.

Therefore, it was necessary to conduct field trials in order to measure absolute efficacy, relative efficacy (compared to existing FCM virus and chemical products) and to determine the appropriate dosage and formulation (eg addition of molasses) that should be used. Jukes et al (2016b) demonstrated that a combination of CrpeNPV and CrleGV resulted in a significantly improved virulence against FCM neonate larvae, due to a synergistic effect. The most promising ratio of the two viruses was also tested in the field. Simultaneously, other novel products and combinations of products were field tested against FCM.

Materials and methods

Seven field trials were conducted in the Eastern Cape and two in the Western Cape. All trials were laid out in a single tree randomised block format, replicated 10 times. Trial site details are given in Table 3.2.3.1.

Table 3.2.3.1. Details of trial sites

Province	Region	Farm	Orchard	Cultivar	Year planted	Spacing (rows x trees) (m)
Eastern Cape	Sundays River Valley	Far Away	54	Newhall Navel	2007	6 x 3
		Sackville	20	Lane Late Navel	2006	5.5 x 3
		Boerboon	22B	Powell Navels	2000	6 x 3
		Scheepersvlakte	N02	Palmer Navel	1998	5.8 x 2.5
		Sur Le Sun	10	Autumn Gold Navel	2003	6 x 4
		Mistkraal	56	Palmer Navels	1999	5.8 x 2.8
Western Cape	Clanwilliam	Kleinvlei	B4	Autumn Gold Navels	2002	5 x 3

All trials were applied using hand guns. All virus treatments were applied after 17h00 so as not to be immediately affected by UV-irradiation.

Treatments applied in each of the trials were as per Table 3.2.3.2. The stock concentration of both of the viruses, CrleGV (Cryptogran) and CrpeNPV was 5×10^{10} OBs/ml. Several experimental insecticides were tested for a couple of different commercial companies and these have been coded as VI, VM, three MBI codes and Combo 1 and 2. Molasses and BreakThru were used with most of the virus treatments at concentrations of 250 ml and 5 ml respectively per 100 L water. However, at Kleinvlei, two of the virus treatments was applied with BreakThru but not with molasses, as indicated in the table. The three MBI experimental treatments were applied with BreakThru, also at 5 ml per 100 L water.

Table 3.2.3.2. Treatment details for nine field trials

Trial site	Application date	Average L of spray mix per tree	Treatment	Concentration in 100 L water
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Far Away	6 and 7 December 2016	23.5	VI	20 g
			VI	25 g
			VI	30 g
			VI	60 g
			Cryptogran**	10 ml
			CrpeNPV**	10 ml
			Cryptogran + CrpeNPV**	7.5 ml + 2.5 ml (3:1)
			Cryptogran + CrpeNPV**	2.5 ml + 7.5 ml (1:3)
			Cryptogran**	1 ml
			CrpeNPV**	1 ml
			Cryptogran + CrpeNPV**	0.75 ml + 0.25 ml (3:1)
			Cryptogran + CrpeNPV**	0.25 ml + 0.75 ml (1:3)
Sackville	29 March 2017	19.5	VI	30 ml
			VM	60 ml
			VI + VM	30 ml + 60 ml
			VI + VM	15 ml + 30 ml
			Runner	60 ml
			MBI-206*	1.2 L
			MBI-203 DF*	200 g
			MBI-203 WDG*	200 g
			Cryptogran**	10 ml
			CrpeNPV**	10 ml
			Cryptogran + CrpeNPV**	0.25 ml + 0.75 ml
Kleinvlei	25 May 2017	12.2	CrpeNPV**	1 ml
			CrpeNPV*	1 ml
			CrpeNPV**	10 ml
			CrpeNPV*	10 ml
			Cryptogran**	10 ml
Boerboon	6 and 7 December 2017	18.2	Cryptogran **	10 ml
			NPV **	10 ml
			NPV **	1 ml
			Cryptogran + NPV **	2.5 ml + 7.5 ml
			Runner	60 ml
			Indoxacarb	30 g
			Indoxacarb	60 g
			Combo 1	30 g + 60 ml
			Combo 2	15 g + 30 ml
Scheepersvlakte	26 and 27 February 2018	18.8	Cryptogran **	10 ml
			NPV **	113.9 ml
			NPV **	11.39 ml
			Cryptogran + NPV **	2.5 ml + 85.4 ml
			MBI206 (Venerate) *	400 ml
			MBI206 (Venerate) *	800 ml
			MBI206 (Venerate) *	1200 ml

			MBI203 DF (Grandevo) *	150 g
			MBI203 DF (Grandevo) *	200 g
			MBI203 WDG (Grandevo) *	150 g
			MBI203 WDG (Grandevo) *	200 g
			Broadband *	50 ml
			EcoBb	10 g
			Cryptogran + Broadband **	10 ml + 50 ml
Sur Le Sun	28 May 2018	19.5	Cryptogran **	10 ml
			NPV **	10 ml
			NPV **	1 ml
			Cryptogran + NPV **	2.5 ml + 7.5 ml
			Methoxyfenozide	60 ml
			Combo 1	30 g + 60 ml
			Combo 2	15 g + 30 ml
			Chlorantraniliprole	10 g
			Chlorantraniliprole	17 g
			Chlorantraniliprole	34 g
			Emamectin benzoate	100 ml
Kleinvlei 2018	24 April 2018	16.0	CrpeNPV **	1 ml
			CrpeNPV **	10 ml
			Cryptogran + NPV ****	2.5 ml + 85.4 ml
			Cryptogran **	10 ml
Mistkraal 2018-9	13 December 2018	21.7	Cryptogran **	10 ml
			Cryptogran Dual Isolate**	10 ml
			CrpeNPV	3 ml
			CrpeNPV **	3 ml
			Cryptogran + NPV **	2.5 ml + 7.5 ml
			Cryptex	3.3 ml
			Cryptex***	3.3 ml
Mistkraal 2019	18 and 19 March 2019	24.4	Methoxyfenozide	60 ml
			Indoxacarb	30 g
			Indoxacarb	60 g
			Combo 1	30 g + 60 ml
			Combo 2	15 g + 30 ml
			Emamectin benzoate	100 ml
			Cryptogran **	10 ml
			Cryptogran + Broadband **	10 ml + 50 ml
			Broadband *	50 ml
			Eco-Bb	100 g

*Treatments which included BreakThru

**Treatments which included molasses and BreakThru

***Treatments which included molasses

Trials were evaluated by picking up the fallen fruit from under all trial trees on the same day each week and inspecting them (including dissecting them) to determine cause of drop. This was completed for all trial sites except Scheepersvlakte. This was initiated three weeks after spraying and was continued for several weeks, either until there was no longer any meaningful difference in FCM infestation between treatments or until harvest. FCM infestation at Scheepersvlakte was severely high thus an alternative evaluation was used whereby two people were given 30 seconds to scout and pick what looked like infested fruit off either side of the tree, this was repeated three times.

Results and discussion

Although the trial was evaluated for seven weeks, there was complete breakdown in the efficacy of all but one of the chemical treatments by the fourth week of evaluation. There was complete breakdown in the efficacy of the virus treatments by the sixth week of evaluation, but already a marked demise in efficacy of most of the treatments by the fifth week of evaluation. Consequently, the results for the first four weeks of evaluation have been tabulated (Table 3.2.3.3.). Efficacy of the experimental chemical treatments over this time ranged from 18-45% reduction in FCM infestation. On the whole, the virus treatments were more effective averaging between 27 and 59% efficacy, Cryptogran being the most effective treatment. Despite a combination of CrpeNPV and CrleGV at a ratio of 3:1 demonstrating some synergy (thus improved virulence) in laboratory trials (Jukes et al., 2017), this was not evident in the field trial. It must be stated that FCM infestation was extremely and unusually high, due mainly to climatic conditions, which caused a very high degree of fruit (navel-end) splitting. This appeared to not only attract FCM to the fruit, but provided much easier access for hatching larvae into the fruit, thus resulting in poor efficacy with most treatments.

Table 3.2.3.3. Comparative efficacy against FCM of the various treatments used in the trial at Far Away.

Treatment	Concentration in 100 L water	FCM infestation per tree per week*	Reduction in infestation (%)
Control		6.6a	-
VI	20 g	4.8a	27
VI	25 g	5.4a	18
VI	30 g	3.6b	46
VI	60 g	4.6a	30
Cryptogran	10 ml	2.7b	59
CrpeNPV	10 ml	3.8a	42
Cryptogran + CrpeNPV	7.5 ml + 2.5 ml (3:1)	3.7a	44
Cryptogran + CrpeNPV	2.5 ml + 7.5 ml (1:3)	3.3b	50
Cryptogran	1 ml	3.4b	49
CrpeNPV	1 ml	4.4a	33
Cryptogran + CrpeNPV	0.75 ml + 0.25 ml (3:1)	4.8a	27
Cryptogran + CrpeNPV	0.25 ml + 0.75 ml (1:3)	4.2a	36

*Values followed by the same letter are not significantly different ($P>0.05$).

The trial at Sackville was evaluated for a period of five weeks. For most of that period, most of the experimental products showed no efficacy at all against FCM (Table 4). Runner, which has consistently proved to be marginally the most effective chemical product against FCM in previous trials, was completely ineffective. However, Cryptogran and CrpeNPV remained relatively effective, still showing some efficacy after five weeks of evaluation. The possible reason for this peculiar result again may lie in the unprecedented level of fruit splitting. Moths would in all likelihood have laid their eggs immediately adjacent to the split on the fruit (Newton, 1989). Hatching larvae would not have had to wander around in search of a suitable penetration point, but would have entered at the split immediately adjacent to their egg, consequently dramatically reducing the chance of them becoming exposed to

a spray residue. On the other hand, the viruses were applied with molasses, which is a feeding attractant (Mwanza et al., 2016), potentially luring the larva to feed on the molasses coated virus and dying, rather than immediately penetrating the fruit.

Table 3.2.3.4. Comparative efficacy against FCM of the various treatments used in the trial at Sackville.

Treatment	Concentration in 100 L water	FCM infestation per tree per week	Reduction in infestation (%)
Control		1.2a*	-
VI	30 ml	1.7a	-42
VM	60 ml	1.6a	-33
VI + VM	30 ml + 60 ml	1.1a	8
VI + VM	15 ml + 30 ml	1.3a	-8
Runner	60 ml	2.0a	-67
MBI-206*	1.2 L	1.1a	8
MBI-203 DF*	200 g	1.2a	0
MBI-203 WDG*	200 g	1.4a	-17
Cryptogran**	10 ml	0.5a	58
CrpeNPV**	10 ml	0.6a	50
Cryptogran + CrpeNPV**	0.25 ml + 0.75 ml	1.4a	-17

*Values followed by the same letter are not significantly different (P>0.05).

The trial at Kleinvlei could only be evaluated for three weeks, as the fruit was harvested earlier than expected. Surprisingly, Cryptogran showed no efficacy during this time (Table 3.2.3.5). The higher concentration of CrpeNPV, applied with molasses, was the most effective treatment, reducing fruit infestation by 56%. However, it was peculiar that the lower concentration with molasses showed very little efficacy, even relative to the same concentration without molasses. This is probably simply the sort of variability that is unavoidable in biological trials.

Table 3.2.3.5. Comparative efficacy against FCM of the various treatments used in the trial at Kleinvlei.

Treatment	Concentration in 100 L water	FCM infestation per tree per week	Reduction in infestation (%)
Control		0.5	-
CrpeNPV**	1 ml	0.3	38
CrpeNPV*	1 ml	0.3	38
CrpeNPV**	10 ml	0.5	6
CrpeNPV*	10 ml	0.2	56
Cryptogran**	10 ml	0.6	-13

The trial at Boerboon was evaluated for eight weeks. The most effective treatments during this trial was Combo 1 and Combo 2, followed by Runner (Table 3.2.3.6), which all reduced infestation significantly. Cryptogran was also found to be relatively effective. The lower dose of CrpeNPV was found to be more effective than the higher dose. The combination of Cryptogran and NPV was not very effective.

Table 3.2.3.6. Comparative efficacy against FCM of the various treatments used in the trial at Boerboon.

Treatment	Concentration in 100 L water	FCM infestation per tree per week	Reduction in infestation (%)
Control	-	0.19a*	-

Cryptogran **	10 ml	0.09a	53.3
NPV **	10 ml	0.23a	-20.0
NPV **	1 ml	0.16a	13.3
Cryptogran + NPV **	2.5 ml + 7.5 ml	0.18a	6.7
Runner	60 ml	0.05b	73.3
Indoxacarb	30 g	0.14a	26.7
Indoxacarb	60 g	0.14a	26.7
Combo 1	30 g + 60 ml	0.03b	86.7
Combo 2	15 g + 30 ml	0.03b	86.7

*Values followed by the same letter are not significantly different (P>0.05).

Scheepersvlakte was evaluated using the same method as for all spray trials (collecting dropped fruit) for three weeks. However, FCM infestation and fruit splitting was incredibly high during this time thus an alternative method (tree scouting) was used to evaluate the fruit for two weeks before the orchard was harvested (Table 3.2.3.4 and 3.2.3.5). Results obtained from the first three-week evaluation show all treatments to be relatively ineffective (Table 3.2.3.7). Results obtained from the second evaluation were slightly better than the first evaluation (Table 3.2.3.5). The higher dose of NPV was found to be the most effective treatment with a 50 % reduction in FCM. The Cryptogran and NPV combination was also effective when compared to the high dose NPV results. The lower dose of NPV and EcoBB was completely ineffective during this trial. The abnormal results obtained in this trial could be explained by the high number of split fruit found in the orchard. This could have resulted in FCM entering the fruit before been exposed to the treatments for the ideal amount of time.

Table 3.2.3.7. Comparative efficacy against FCM of the various treatments used in the trial at Scheepersvlakte (Evaluation method: picking up dropped fruit for 3 weeks).

Treatment	Concentration in 100 L water	FCM infestation per tree per week	Reduction in infestation (%)
Control	-	2.17a*	-
Cryptogran **	10 ml	2.20a	-2
NPV **	113.9 ml	2.17a	0
NPV **	11.39 ml	2.10a	3
Cryptogran + NPV **	2.5 ml + 85.4 ml	1.57a	28
MBI206 (Venerate) *	400 ml	2.00a	8
MBI206 (Venerate) *	800 ml	2.20a	-2
MBI206 (Venerate) *	1200 ml	2.27a	-5
MBI203 DF (Grandevo) *	150 g	1.97a	9
MBI203 DF (Grandevo) *	200 g	2.60a	-20
MBI203 WDG (Grandevo) *	150 g	1.93a	11
MBI203 WDG (Grandevo) *	200 g	1.80a	17
Broadband *	50 ml	1.40a	35
EcoBb	10 g	2.80a	-29
Cryptogran + Broadband **	10 ml + 50 ml	2.13a	2

*Values followed by the same letter are not significantly different (P>0.05).

Table 3.2.3.8. Comparative efficacy against FCM of the various treatments used in the trial at Scheepersvlakte (Evaluation method: 30 second scouting and picking fruit).

Treatment	Concentration in 100 L water	FCM infestation per tree per week	Reduction in infestation (%)
Control	-	2.3a	-

Cryptogran **	10 ml	1.85a	20
NPV **	113.9 ml	1.15b	50
NPV **	11.39 ml	2.7a	-17
Cryptogran + NPV **	2.5 ml + 85.4 ml	1.35b	41
MBI206 (Venerate) *	400 ml	2.25a	2
MBI206 (Venerate) *	800 ml	2.05a	11
MBI206 (Venerate) *	1200 ml	2.1a	9
MBI203 DF (Grandevo) *	150 g	1.4b	39
MBI203 DF (Grandevo) *	200 g	1.65a	28
MBI203 WDG (Grandevo) *	150 g	2.1a	9
MBI203 WDG (Grandevo) *	200 g	1.55a	33
Broadband *	50 ml	1.75a	24
EcoBb	10 g	2.45a	-7
Cryptogran + Broadband **	10 ml + 50 ml	1.5a	35

*Values followed by the same letter are not significantly different (P>0.05).

The trial at Sur le Sun could only be evaluated for two weeks, as the fruit was harvested earlier than expected. All products except for Combo 1 and Combo 2 and emamectin benzoate showed no efficacy (Table 3.2.3.9).

Table 3.2.3.9. Comparative efficacy against FCM of the various treatments used in the trial at Sur le Sun.

Treatment	Concentration in 100 L water	FCM infestation per tree per week	Reduction in infestation (%)
Control	-	0.5a*	-
Cryptogran **	10 ml	0.65a	-30
NPV **	10 ml	0.6a	-20
NPV **	1 ml	0.55a	-10
Cryptogran + NPV **	2.5 ml + 7.5 ml	0.65a	-30
Methoxyfenozide	60 ml	0.65a	-30
Indoxacarb + methoxyfenozide	30 g + 60 ml	0.35a	30
Indoxacarb + methoxyfenozide	15 g + 30 ml	0.35a	30
Chlorantraniliprole	10 g	0.8a	-60
Chlorantraniliprole	17 g	0.8a	-60
Chlorantraniliprole	34 g	0.7a	-40
Emamectin benzoate	100 ml	0.4a	20

*Values followed by the same letter are not significantly different (P>0.05).

The trial at Kleinvlei was evaluated for 7 weeks. Cryptogran was the only product that reduced FCM infestation (Table 3.2.3.10). Both NPV doses and the NPV:GV mix were ineffective at reducing the FCM.

Table 3.2.3.10. Comparative efficacy against FCM of the various treatments used in the trial at Kleinvlei (2018).

Treatment	Concentration in 100 L water	FCM infestation per tree per week	Reduction in infestation (%)
Control	-	0.29	-
CrpeNPV **	1 ml	0.30	-5
CrpeNPV **	10 ml	0.30	-5

Cryptogran + NPV ****	2.5 ml + 85.4 ml	0.30	-5
Cryptogran **	10 ml	0.21	25

The first trial at Mistkraal was evaluated for 5 weeks. Cryptogran, Cryptogran Dual Isolate and CrpeNPV with molasses and Breakthru were the only treatments that reduced FCM infestation (Table 3.2.3.11), but the differences were not significant.

Table 3.2.3.11. Comparative efficacy against FCM of the various treatments used in the trial at Mistkraal 2018-9.

Treatment	Concentration in 100 L water	FCM infestation per tree per week	Reduction in infestation (%)
Control	-	0.18a*	-
Cryptogran **	10 ml	0.14a	22
Cryptogran Dual Isolate**	10 ml	0.08a	56
CrpeNPV	3 ml	0.18a	0
CrpeNPV **	3 ml	0.14a	22
Cryptogran + NPV **	2.5 ml + 7.5 ml	0.28a	-56
Cryptex	3.3 ml	0.24a	-33
Cryptex***	3.3 ml	0.22a	-22

*Values followed by the same letter are not significantly different (P>0.05).

The first second at Mistkraal was evaluated for 4 weeks. Indoxacarb, Emamectin benzoate and Broadband were the only treatments that reduced FCM infestation (Table 3.2.3.12), but the differences were not significant.

Table 3.2.3.12. Comparative efficacy against FCM of the various treatments used in the trial at Mistkraal 2019.

Treatment	Concentration in 100 L water	FCM infestation per tree per week	Reduction in infestation (%)
Control	-	0.75a*	-
Methoxyfenozide	60 ml	0.78a	-3
Indoxacarb	30 g	0.70a	7
Indoxacarb	60 g	0.68a	10
Combo 1	30 g + 60 ml	0.90a	-20
Combo 2	15 g + 30 ml	1.10a	-47
Emamectin benzoate	100 ml	0.45a	40
Cryptogran **	10 ml	1.13a	-50
Cryptogran + Broadband **	10 ml + 50 ml	0.88a	-17
Broadband *	50 ml	0.68a	10
EcoBb	100 g	0.83a	-10

*Values followed by the same letter are not significantly different (P>0.05).

Conclusion

The novel virus, CrpeNPV, demonstrated potential in the field for control of FCM. However variable results were observed for virus, fungal, chemical and experimental products. In the majority of the field trials, the virus products produced slightly better results than the experimental products, other than the experimental combinations, which appeared very promising. Field conditions were challenging, thus producing variable and inconclusive results. Consequently, no final conclusions can be drawn on any of the products, even those that appeared to be ineffective. Although this project is now terminated, further such field trials will be conducted within a new approved project.

Future Research

This project is now terminated. Further work on field trials with various products for FCM control will be conducted within a new project, no. 1225.

Technology Transfer

None.

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3.2.4 FINAL REPORT: Novel approaches to mating disruption of FCM

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

Summary

False codling moth (FCM) population pressure varies between different farms in the Citrusdal and Clanwilliam areas. Factors which may hinder the successful application of SIT e.g. mountainous terrain and proximity to abandoned citrus orchards, should be considered when deciding which FCM control programme to follow. Within the Citrusdal Sterile Insect Technique (SIT) area, on farms where FCM is a particularly severe pest, this project showed that the use of additional chemical mating disruption products such as Isomate and Checkmate can play a valuable role in the further reduction of fruit losses due to this pest. Aerial applications of Checkmate significantly reduced fruit fall due to FCM. This combined approach of SIT and chemical mating disruption can be recommended on farms with a particular problem with FCM. The artificial manufacturing process for FCM-pheromone was also investigated, but it was found that this would not be an economic proposition at present usage levels. Nevertheless, the chemical processes involved in production were researched and summarized for possible future use. The application of increased dosages of mating disruption products beyond present registration levels could not be shown to be advantageous in terms of reducing fruit-drop although reduced moth captures in traps did result. New mating disruption products such as Splat and X-Mate were tested against Isomate. However, FCM levels at the experimental site were too low for comparisons in terms of fruit fall. Isomate blocks did, however, show the lowest levels of moth activity when measured by pheromone traps. X-Mate tended to show the most moth activity perhaps due to the relatively low numbers of units placed out per hectare. Further work in connection with this should be considered.

Opsomming

Valskodlingmot (VKM) populasie druk wissel tussen verskillende plase in die Citrusdal en Clanwilliam gebiede. Faktore wat die suksesvolle toepasing van die SIT tegniek negatief beïnvloed sluit bergagtige terrein en ou verlate sitrusboorde in, en moet in ag geneem word wanneer daar besluit word oor watter VKM beheer program te volg. Binne die Citrusdal Steriele Insek Tegniek (SIT) area, op plase waar VKM 'n ernstige plaag bly, het die projek gewys dit aanvulende chemiese paring ontwrigting produkte by voorveeld Isomate en Checkmate kan 'n waardevolle rol speel in die verlaging van vrug verliese. Lugtoedienings van Checkmate het 'n betekenisvolle vermindering van vrugverliese as gevolg van VKM veroorsaak. Hierdie gekombineerde benadering van SIT en chemiese paringsontwrigting kan op plase met 'n ernstige VKM probleem aanbeveel word. Die kunsmatige vervaardiging van VKM feromoon is ook nagegaan, maar dit was gevind dat dit nie ekonomies lewensvatbaar is as gevolg van lae volumes wat benodig sou word. Nogtans, is die betrokke chemiese prosesse nagegaan en opgesom vir moontlike toekomstige gebruik. Die toediening van verhoogde dosise van paringsontwrigting produkte, verby huidige registrasie vlakke, kon nie as 'n voordelige praktyk in terme van verminderde vrug verlies beskou word nie, alhoewel verminderde mot tellings het in lokvalle voorgekom. Nuwe paringsontwrigting produkte soos byvoorbeeld Splat en X-Mate, was teen Isomate gemeet. Nogtans was VKM getalle by die eksperimentele blokke te laag om vergelykings in terme van vrug verlies te meet. Isomate behandelde blokke het die laagste mot aktiwiteit getoon. X-Mate behandelde blokke was geneig om die meeste mot aktiwiteit te wys miskien as gevolg van die relatief lae hoeveelheid eenhede wat aanbeveel is per hektaar. Verdere werk in hierdie verband moet oorweeg word.

Introduction

False codling moth (FCM), *Thaumatotibia leucotreta* Meyrick, is a serious phytosanitary pest of citrus in South Africa. With respect to this insect, the regulations regarding the export of fruit, particularly to the EU and USA, will become more problematical in the near future. A number of different methods are employed to combat *T. leucotreta*, e.g. orchard sanitation, mating disruption (MD), sterile insect technique (SIT) and chemical control, but none of these methods on their own are able to bring about complete control of the pest. Mating disruption is an established method of control for FCM (Moore & Kirkman 2012) and other moths (Sanders 1997, Weakley *et al.* 1987). A number of different chemical products have been registered, including pheromones, and the Sterile Insect Technique is being applied successfully against FCM (Boersma *et al.* 2017, Hofmeyr *et al.* 2015, Schoeman & de

Beer 2008). At present, the cost of mating disruption chemicals is very high. Registered products are slotted into the market so as to remain affordable and not necessarily for maximum effect. Registered and experimental chemicals have been included in the trial as well as aerial applications of Checkmate.

Stated objectives

The aim of this project is to investigate FCM control by the increased use of mating disruption (MD) products beyond levels that are presently registered. Another aspect of this is the use of mating disruption products in combination with Sterile Insect Technique (SIT). Both of these aspects, if proved to have merit, would make the possibility of local FCM pheromone synthesis more viable by increasing the potential demand for the pheromone.

- To investigate whether increased applications of FCM mating disruption products will boost the degree of efficacy significantly. The evaluation of new and existing MD products is also included in the study.
- If control is improved, then ways of lowering production costs by, for example, mass chemical synthesis would be investigated (Moore & Kirkman 2012).

Materials and methods

Season 2013/14

A suitable site was identified in the Citrusdal area for a comparison of a new River Bioscience (RB) mating disruption (MD) product. A single application of the River Bioscience product was applied using special machinery supplied by RB for the trial. Due to the extremely low levels of FCM in the Citrusdal area, and the poor application machinery it was decided not to apply another treatment.

Season 2014/15

During 2014 / 15 two farms, in the Citrusdal area, that had been identified by Xsit as hotspots for FCM, were chosen as experimental sites for the application of mating disruption products additional to the release of sterile FCM carried out routinely by Xsit during the spring to autumn months. Clearly the release of sterile FCM alone was not sufficient to bring about satisfactory control of FCM on these two farms hence their perennial identification as hotspots for the pest. In the case of Bo-Berg Vlei, mountainous terrain made release of sterile FCM quite hazardous for the applicator and may have contributed to a relatively poor FCM control in relation to many farms at lower altitude within the central Citrusdal valley. Vrede farm, although located at lower altitude, is adjacent to an old citrus research station where there are a number of orchards that are untended. These orchards therefore can serve as a source of pests including FCM.

At Bo-Bergvlei farm, (32° 27' 53.63" S, 18° 47' 52.15" E) the experimental FCM control programme was to consist of two applications of Isomate, eight weeks apart, as per registration. At Vrede farm, (32° 32' 55.35" S, 18° 58' 45.39" E) the efficacy of Checkmate^R (Chempac Pty.) applied by air was to be tested as an additional control measure and compared to the routine Xsit aerial sterile release programme.

At both farms aerial releases of sterile FCM were carried out as usual by Xsit as part of their commercial programme. At Bo-Bergvlei one half of a 4 ha block of Washington navels also received two applications of Isomate as per registration. The first application was in mid-November and the second was in mid-January @ 400 units per ha.

At Vrede, four applications of Checkmate were applied by means of an Xsit autogyro at 132 ml per ha at 4-weekly intervals, weather permitting. At both farms, FCM yellow delta traps (Chempac, Symondium) were placed out in

the treated and untreated (SIT only) blocks and monitored weekly. In addition, 10 trees per treatment (& controls) were marked and all fallen fruit were collected weekly and examined for the presence of FCM.

Season 2015/16

During 2015/16 work continued at Bo-Bergvlei in the Citrusdal area as this is an FCM hotspot. At this farm one half (2 ha) of a 4 ha block of Washington navels received two applications of Isomate as per registration. This was followed up by three applications of Checkmate at 150 ml per ha. One hectare received a single Isomate at the end of January. A further hectare received only SIT treatments. Weekly aerial releases of sterile FCM were carried out as usual over the whole block by XSIT as part of their commercial programme. The experimental navel block is surrounded by rocky outcrops and so is difficult for aerial releases of sterile FCM. This was particularly so as releases by autogyro were no longer permitted. The fixed-wing aircraft in use at the time would have found it more difficult to turn within the limited airspace between the mountainous terrain. Nevertheless, the conditions were the same for all treatments and so a valid comparison could be made. Yellow delta traps loaded with Chempac FCM pheromone were placed out in the treated and untreated (SIT only) blocks and monitored weekly. In addition, 10 trees per treatment (including SIT control) were marked and all fallen fruit were collected weekly and examined for the presence of FCM.

Season 2016/17

In 2016/17, the quantity of Isomate needed for use in this trial was unavailable 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 with the same chemical composition albeit in different forms / concentrations. Three treatments were used: a 1x treatment (110 ml Checkmate per hectare per application), a 3x treatment (330 ml Checkmate per hectare per application) 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.

Season 2017/18

A trial site (Rietvlei farm, 32° 06' 49.45" S, 18° 51' 08.35 E) with reportedly high FCM population levels was selected in Clanwilliam for the 2017/18 season to compare X-Mate, Isomate and Splat, along with an untreated control block. Each mating disruption product was applied to Midknights, Bahianinha Navels and Cambria Navels, with treatment blocks being at least 1 ha in size. Splat was applied three times during the season, at the end of November 2017, toward the end of February 2018 and towards the end of May 2018. Isomate was applied twice as per the registration, first in mid-December 2017 and the second in mid-February. X-mate was applied once as per registration in mid-December 2017. Trap monitoring was initiated from 13 December 2017 with yellow Delta traps and Chempac FCM pheromone lures. Monitoring was done every second week initially, but increased to every week from May until the beginning of June, after which monitoring frequency returned to every second week. This farm was part of the XSIT sterile FCM release programme, therefore both wild and sterile FCM males were monitored. Data stations of five trees in each treatment block were marked out in February 2018, with fruit infestation being monitored.

Results and discussion

Season 2013/14

FCM pressure in the Citrusdal area was extremely low with no meaningful moth catches or fruit drop occurring. In the authors' opinion a more even application of the River Bioscience material was needed.

Season 2014/15

At Bo-Bergvlei from early January onwards, no wild FCM were caught in traps where Isomate + SIT were applied. In the control block, sporadic catches of wild FCM still occurred after early January although at low levels, (Figures 3.2.4.1 and 2).

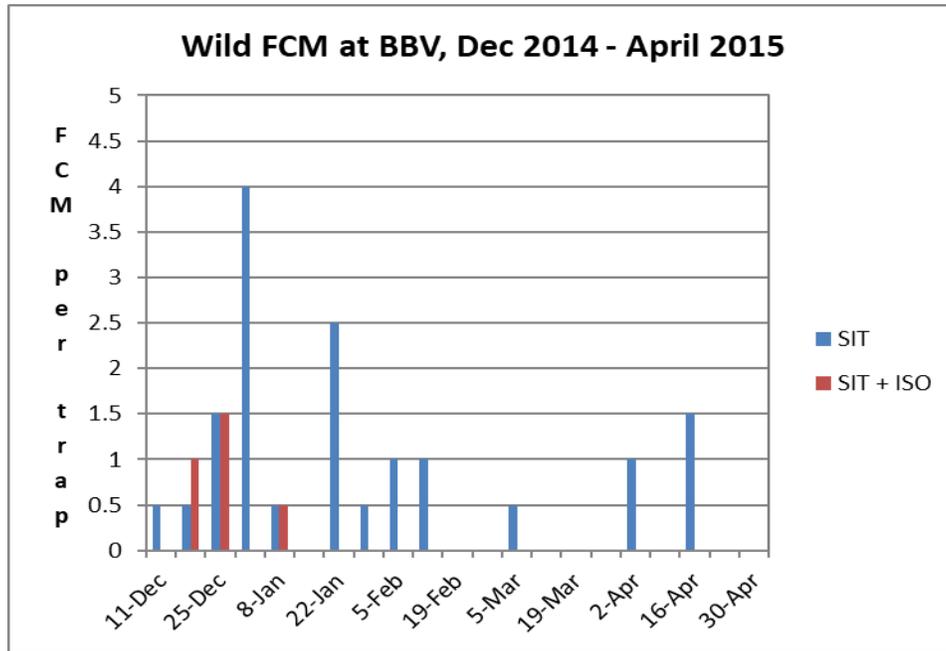


Figure 3.2.4.1. Wild FCM caught in yellow delta traps at Bo Berg Vlei Farm, Citrusdal: December 2014 to April 2015. Control block = SIT only. Isomate block = SIT + 2 x Isomate applications.

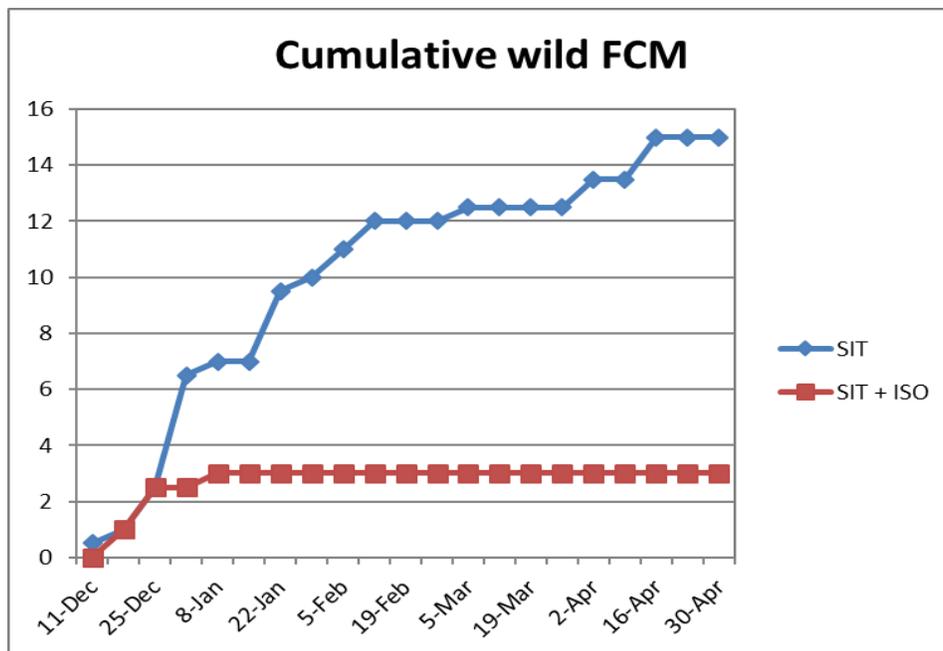


Figure 3.2.4.2. Cumulative catches of wild FCM at Bo-Berg Vlei farm, Citrusdal: December 2014 to April 2015.

With regard to fruit infestation, at Bo-Berg Vlei, 12 extra fruit per tree were lost due to FCM in the control block (SIT only) as compared to the SIT + Isomate block (Figure 3.2.4.3).

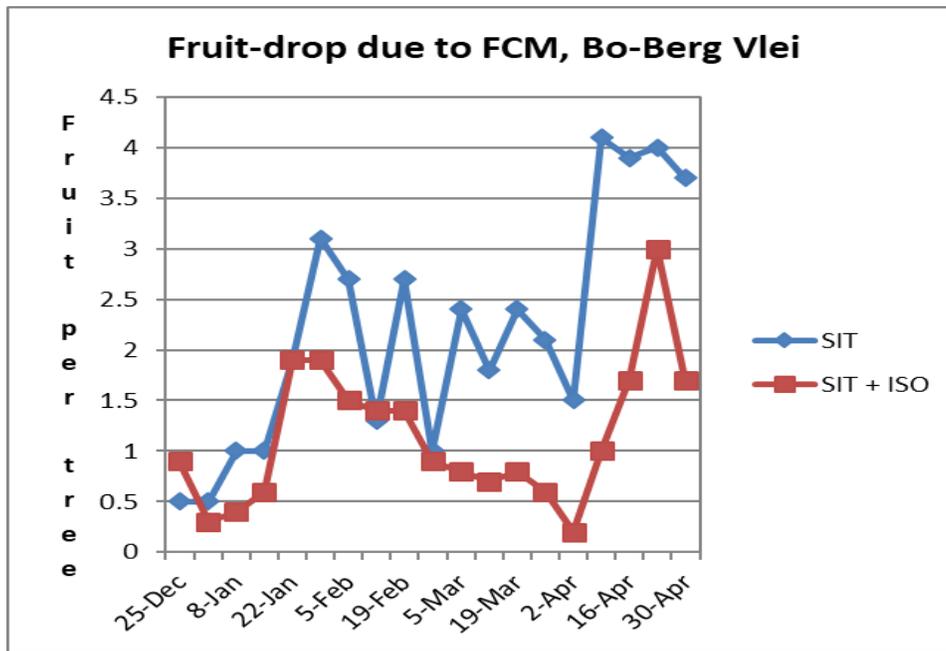


Figure 3.2.4.3. Fruit-drop due to FCM at Bo-Berg Vlei, December 2014 to April 2015

At Vrede, during January and February, fruit loss due to FCM was lower than at Bo-bergvlei. Nevertheless, the Checkmate block lost a total of 3.5 fewer fruit per tree to FCM during these two months, (Figure 3.2.4.4).

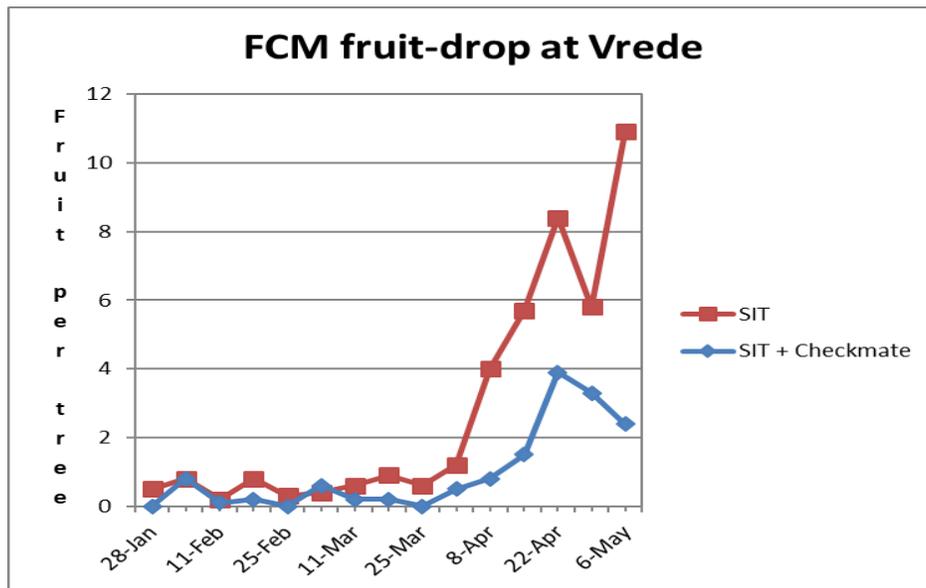


Figure 3.2.4.4. Fruit-drop due to FCM at Vrede, January to May 2015.

During April 2015, at both farms, fruit loss due to FCM increased considerably. By the middle of April, fruit loss during the previous 3-month period in control blocks (SIT only) at Bo-Bergvlei had accumulated to nearly 18 extra fruit per tree and, at Vrede, was 11 extra fruit per tree, in comparison to the SIT + mating disruption blocks.

Mating disruption products can therefore clearly enhance the results of SIT and improve the overall control of FCM particularly in orchards / on farms with high moth numbers. Experiments will also be done in the future on farms that are not included in the XSIT programme.

As regards the local synthesis of FCM pheromone, research was not encouraging due to too small a volume being estimated as being required by the industry. However, contact was made with an experienced organic chemist, Dr. Hennie Jordaan of Imagichem in the Eastern Cape who researched suitable chemical pathways by way of which the pheromone could be synthesised. See Appendix A for the report compiled by Dr. Jordaan in July 2015, at no cost to CRI. This report may be of use in future research.

Season 2015/16

Despite the multiple extra MD treatments, under these high pressure conditions, fruit loss could not be reduced to below 0.7 per fruit per tree per week by late April (with a mean of 1.9 fruit per tree per week). Indeed, there was very little difference in fruit loss between the single Isomate applied at end of January and the treatment that included 2 x Isomate + 3 Checkmates. It was only in the final two weeks that fruit loss in the multiple treatment block started to be significantly less than that in the 1 x Isomate block. In this experiment, it was not possible to drive fruit loss down to very low levels (<0.5 fruit per tree per week). This might be possible under conditions of lower FCM pressure. Under these high FCM conditions, total control of FCM could not be achieved even by the excessive amount of MD products that were applied.

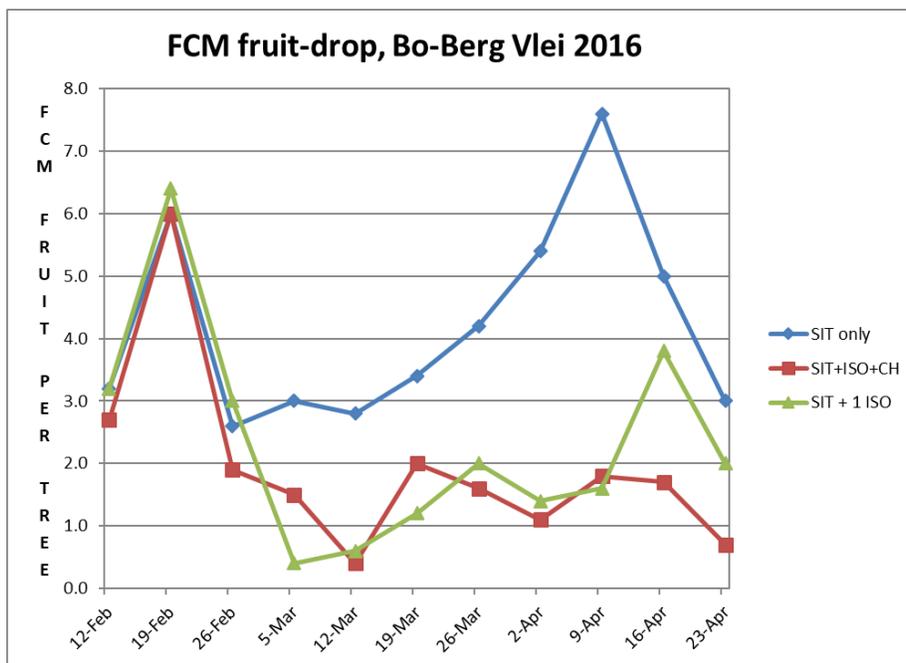


Figure 3.2.4.5. Fruit-drop due to FCM at Bo-Berg Vlei, February to April 2016, when comparing three different mating disruption programmes.

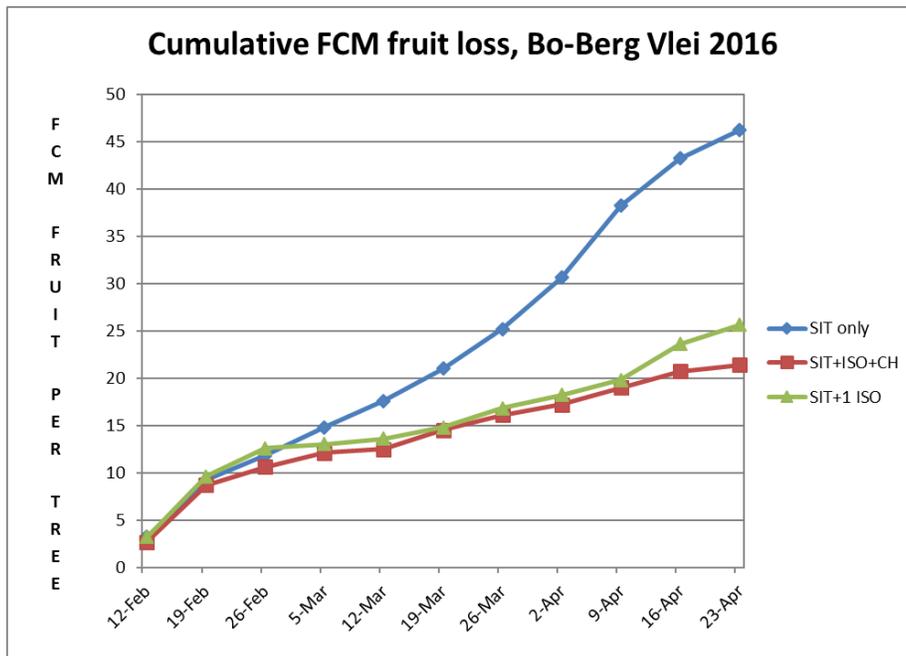


Figure 3.2.4.6. Cumulative fruit-drop due to FCM at Bo-Berg Vlei, February to April 2016, when comparing three different mating disruption programmes.

Season 2016/17

For the sterile moths, the trap catches in the 1x and control treatment followed a similar trend and 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 (Figure 3.2.4.7). This same trend of the 3x treatment trapping the fewest 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 (Figure 3.2.4.8).

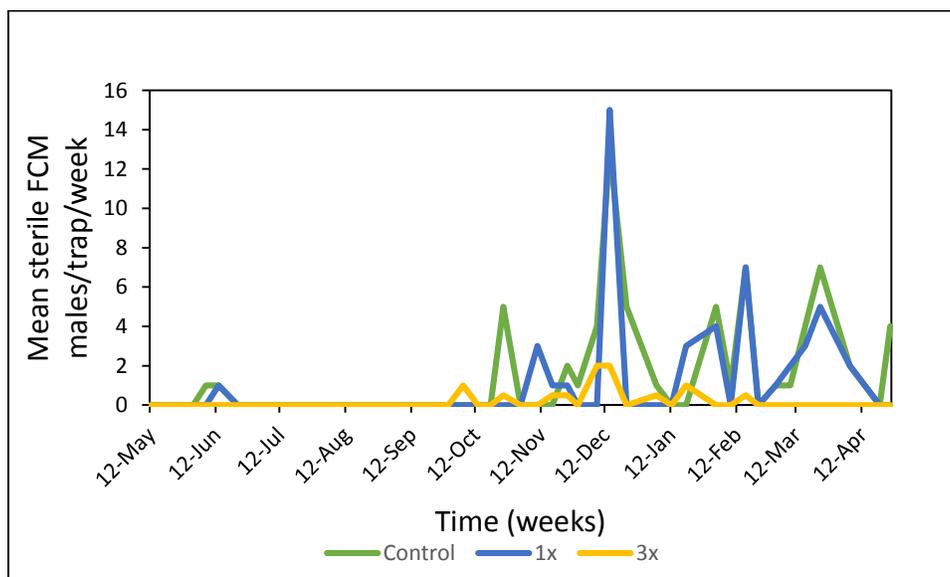


Figure 3.2.4.7. Mean number of sterile FCM caught per trap per week in Citrusdal in citrus treated with Checkmate from May 2016 – April 2017

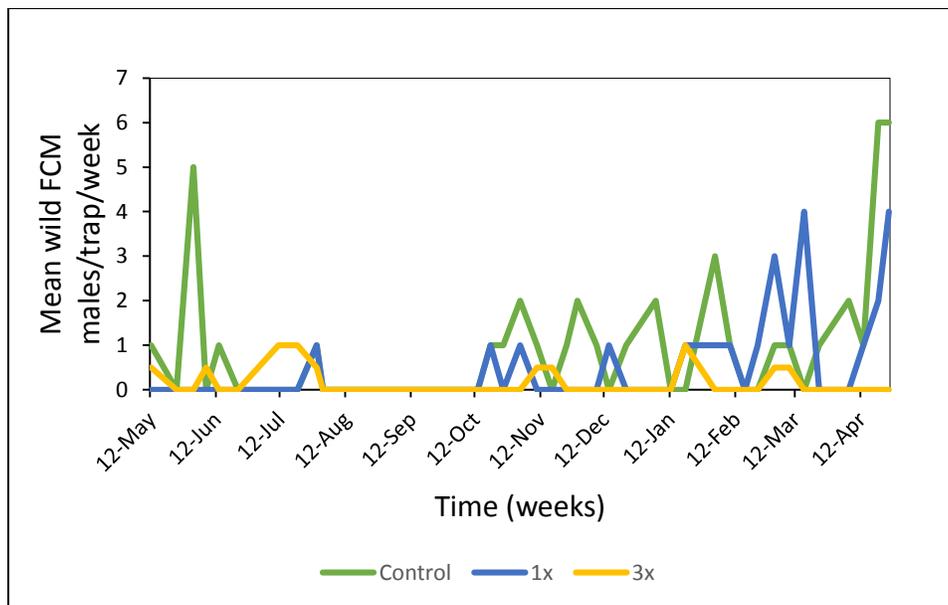


Figure 3.2.4.8. Mean number of wild FCM caught per trap per week in Citrusdal in citrus treated with Checkmate from May 2016 – April 2017

This trend of progressive reduction of moth catches with increasing dosage of Checkmate did not persist into the fruit infestation part of the experiment, which was carried out 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 infestation per tree per week did not show clear differences between treatments and fruit infestation was not noticeably lower in the 3x treatment (Figure 3.2.4.9).

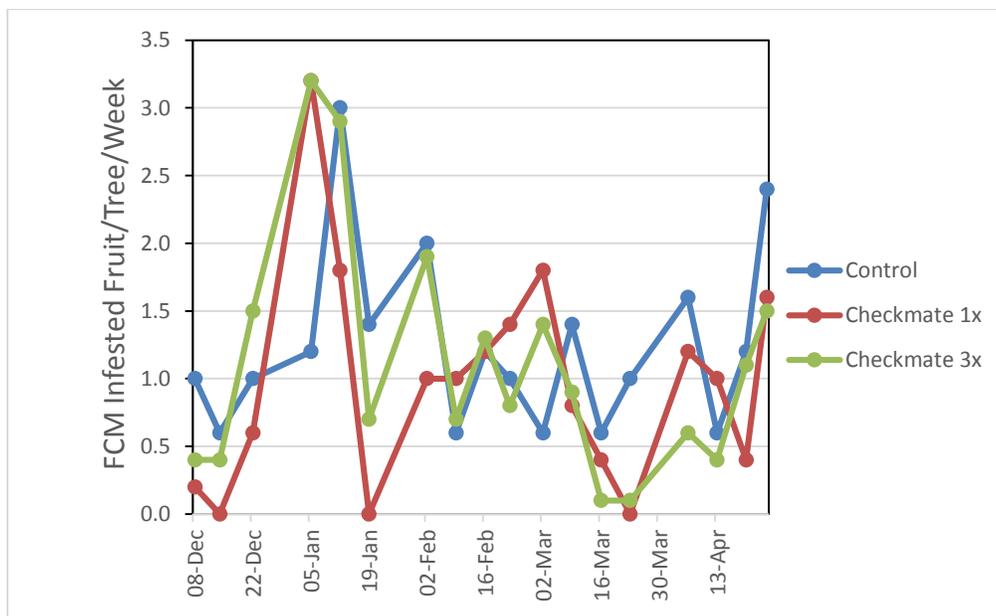


Figure 3.2.4.9. Weekly comparison of FCM infested fruit from citrus blocks at Citrusdal treated with Checkmate 3x dosage, Checkmate 1x dosage and control (untreated).

Season 2017/18

In the Midnight orchard, sterile FCM trap catches were generally higher for the untreated control block than the blocks treated with mating disruption. Isomate was the most consistent of the three mating disruption products applied, with an average of one moth/trap/week being recorded in the first week, followed by zero trap catch for the remainder of the experiment. Splat did show a small increase in sterile moth catches to an average of 2/trap/week in early February, but this may be due to reduced efficacy as the second Splat application was scheduled for the end of February. The X-Mate treated block had the highest sterile FCM trap catch of the three mating disruption treated blocks, but trap catch was generally lower here than the control block. Wild FCM trap catch was very low in the Midnights, with a single wild male being caught in the X-Mate block in mid-January and a single wild male being caught in the control trap at the end of March.

The Bahianinha Navels showed the highest amount of sterile FCM activity, with the control block recording a peak of 23 moths/trap/week at the end of March. The X-Mate trap once again recorded fewer sterile FCM trap catches than the control treatment, but more than both Splat and Isomate. Sterile moth activity was higher in the Splat-treated Bahianinha orchard than had been observed in the Midnight orchard. The lowest trap catch was recorded in the Isomate-treated block and this was the case throughout the experiment. Wild FCM trap catches were once again very low, never exceeding an average of 0.5 moths/trap/week and were only caught in the X-Mate traps.

The Cambria orchard was the youngest of all the trees and showed the lowest sterile and wild trap catches of all three cultivars. In this case, the untreated control block did not always show higher trap catch than the mating disruption treated blocks, with both Splat and X-Mate traps catching higher numbers of sterile moths than the control trap during the experiment. However, the young tree age and the low sterile trap catches overall, which never exceeded an average of 1.5 moths/trap/week may explain the lack of clear trends in the Cambria orchards. The Isomate treatment was the lowest of all three mating disruption blocks, with no sterile FCM being trapped throughout the duration of the experiment. The wild FCM trap catch was also the lowest of the three cultivars, with only a single wild FCM male being trapped in the X-Mate treated block in early February.

Although fruit infestation was monitored from the end of February 2018, no infested fruit was found. The low wild FCM trap catch data does provide some support for this lack of infestation and it appears that this was a low activity year for FCM in these orchards as high FCM population levels have previously been observed on this farm.

Conclusion

False Codling Moth population density varies considerably between different farms in the Citrusdal and Clanwilliam area. Experience gained during this project highlighted some perennial hotspots for FCM activity which would benefit from a more vigorous approach to control of the pest. It is unfair to expect SIT alone to be equally effective on farms which have a particular disadvantage. In the case of Bo-Berg Vlei, mountainous terrain made the aerial release of sterile FCM hazardous for the operator when autogyro or fixed-wing aircraft were being used. Nowadays helicopters are used for sterile releases which will improve the distribution of released moths in problematic areas. In the case of Vrede farm, the proximity of adjacent abandoned citrus orchards led to an increased FCM problem in the commercial blocks.

Under these circumstances it was shown that a combination of SIT releases combined with application of chemical mating disruption products could significantly reduce the amount of fruit lost to FCM. This approach should be recommended on farms with perennially high levels of FCM damage.

The local mass production of FCM pheromone was not seen to be economically viable at the present time due to the relatively small amount that the industry would require and the high cost of production. Nevertheless, the chemical pathways involved in production were elucidated and are summarized in the appended report (A) should further research need to be done in the future.

The use of increased dosages beyond registration of mating disruption products did lead to further suppression of moth activity as measured in pheromone traps in comparison to the registered dosage. However, benefits of this approach were not seen when fruit fall was measured and so such an approach has little to support it at this time.

Various new mating disruption products are becoming available e.g. Splat and X-Mate. A comparative trial with Isomate was carried out. Isomate was the most successful in suppressing FCM flight activity but low pest pressure prevented meaningful results when measuring fruit fall.

Future research

Further work needs to be done on the relative efficacy of the new mating disruption materials that are becoming available especially considering the vastly differing number of units that are recommended to be applied per hectare (as few as 40 in the case of X-mate).

Technology transfer

Talk presented at Hortgro FCM seminar, Stellenbosch, October 2016.

Poster presented at ESSA Congress, University of Pretoria, 2-7 July, 2017.

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

Project 1060 (2013/4, 2015/6-2018/9) by T G Grout, P R Stephen and K C Stoltz (CRI)

Summary

This project was suspended during 2017/8 in order to attend to important *ad hoc* phytosanitary research.

Opsomming

Hierdie projek is gedurende 2017/8 uitgestel om aandag te gee aan belangrike *ad hoc* fitosanitêre navorsing.

3.2.6 PROGRESS REPORT: Semi-commercial control of FCM using sequential CO₂ fumigation and a short cold treatment

Project 1197 (2018/9 – 2019/20) by T G Grout and K C Stoltz (CRI)

Summary

This research was planned as a verification of earlier results with carbon dioxide fumigation followed by 13 days at 2°C. However, the IPM research committee requested the inclusion of warmer temperatures. These warmer temperature cold periods of 3°C for 13 days and 4°C for 17 days have not given the required degree of control of FCM fifth instars and the impact of the prior fumigation appears to be lost. Previous research showed that the commercially used FCM diet was unsuitable for fumigation trials so the more porous fruit fly diet was evaluated in this report period, but found to be not much better. The mortality for fifth instar FCM in Valencia fruit stored at 4°C for 17 days without fumigation was 100% and for the same cold period after CO₂ fumigation at 70% in air was 98.2%. In comparison, the same treatments for fifth instar FCM in fruit fly medium gave mortalities of 41.0% and 66.1%, respectively. Further research therefore had to be conducted in fruit which limited the numbers of larvae exposed per treatment. After three trials were conducted with a post-fumigation cold treatment of 12 days at 2°C that involved treating 5 785 fifth instars, a larval mortality of 99.20% was obtained based on movement when prodded. A further seven replicates were conducted with a post-fumigation cold treatment of 14 days at 2°C. These trials involved treating a total of 15 829 fifth instars and the mortality obtained was 99.92% on prodding. Larvae showing movement after treatment were placed on fruit fly medium to determine whether any would pupate and survive to adult. Only one live adult was obtained, giving a final mortality of 99.99% whereas with the 12 day cold period after fumigation nine moths were obtained, giving a final mortality of 99.84%. Research is continuing with more trials against FCM using 14 days at 2°C after simultaneous fumigation of packed fruit in cartons in a pallet, versus fumigation of loose fruit in crates. However, the first replicate had to be scrapped due to frequent power cuts. Future research is planned with Medfly larvae in oranges to confirm that 14 days at 2°C after fumigation is equally effective against this fruit fly.

Opsomming

Hierdie navorsing is as 'n verifikasie beplan van vroeër resultate met koolstofdioksied beroking gevolg deur 13 dae by 2°C. Die IPM navorsingskomitee het egter die insluit van warmer temperature versoek. Hierdie warmer temperatuur koue-periodes van 3°C vir 12 dae en 4°C vir 17 dae het nie die vereiste mate van beheer van VKM vyfde instars gegee nie en die impak van die vooraf beroking blyk verlore te wees. Vorige navorsing het getoon dat die kommersieel gebruikte VKM dieet nie geskik vir berokingsproewe was nie, so die meer poreuse vrugtevliegdieet is in hierdie verslagperiode geëvalueer, maar is gevind om nie veel beter te wees nie. Die sterftes vir vyfde instar VKM in Valencia vrugte gestoor by 4°C vir 17 dae sonder beroking, was 100%, en vir dieselfde koue-periode ná CO₂ beroking teen 70% in lug was die sterftes 98.2%. In vergelyking, het dieselfde behandelings vir vyfde instar VKM in vrugtevliegmedium sterftes van 41.0% en 66.1% onderskeidelik gegee. Verdere navorsing moes dus in vrugte uitgevoer word wat die aantal blootgestelde larwes per behandeling verminder. Nadat drie proewe met 'n ná-beroking koue-behandeling van 12 dae by 2°C uitgevoer is, wat die behandeling van 5 785 vyfde instars behels het, is 'n larwe sterftesyfer van 99.20% verkry, gebaseer op beweging wanneer geprikkel word. 'n Verdere sewe herhalings is met 'n ná-beroking koue-behandeling van 14 dae by 2°C uitgevoer. Hierdie proewe het die behandeling van 'n totaal van 15 829 vyfde instars behels, en die sterftes verkry was 99.92% ná prikkeling. Larwes wat beweging getoon het ná behandeling, is op vrugtevliegmedium geplaas om vas te stel of enige in papies sal ontwikkel en tot volwassenes oorleef. Slegs een lewendige volwassene is verkry, wat 'n finale sterftesyfer van 99.99% gee, terwyl met die 13 dag koue-periode ná beroking, nege motte verkry is, wat 'n finale sterftesyfer van 99.84% gee. Navorsing gaan voort met meer proewe teen VKM, deur gebruik te maak van 14 dae by 2°C ná gelyktydige beroking van gepakte vrugte in kartonne in 'n palet, teenoor beroking van los vrugte in kratte. Die eerste herhaling moes egter geskrap word weens gereelde krag-onderbrekings. Toekomstige navorsing word beplan met Mediterreëse vrugtevlieglarwes in lermoene ten einde te bevestig dat 14 dae by 2°C ná beroking net so effektief teen hierdie vrugtevlieg is.

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

Summary

As of 15 July 2017, the European Union published a new regulation regarding FCM and affecting exports of citrus from southern Africa to the European Union. The regulation requires the fruit to be sourced from an FCM-free area or place of production, or to receive a cold treatment or any other treatment that can ensure the exported consignment is free of FCM. Cold disinfestation of citrus fruit from South Africa to Europe is not feasible, due to the relatively short shipping time, particularly from Cape Town port, but more importantly due to the large volumes of fruit exported to Europe and the inadequate infrastructure to facilitate cold treatment of these large volumes. Consequently, a multi-step systems approach has been developed as an alternative treatment to cold sterilisation. The final stage in the systems approach is a time-temperature shipping protocol. Although this is a cold treatment, it is not a complete disinfestation treatment. The precise level of mortality required by this treatment is determined by the measured efficacy of the preceding steps in the systems approach. To date, the mortality of the most cold tolerant larval stages of FCM, using several time-temperature combinations, have been determined. Research during the year in question was significantly delayed, due to CRI PE having to relocate to new premises. Despite this, further work on incomplete cold treatments, as a component in the systems approach, was resumed. The efficacy of each of 3°C, 4.5°C and 5°C for periods ranging from 16 to 26 days was measured. Mortality of larvae at 3°C ranged from 96.3% to 100% for the durations of 16 to 26 days. Mortality at 4.5°C ranged from 89.35 to 96.88%. Mortality at 5°C ranged from 39.8 to 71.6% for durations of 19 to 22 days, and from 93.95 to 96.38% for durations of 23 to 26 days. Results from these new time-temperature combinations have been included in the FCM systems approach for chilling sensitive cultivars. Studies on the behaviour and survival/development of larvae surviving cold treatments were also conducted, due to reported interceptions of “live” FCM in South African citrus fruit in the EU. Fourth and fifth instars in artificial diet were exposed to 4°C for 16, 18 and 20 days, 3°C for 16 and 18 days, and 2°C for 14 and 16 days. Survivors were placed in the centre of a 10cm radius circle, with 1 cm gradations and speed and distance of movement monitored over a period of several hours. This was compared with untreated control larvae from the same batches. There was no clear relationship between movement and survival to adulthood at 3 and 4°C. However, after 2°C for 14 days, there was a clear relationship, but unfortunately there are some exceptions to the rule e.g. 9% of larvae moving zero distance still developed to adulthood. Finally, experiments on the effect of cold treatments on eggs were initiated. Firstly, susceptibility of eggs on wax sheets and eggs on fruit surfaces to a temperature of 1°C was compared. After 3 days, 47% of eggs on wax paper were dead, whereas only 27% of eggs on fruit were dead. After 5 days, 81% of eggs on wax paper were dead and only 67% of eggs on fruit were dead. Thereafter, the cold susceptibility of newly oviposited and mature eggs (3 days old) was compared. After 4 days at 1°C, mortality of the two ages of eggs was almost identical.

Opsomming

Vanaf 15 Julie 2017 het die Europese Unie 'n nuwe regulasie rakende VKM gepubliseer, wat uitvoere van sitrus van suidelike Afrika na die Europese Unie beïnvloed. Die regulasie eis dat die vrugte van 'n VKM vrye streek of plek van produksie verkry word, of aan 'n koue behandeling blootgestel word of enige ander behandeling wat kan verseker dat die besending vry is van VKM. Koue-ontsmetting van sitrusvrugte van Suid-Afrika tot Europa is nie haalbaar nie, as gevolg van die relatiewe kort verskepingstydperk, veral van Kaapstad hawe, maar meer belangrik as gevolg van die groot volumes vrugte wat Europa toe uitgevoer word en die onvoldoende infrastruktuur om kouebehandeling van hierdie groot volumes te fasiliteer. Gevolglik is 'n multi-stap stelselsbenadering as 'n alternatiewe behandeling vir koue sterilisasie ontwikkel. Die finale stap in die stelselsbenadering is 'n tyd-temperatuur verskepings protokol. Al is hierdie wel 'n koue behandeling, is dit nie 'n volledige ontsmettings behandeling nie. Die presiese vlak van mortaliteit wat deur hierdie stap benodig word, word bepaal deur die gemete doeltreffendheid van die vorige stappe in die stelselsbenadering. Tot op hede is die mortaliteit van die mees kouetolerante lewensstadiums van VKM deur gebruik van verskeie tyd-temperatuur kombinasies bepaal. Gedurende die betrokke navorsingsjaar is werk beduidend vertraag omdat die CRI PE kantore na 'n ander perseel

toe moes skyf. Ondanks hierdie vertraging, is verdere werk met onvoldoende kouebehandelings, as 'n komponent in 'n stelselsbenadering, voortgesit. Die doeltreffendheid van elk van 3°C, 4.5°C en 5°C vir tydperke wat van 16 tot 26 dae geduur het is gemeet. Mortaliteit van larwes teen 3°C het van 96.3% tot 100% gewissel vir tye van 16 tot 26 dae. Mortaliteit van larwes teen 4°C het gewissel van 75.76% tot 99.84% vir tye van 16 tot 26 dae. Mortaliteit teen 4.5°C het gewissel van 89.35 tot 96.88%. Mortaliteit teen 5°C het gewissel van 39.8 tot 71.6% vir tye van 19 tot 22 dae, en vanaf 93.95 tot 96.38% vir tye van 23 tot 26 dae. Resultate van hierdie nuwe tyd-temperatuur kombinasies is nou ook in die VKM stelselsbenadering ingesluit vir koue gevoelige kultivars. Studies is gedoen op die gedrag en oorlewing/ontwikkeling van larwes wat koue behandelings oorleef het omrede verslae van onderskeppings van lewendige VKM in Suid-Afrikaanse sitrus vrugte in die EU. Vierde en vyfde instars in kunsmatige diete is aan 4°C vir 16, 18 en 20 dae, 3°C vir 16 en 18 dae, en 2°C vir 14 en 16 dae blootgestel. Oorlewendes is in die middel van 'n 10 cm radius sirkel, met 1 cm gradasies en spoed en afstand van beweging is oor 'n tydperk van 'n paar ure gemonitor. Hierdie is met onbehandelde kontrole larwes van dieselfde eierbondels vergelyk. Teen 3 en 4°C is daar was geen duidelike verhouding tussen beweging en oorlewing tot volwasendheid. Na 2°C vir 14 dae was 'n duidelike verhouding egter gekry, maar ongelukkig is daar uitsonderings b.v. 9% van larwes wat glad nie beweeg het nie het nogsteeds tot volwassendheid ontwikkel. Laastens is eksperimente op die effek van koue behandelings op eiers begin. Eerstens is vatbaarheid van eiers op waksvelle en eiers op vrug opervlakke vir 'n temperatuur van 1°C vergelyk. Na 3 dae, is 47% van eiers op waksvelle dood en net 27% van eiers op vrugte is dood. Na 5 dae is 81% van eiers op waksvelle dood en net 67% van eiers op vrugte is dood, daarom moes alle verdere proewe op vrugte gedoen word. Daarna is die koue gevoeligheid van vars eiers en ou eiers (3 dae oud) vergelyk. Na 4 dae teen 1°C, is mortaliteit van die twee eier ouderdoms amper identies.

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

Project 1090 (April 2014 – March 2021) by Wayne Kirkman, Sean Moore (CRI), Martin Hill, Rui Krause and Roman Tandlich (RU)

Summary

Previous studies showed that a Solid Phase Micro-extraction (SPME) probe effectively trapped and concentrated headspace volatile compounds surrounding intact citrus fruit. Volatile compound detection was then achieved by using a Gas Chromatography-Mass Spectrometry (GCMS) system. GCMS analysis was conducted on five major volatile compounds of citrus; D-limonene, 3,7-dimethyl-1,3,6-octatriene, (E)-4,8-dimethyl-1,3,7-nonatriene, caryophyllene and naphthalene. In trials conducted in 2016 on infested Witkrans Navel oranges, D-limonene levels decreased with time after infestation, while levels of naphthalene increased. The ratio of these compounds was significantly different between healthy and infested fruit for all time periods. In similar trials conducted in 2017 on Mor Mandarins, Washington and Witkrans Navel oranges and Midnight and Delta Valencia oranges, these same trends were not observed. This was mainly due to variability in D-limonene levels in all cultivars as a result of extremely unusual climatic conditions in the Eastern Cape which resulted in excessive splitting and fruit drop, as well as scorching of Valencia orchards by uncharacteristic berg winds. In the trials conducted in 2018 on infested Washington and Witkrans Navel oranges, as well as Midnight and Delta Valencia oranges, D-Limonene levels decreased significantly and naphthalene levels increased with time after infestation, and the ratio between the two compounds (D-Limonene/naphthalene) was significantly lower than with healthy fruit. In Clementine Mandarins there was a significant increase in beta-Ocimene levels with time after infestation, where levels were undetectable in the control fruit. Infested Midnight Valencia oranges also showed significantly lower levels of caryophyllene with time after infestation. The ability of an electronic nose to detect FCM infested fruit was investigated. In the trial conducted on Washington Navel oranges, 80%, 90% and 90% were correctly detected for 2, 6 and 10 days after infestation. Twenty percent of the control fruit were incorrectly classed as infested. A Selected Ion Flow Tube Mass Spectrometry (Sift-MS) unit at the University of Leuven in Belgium could differentiate between healthy fruit and fruit injured 24 hours earlier after a few seconds of real-time volatile detection and analysis.

Opsomming

Vorige studies het getoon dat 'n "Solid Phase Micro-extraction (SPME) probe" (SPME) "headspace" vlugtige stowwe wat skoon vrugte omring, effektief kan opvang en konsentreer. Opsporing van vlugtige verbindings is met 'n Gas chromatografie-Massaspektrometrie (GCMS) sisteem gedoen. GCMS-analises is op vyf belangrike vlugtige verbindings van sitrus gedoen: D-limonien, 3,7-dimethyl-1,3,6-octatriene, (E)-4,8-dimethyl-1,3,7-nonatriene, caryophyllene en naftaleen. In proewe wat in 2016 op besmette Witkrans Nawellemoene uitgevoer is, het die D-limonien vlakke met tyd na besmetting afgeneem, terwyl vlakke van naftaleen toegeneem het. Daar was ten alle tye 'n beduidende verskil in die verhouding van hierdie verbindings in gesonde en besmette vrugte. In soortgelyke proewe in 2017 op Mor Mandaryne, Washington en Witkrans Nawellemoene en Midnight en Delta Valencia lemoene is hierdie tendense nie waargeneem nie. Dit was hoofsaaklik weens die variasie in die D-limonien vlakke in alle kultivars, wat veroorsaak is deur uiters ongewone klimaatstoestande wat gelei het tot grootskaalse vrugbars en vrugval in die Oos-Kaap, asook Valencia boorde wat geskroei het weens ongewone bergwinde. In proewe wat 2018 op besmette Washington en Witkrans Nawellemoene, asook Midnight en Delta Valencia lemoene uitgevoer is, het D-limonien vlakke met tyd na besmetting betekenisvol afgeneem en naftaleen vlakke het toegeneem. Die verhouding tussen die twee verbindings (D-limonien/naftaleen) was ook betekenisvol laer as in onbesmette vrugte. In Clementine Mandaryne was daar 'n betekenisvolle toename in beta-osimeen vlakke met tyd na besmetting met onopspoorbare vlakke in die kontroles. Besmette Midnight Valencia lemoene het ook beduidende laer vlakke van caryophyllene met tyd na besmetting getoon. Die vermoë van 'n elektroniese neus om VKM besmette vrugte op te spoor, is ondersoek. In proewe op Washington Nawellemoene wat 2, 6 en 10 dae vroeër besmet is, kon die elektroniese neus onderskeidelik 80%, 90% en 90% van die vrugte as besmet klassifiseer. Twintig persent van die kontroles is as vals-positief geklassifiseer. 'n "Selected Ion Flow Tube Mass Spectrometry" (Sift-MS) eenheid by die Universiteit van Leuven in België kon tussen gesonde vrugte en vrugte wat 24 uur vroeër beseer is, onderskei binne 'n paar sekondes van "real-time" opsporing en analise van vlugtige stowwe.

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

Project 1117 (April 2015 – March 2019) by Patrick Mwanza, Gill Dealtry, Michael Lee (NMU) and Sean Moore (CRI)

Summary

Baculovirus biopesticides offer a more environmentally friendly approach to combat crop pests and are used as part of an integrated pest management programme (IPM) to combat these crop pests. On citrus, formulations of the baculovirus *Cryptophlebia leucotreta* granulovirus (CrleGV) are used in the control of the false codling moth (FCM). Ultraviolet (UV) radiation is the most important factor affecting the persistence of baculovirus biopesticides in the field. Under field conditions the half-life of baculoviruses, varies from 10 h to 10 d and in the absence of any form of UV protection, the average half-life is around 24 h. In this study, CrleGV-SA resistant to UV has been isolated by repeated exposure of virus to UV in a Q-SUN Xe-3HC Xenon Test Chamber that simulates normal sunlight, followed by propagation in FCM fifth instar larvae for a total of five exposure cycles. Bioassays with control and UV exposure cycles 1-5 have shown more than 1000-fold improvement in virulence (LC_{50}) on exposure to UV light after 5 selection cycles, indicating selection of UV-resistant virus. UV damage to the virion in the original CrleGV-SA sample has been observed by TEM imaging. After 5 cycles of selection only 11% of virus shows UV damage, with the remainder appearing intact. The 1st and 5th cycle virus genomes have been sequenced and compared with the original CrleGV-SA sequence using the Geneious 7.1.7 software to identify mutations in known regions of the genome. Three UV-protectants have been tested (lignin, OE446 and Uvinuyl-Easy) and shown not to reduce larval ingestion of formulation containing protectant and virus. Furthermore, all of the protectants confer some limited UV protection, as indicated in bioassays. CrleGV-SA samples from the 5th cycle of selection for UV-resistance, mixed with each of these UV-protectants and exposed to UV radiation are currently being tested in bioassays to determine whether the combination provides greater UV protection and enhanced virus virulence. Completion date for this project was to be March 2019; however, completion has been delayed. Consequently, a final report will only be submitted in 2020.

Opsomming

Bakulovirus bio-insekdoders bied 'n meer omgewings vriendelike benadering om gewas plae te bestry en word gebruik as deel van 'n geïntegreerde plaagbestuur program (IPM) om hierdie gewas plae te bestry. Op sitrus word formulasies van die bakulovirus *Cryptophlebia leucotreta* granulovirus (CrleGV) gebruik om valskodlingmot (VKM) te beheer. Ultraviolet (UV) bestraling is die mees belangrike faktor wat die nawerking van bakulovirus bio-insekdoders in die veld beïnvloed. Onder veld toestande varieer die halflewe van die bakulovirus vanaf 10 h tot 10 d en in die afwesigheid van enige vorm van UV beskerming is die gemiddelde halflewe ongeveer 24 h. In hierdie studie is CrleGV-SA bestand teen UV geïsoleer deur herhaalde blootstelling van die virus tot UV in 'n Q-SUN Xe-3HC Xenon Test Chamber wat normale sonlig namaak, gevolg deur vermeerdering in VKM vyfde instar larwes vir 'n totaal van vyf blootstelling siklusse. Biototse met kontrole en UV blootstelling siklusse 1-5 het meer as 'n 1000-voudige verbetering in virulensie (LC_{50}) getoon tydens blootstelling tot UV lig na 5 keurings siklusse, wat keuring van die UV-weerstandbiedende virus aandui. UV skade aan die virion in die oorspronklike CrleGV-SA monster is waargeneem deur TEM beelde. Na 5 siklusse van keuring het slegs 11% van die virus UV-skade getoon, terwyl die res ongeskonde voorkom. Die 1^{ste} en 5^{de} siklus genome se nukleotied volgorde is bepaal en vergelyk met die oorspronklike CrleGV-SA nukleotied volgorde deur die Geneious 7.1.7 sagteware te gebruik om die mutasies te identifiseer in bekende streke van die genoom. Drie UV-beskermers is getoets (lignien, OE446 en Uvinuyl-Easy) en het nie die larvale inname van formulasies met beskermer en virus verminder nie. Verder verleen die beskermers taamlike beperkte UV-beskerming, soos aangedui in die biototse. CrleGV-SA monsters vanaf die 5^{de} siklus van keuring vir UV-weerstand, gemeng met elk van hierdie UV-beskermers en blootgestel aan UV bestraling word tans getoets in biototse om te bepaal of die kombinasie meer UV-beskerming bied en dus virulensie verbeter. Die voltooiingsdatum vir hierdie projek was Maart 2019, maar voltooiing is egter vertraag. Gevolglik sal 'n finale verslag eers in 2020 ingedien word.

3.2.10 PROGRESS REPORT: Identification and development of an attractant for monitoring FCM adult females

Project 1162 (April 2017 – March 2019) by Sean Moore, Wayne Kirkman, Mellissa Peyper (CRI) and Tamryn Marsberg (RU)

Summary

FCM is currently monitored in the field using traps baited with synthesised female moth sex pheromone, thus attracting only male moths. However, such a system can never be adequately accurate, as it is the females (not the males) that lay eggs on the fruit, leading to the larvae that do the damage. Additionally, male moths are believed to fly greater distances than females and are therefore not necessarily representative of the female population in the area. Identification of an attractant for females would enable a far more accurate monitoring technique. Previous work identified a few volatile compounds emitted by citrus fruit, and blends of compounds, that may have potential for attracting adult female FCM. A trial conducted last season to test the attractiveness of these compounds resulted in only one trap catching one female. Subsequently more volatiles with potential for attracting female moths were identified, and the release rate of these in customised dispensers determined in the laboratory. A series of no-choice field cage trials are currently being conducted by releasing 50 virgin female FCM into a cage. A yellow delta trap with a sticky floor, loaded with the volatile dispenser is placed upwind from the moths. Trials are currently under way, with none of the volatiles tested thus far demonstrating any attractiveness. The next step will be to test the same volatiles with mated female moths.

Opsomming

VKM word tans in die veld gemonitor deur gebruik van lokvalle met 'n lokaas van gesintetiseerde wyfie mot seksferomoon, en lok dus net mannetjie motte. So 'n stelsel kan egter nooit akkuraat genoeg wees nie, want dit is die wyfies (nie mannetjies nie) wat eiers op die vrugte lê, wat lei tot die larwes wat die skade doen.

Daarbenewens, blyk dit dat mannetjie motte groter afstande as wyfie motte vlieg en is daarom nie noodwendig verteenwoordigend van die wyfie populasie in the area nie. Identifikasie van 'n lokmiddel vir wyfies sal 'n meer akkurate moniterings tegniek moontlik maak. Vorige werk het 'n paar vlugtige stowwe en mengsels van verbindings geïdentifiseer wat belofte inhou vir aanlokking van volwasse wyfie VKM. 'n Proef gedurende die vorige seisoen om die aanloklikheid van die verbindings te toets het daartoe gelei dat een wyfie mot in een lokval gevang is. Daarna is meer vlugtige stowwe geïdentifiseer, en die loslatings tempo daarvan in aangepaste vrystellers verkry in die laboratorium. 'n Reeks hok proewe word tans gedoen deur 50 ongepaarde wyfie motte in die hok vry te laat. 'n Geel delta lokval met 'n taai vloer, gelaai met die gevulde vlugtige stof vrysteller word windop van die motte geplaas. Proewe is tans onderweg, met geen van die vlugtige stowwe wat sover 'n aanloklikheid toon nie. Die volgende stap sal wees om die proewe te herhaal met gepaarde wyfie motte.

3.2.11 **PROGRESS REPORT: Improvement of the quality and quality control testing of sterile moths for FCM SIT**

Project 1164 (April 2017 – March 2019) by Sean Moore, Wayne Kirkman (CRI), Mellissa Peyper, Clarke van Steenderen, Tammy Marsberg, Martin Hill (RU), Ciska Kruger, Clarissa Mouton and Craig Chambers (Xsit)

Summary

The sterile insect technique (SIT) for FCM has been commercially implemented in citrus in South Africa since 2007 with generally good success. However, a few possible problems have been identified and there is a continual pursuit to improve the quality and performance of the sterile moths. Thus, this study focused on various aspects. Firstly, the lack of activity of sterile moths at cooler temperatures compared to wild moths. Previous work identified trehalose as an effective cryoprotectant for sterile moths, if added to the larval diet. Consequently, a field trial was conducted during autumn and winter of 2018, comparing recaptures of moths reared on a trehalose augmented diet with those reared on the normal diet. Recaptures were 18% higher for trehalose moths. Additionally, trap catches indicated that trehalose-fed moths survived longer in the field than control moths. Furthermore, a reliable quality control test to measure the mating competitiveness of sterile male moths was required. This was investigated in laboratory trials by comparing the spermatophore transfer of sterile males to wild females vs that of wild males to wild females. Simultaneously, choice-trials were conducted in a field net to compare the mating incidents between sterile and wild moths. Wild males were found to have a significantly higher spermatophore transfer than sterile males, with sterile males showing no preference for sterile females. A statistically significant correlation was also recorded between mating incidents in cages involving sterile males and spermatophore transfer in laboratory trials. Therefore, laboratory based spermatophore transfer trials can be used as a reliable quality control measure for sterile males. Finally, AFLP tests are being developed as a means to differentiate between wild larvae and Xsit larvae, in order to determine whether any larvae infesting fruit in the field are F1 steriles.

Opsomming

Die sterile insek tegniek (SIT) vir VKM is in sitrus in Suid-Afrika kommersieel geïmplementeer sedert 2007 met algemene goeie sukses. 'n Paar probleme is egter geïdentifiseer en daar is 'n voortdurende poging om die gehalte en vertoning van die motte te verbeter. Hierdie studie fokus dus op verskeie aspekte. Eerstens, die vermindering in aktiwiteit van steriele motte teen koeler temperature in vergelyking met die wilde motte. Vorige navorsing het trehalose geïdentifiseer as 'n doeltreffende kouebeskermer vir motte, as dit by die larwe dieet bygevoeg word. Gevolglik is 'n proef gedurende herfs en winter van 2018 uitgevoer wat die hervangs van motte wat op 'n trehalose dieet geteel is vergelyk met motte wat op die gewone dieet geteel is. Hervangste was 18% hoër vir die trehalose motte. Daarbenewens, vangs het aangedui dat trehalose motte langer in die veld oorleef as beheermotte. Verder, is 'n betroubare gehalte beheer toets om paringsmededingendheid van steriele motte te meet nodig. Hierdie toets is ondersoek in laboratorium proewe deur te bepaal wat die spermatofoor oordrag tussen steriele mannetjies en wilde wyfies, en wilde mannetjies en wilde wyfies is. Gesamentlik is keuse-proewe uitgevoer in 'n veld net om die

parings voorvalle tussen steriele en wilde motte te vergelyk. Wilde mannetjies het aansienlik hoër spermatofoor oordrag ervaar in vergelyking met steriele mannetjies, met steriele mannetjies wat geen voorkeur vir steriele wyfies toon nie. 'n Statisties beduidende korrelasie was ook aangeteken tussen parings voorvalle in hokke wat steriele mannetjies en spermatofoor oordrag in laboratoriumtoetse behels. Daarom kan laboratorium proewe van spermatofoor oordrag 'n betroubare gehaltebeheer toets vir steriele mannetjies wees. Laastens, word AFLP toetse ontwikkel as 'n manier om te onderskei tussen wilde en XSit larwes, om te bepaal of enige larwes wat vrugte in die veld besmet, F1 steriel is.

3.2.12 **PROGRESS REPORT: Development of a remote vapour detection system using a trained sniffer dog to detect FCM infested fruit**

Project 1175 (April 2017 – April 2019) by Stan Gillham, Pierre Olivier (Citrus Pest Detection Dogs) and Sean Moore (CRI)

Summary

The ability of a sniffer dog to detect FCM-infested fruit has been demonstrated with a 98.9% accuracy within a previously registered research project. This is substantially more accurate than the currently used human inspection system, which has a maximum potential of 77.8%. However, there are a couple of potential hurdles to the successful implementation of this within citrus packhouses. Firstly, inspection of cartons or pallets in the packhouse could be extremely labour intensive and may require several dogs and dog handlers in a large packhouse. Secondly, there may be regulatory restrictions on the presence of dogs in the packhouse. Both of these can be overcome through the development of a remote vapour detection system involving a vacuum pump and absorbent filters for collection of odours. Odours can be collected from numerous cartons and/or pallets, marked and presented to a sniffer dog in any convenient location thereafter. A battery operated vacuum pump, with sufficient suction capacity, was developed for the purpose. Appropriate filters were selected for absorbing volatiles extracted from infested and healthy fruit, and the sniffer dog, Max was imprinted on the former. Sean Moore paid a visit to the dog handlers and observed and recorded a training session with the dog. It was clear that the dog found detection of odours associated with infested fruit more challenging when doing so from a remote filter than from the fruit itself. Consequently, filters and filter holders, which had absorbed odours from healthy and infested fruit and negative controls were tested for odours using GCMS. Unfortunately, no odours could be detected, revealing the need to store filters frozen between collection of odours and exposure to the dog. This method will be implemented when training resumes in April when ripe fruit are again available.

Opsomming

Die vermoë van 'n snufferhond om VKM-besmette vrugte is in 'n vorige geregistreerde navorsingsproef met 'n 98.9% akuraatheid bewys. Hierdie is beduidend meer akuraat as die huidige menslike inspeksie sisteem wat gebruik word wat 'n maksimum vermoë van 77.8% het. Daar is egter twee potensiele struikelblokke tot die suksesvolle toepassing hiervan in sitruspakhuis. Eerstens kan inspeksie van kartonne of pallette in die pakhuis uiters arbeids intensief wees en mag dalk verskeie honde en hondehandteerders benodig, veral in 'n groot pakhuis. Tweedens, mag daar dalk regulatoriese beperkings op die teenwoordigheid van honde in die pakhuis wees. Albei van hierdie kan deur die ontwikkeling van 'n afgeleë vlugtigestof opsporingstelsel oorkom word, wat 'n vakuumpomp en absorberende filters gebruik vir die versameling van die vlugtige stowwe. Vlugtigestowwe kan van verskeie kartonne en/of pallette versamel word en gemerk word en daarna aan 'n snufferhond in enige geskikte perseel voorgestel word. Tot op hede is verskeie opsies vir die versameling van vlugtigestowwe ondersoek, soos onderdrukte lug met 'n verstelbare drukklep wat die onderdrukte lug kan insuig en ook die druk kan beheer. 'n Battery-krag vakuumpomp, met voldoende suigings vermoë, is vir hierdie doeleinde ontwikkel. Geskikte filters is geselekteer vir absorpsie van vlugtigestowwe wat van besmette en gesonde vrugte geëkstrakteer word, en die snufferhond, Max, is op die eersgenoemde ingeprint. Sean Moore het 'n besoek gemaak aan die honde hanteerders en het 'n opleidings sessie met die hond waargeneem en aangeteken. Dit was duidelik dat dit meer uitdagend vir die hond was om besmette vrug reuke van die afgeleë sensor op te spoor

as van die vrugte self. Gevolglik is filters en filter-houers, wat reuke van gesonde en besmette vrugte geabsorbeer het en negatiewe kontroles is getoets vir reuke deur gebruik van GCMS. Ongelukkig kon geen reuke opgespoor word nie, wat aangedui het dat filters tussen versameling en blootstelling aan die hond gevries moet word. Hierdie metode sal geïmplementeer word wanneer opleiding in April 2019 hervat word, wanneer ryp vrugte weer beskikbaar is.

3.2.13 **PROGRESS REPORT: FCM control under nets – is pest-freedom possible?**

Project 1189 (October 2017 – July 2019) by Sean Moore, Wayne Kirkman, Paul Cronje (CRI), Mellissa Peyper, Tammy Marsberg and Sonnica Albertyn (RU)

Summary

Nets were erected over various orchards in the Sundays River Valley, Eastern Cape on four different farms in the winter of 2017. These four farms were monitored from the start of the season in 2017 until harvest in 2018. FCM levels were monitored weekly using both trap catches and fruit infestation, inside and outside of nets. By the end of the 2017/2018 season it was recorded that FCM levels were not lower under the nets. This could be due to nets being erected over orchards with previously high levels of FCM. However, where sterile moths were released under and outside of nets, recaptures of sterile moths and consequently sterile to wild moth ratios were also higher under nets, indicating the potential for greater suppression of FCM under nets over time. Red scale and mealybug infestation and thrips damage were monitored once off, approximately four weeks before harvest. Overall results showed that red scale and mealybug presence was higher under nets and thrips damage was higher outside nets. Monitoring has continued during the 2018/2019 season, using three of the same trial sites. Monitoring of FCM has remained the same and to date no FCM infestation has been recorded both inside and outside of nets. Scouting for mealybug, red scale and thrips infestation is being conducted monthly until harvest. Variable results have been recorded to date. Citrus leafhopper trap catches are higher outside of nets and green citrus leafhopper catches are higher under nets. Fruit fly traps are also being monitored.

Opsomming

Nette is opgerig oor verskeie boorde op vier plase in die Sondagsrivier Vallei, Oos-Kaap, gedurende die winter van 2017. Hierdie vier plase is gemonitor van die begin van seisoen in 2017 tot oestyd in 2018. VKM vlakke is weekliks gemonitor met behulp van beide lokvalle en vrugtebesmetting, binne and buite die nette. Aan die einde van die 2017/2018 seisoen, was die VKM vlakke nie laer onder die nette nie. Hierdie kan wees as gevolg van hoër vlakke van VKM in hierdie boorde voor die nette opgerig was. Waar steriele motte egter onder en buite die nette los gelaat was, was herwinnings van steriele motte en gevolglik steriele tot wilde motte verhoudings ook hoër onder nette, wat die potensiaal vir groter onderdrukking van VKM onder nette met verloop van tyd aandui. 'n Eenmalige inspeksie vir rooidopluis en witluis besmetting en blaaspootjie skade is voltooi vier weke voor oes. Algehele resultate het getoon dat rooidopluis en witluis hoër onder die nette was en blaaspootjie skade hoër buite die nette. Monitoring het voortgegaan vir die 2018/2019 seisoen, waar dieselfde boorde gebruik word, vir drie van dieselfde proef plase. Monitoring van VKM besmetting het dieselfde gebly en tot op hede geen VKM is binne en buite nette versamel nie. Inspeksie vir rooidopluis, witluis en blaaspootjie skade is elke maand gedoen tot oestyd. Wisselende resultate is tot nou toe waargeneem. Sitrus bladspringer lokval vangste is hoër buite nette en groensitrus bladspringer is hoër onder nette. Vrugtevlug lokvalle word ook gemonitor.

3.2.14 **PROGRESS REPORT: Selection for improved virulence to FCM or resistance by FCM using a homologous and a heterologous baculovirus**

Project 1199 (2017/8 – 2020/21) by D Taylor, M Jukes, C Knox, S Moore and M Hill (Rhodes University/CRI)

Summary

Cryptophlebia leucotreta granulovirus (CrleGV) has been used as part of an integrated pest management system on citrus in South Africa for 15 years. During this time, CrleGV has been very effective, but there has been no research on whether *Thaumatotibia leucotreta* (Meyr.) (Lepidoptera: Tortricidae) has developed resistance in the field. There have been no studies on the potential of *T. leucotreta* of developing resistance to CrleGV or CrpeNPV. For a long time, it was considered impossible for insects to develop resistance to biocontrol agents because the virus and insect co-evolve, a situation known as the red queen hypothesis. This turned out to be untrue when a resistant *Cydia pomonella* population was discovered in Europe. While this is the only known example of resistance to a baculovirus known in the field, it has been achieved many times and with many species in laboratories. This study will be the first to give some insight into the risk of resistance to CrleGV being developed by *T. leucotreta*. Progress to date includes a written literature review and proposal of planned methodology. As this project is a continuation of work conducted in the previous year, several methods have now been adapted and improved based on difficulties encountered. Stocks of virus have been carefully enumerated to ensure all dosages prepared downstream can be reliably utilised. Pilot experiments are also being conducted to ensure modifications made to the methodology are suitable prior to establishing the resistance colony. Additionally, seven-day bioassays are being conducted on FCM neonate larvae to establish initial LC values. These bioassays are planned to be repeated after the resistance colony has been established to determine whether changes in susceptibility can be measured.

Opsomming

Cryptophlebia leucotreta granulovirus (CrleGV) is vir 15 jaar as deel van 'n geïntegreerde plaagbestuurstelsel op sitrus in Suid-Afrika gebruik. Tot op hede het CrleGV baie doeltreffend gewerk, maar daar is geen navorsing gedoen oor moontlike *Thaumatotibia leucotreta* (Meyr.) (Lepidoptera: Tortricidae) weerstandbiedendheid in die veld nie. Daar is ook nog nie enige studies oor die potensiaal van *T. leucotreta* om weerstand teen CrleGV of CrpeNPV te ontwikkel nie. Dit vir 'n geruime tyd beskou dat insekte nie weerstand teen biologiese middels sal ontwikkel nie omrede die virus en insek saam oor eeu geëvolueer het, 'n situasie bekend as die rooi koningin-hipotese. Dit het duidelike geword dat hierdie onwaar was toe 'n weerstandbiedende *Cydia pomonella*-bevolking (teen CpGV) in Europa ontdek is. Terwyl dit die enigste bekende voorbeeld is van weerstand teen 'n bakulovirus in die veld, is dit gereeld en met 'n verskeidenheid spesies in laboratorium studies bereik. Hierdie studie sal die eerste wees om insig te gee aan die risiko dat weerstand teen CrleGV deur *T. leucotreta* kan ontwikkel. Vordering tot op datum sluit in 'n skriftelike literatuuroorsig en voorstel van beplande metodiek. Aangesien hierdie projek 'n uitbreiding is van werk wat in die vorige jaar gedoen is, is verskeie metodes nou aangepas en verbeter, gebaseer op probleme wat ondervind is. Virus voorrade is noukeurig gekwantifiseer om te verseker dat alle dosisse wat voorberei word, met vertroue gebruik kan word. Loodsproewe word ook uitgevoer om te verseker dat aanpassings aan die metodiek geskik is voordat die weerstande kolonie gestig word. Daarbenewens word sewe-dag biotoetse uitgevoer op pastuitgebroeide VKM larwes om oorspronklike LC-waardes te bepaal. Hierdie biotoetse sal herhaal word nadat die weerstande kolonie gevestig is, om te bepaal of veranderinge in vatbaarheid gemeet kan word.

3.2.15 PROGRESS REPORT: Sexual attraction and mating compatibility between FCM populations and the potential impact on the sterile insect technique

Project 1202 (2018-2019) by J Upfold (RU - MSc student) S D Moore (CRI), M Hill, C Coombes (RU)

Summary

The sterile insect technique (SIT) was developed in the mid-19th century to eradicate screwworm in North America. Sterile insect technique for false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) was initiated in South Africa in 2007. Sterilised male FCM from a culture originating from Citrusdal in the Western Cape are released into orchards to mate with females, resulting in suppressed population numbers in the next generation. The FCM sex pheromone is comprised of three isomers, trans-8-dodecenyl acetate, cis-8-dodecenyl acetate and dodecyl acetate. However, previous research has found that geographically distinct FCM populations appear to have differences in their sex pheromones. For example, the sex pheromone of Ivory Coast FCM has a

ratio of 69%, 23% and 8% respectively, and Malawian FCM has a ratio of 32% 52% and 16% respectively. Recently, choice trials with Citrusdal FCM males and virgin females from different regions were conducted in a controlled environment. The males showed a significant preference for females from their own population. This study is therefore being taken further. Composition of sex pheromones in females from different regions, in South Africa will be compared, as will the attraction of males to females from regionally distinct populations, in both choice and no-choice tests. Finally, mating compatibility will be compared between these different populations. If significant differences are found, an FCM laboratory culture of mixed origin will be developed to try and overcome these differences and improve the efficacy of of not only SIT in all regions of the country, but other semiochemical based technologies, such as mating disruption and monitoring with sex pheromones.

Opsomming

Die steriele insekte tegniek (SIT) is ontwikkel in die middel van die 19de eeu om spykerwurmsiekte uit te wis in Noord-Amerika. Steriele insekte tegniek vir valskodlingmot (VKM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) is in 2007 in Suid-Afrika geïnisieer. Gesteriliseerde mannetjie VKM van 'n kultuur met wat gestig is van VKM uit Citrusdal in die Wes-Kaap, is vrygestel in boorde om te paar met wilde wyfie VKM, wat lei tot die onderdrukking van bevolkingsgetalle in die volgende generasie. Die VKM feromoon bestaan uit drie isomere, trans-8-dodeseniël asetaat, cis-8-dodeseniël asetaat en dodeseniël asetaat. Tog het vorige navorsing bevind dat geografies afgesonderde bevolkings van VKM blyk om verskille in hul feromone te hê. Byvoorbeeld, die feromoon van die Ivoorkus VKM het 'n verhouding van 69%, 23% en 8% onderskeidelik, en Malawiese VKM het 'n verhouding van 32% 52% en 16% onderskeidelik. Onlangs is keuse proewe tussen Citrusdal VKM mannetjies en ongepaarde VKM wyfies vanuit verskillende streke in 'n beheerde omgewing uitgevoer. Die mannetjie VKM het 'n beduidende voorkeur vir wyfies van hul eie bevolking gewys. Hierdie studie word dus verder geneem. Die seksferomone in wyfie VKM uit verskillende streke van Suid-Afrika sal vergelyk word. Die aantrekkingskrag van mannetjie VKM en wyfies uit streeks-duidelike bevolkings sal ook vergelyk word, in beide keuse en nie-keuse proewe. As beduidende verskille gekry word, sal 'n VKM-laboratoriumkultuur van gemengde oorsprong ontwikkel word om hierdie verskille te oorkom en die doeltreffendheid van SIT en ander semiochemies gebaseerde tegnologieë soos paringsontwrigting en monitering met seksferomoon, in alle streke van die land te verbeter.

3.2.16 **PROGRESS REPORT: Yeast-baculovirus synergism: Investigating mixed infections for improved management of the false codling moth, *Thaumatotibia leucotreta*.**

Project 1163 by Caroline Knox, Martin Hill (RU) and Sean Moore (CRI)

Summary

It has previously been reported that there is a mutualistic association between *Cydia pomonella* also known as codling moth, and epiphytic yeasts. Laboratory assays and field trials show that combining yeast with *Cydia pomonella* granulovirus significantly increased larval mortality. We proposed to determine which species of yeast occur naturally in *Thaumatotibia leucotreta* larvae and to examine whether any of these yeasts, when combined with the *Cryptophlebia leucotreta* granulovirus, increase larval mortality. Previously Navel oranges infested with *T. leucotreta* larvae were collected from orchards in Sundays River Valley and analysed for the presence of yeast. A total of four yeasts were isolated from *T. leucotreta* larvae and identified as *Meyerozyma caribbica*, *Pichia kluyveri*, *Pichia kudriavzevii* and *Hanseniaspora opuntiae*. An oviposition preference assays was conducted on *T. leucotreta* females which showed that they prefer specific yeast isolates for ovipositioning, namely *Pichia kudriavzevii* and *Hanseniaspora opuntiae*. A detached fruit bioassay was performed using *Pichia kudriavzevii* to investigate whether combining yeast with CrleGV-SA significantly increased larval mortality. An increase in larval mortality was observed, however this result was determined not to be statistically significant. A detached fruit bioassay to determine the optimal yeast to virus concentration by efficacy of mixing *P. kudriavzevii* with CrleGV-SA was performed. Lowering the yeast concentration from 10^8 cells/ml to 10^6 cells/ml increased larval mortality and it was determined to be statistically significant. Recently the bioprospecting process was expanded to other

geographically distinct citrus producing regions in South Africa. To isolate new and unique yeast isolates that may elicit stronger responses in *T. leucotreta* than those isolated thus far. This led to the isolation and identification of three additional yeast species namely *Pichia fermentans*, *Kluyveromyces marxianus* and *Candida lusitanae*. Currently oviposition, Y-tube and neonate movement assays are being conducted on *T. leucotreta* using the newly isolated yeasts. Additionally, yeasts isolates are being subjected to gas chromatography-mass spectrometry (GCMS) analysis to identify the volatiles responsible for causing attraction. Lastly detached fruit bioassays are being conducted with Break-THRU® S240 and molasses to further increase mortality rates.

Opsomming

Daar is voorheen berig dat daar 'n mutualistiese assosiasie is tussen *Cydia pomonella*, ook bekend as kodlingmot, en epifitiese giste. Laboratorium toetse en veldproewe toon dat 'n kombinasie van gis met *Cydia pomonella* granulovirus aansienlik die larwe mortaliteit verhoog. Ons het voorgestel om te bepaal watter spesies gis natuurlik in *Thaumatotibia leucotreta* larwes voorkom en om te ondersoek of enige van hierdie giste, wanneer dit gekombineer word met die *Cryptophlebia leucotreta* granulovirus, larwe mortaliteit verhoog. Voorheen was Nawelmoene wat met *T. leucotreta*-larwes besmet is, van boorde in Sondagsriviervallei afgehaal en geanaliseer vir die teenwoordigheid van gis. Altesaam vier giste is van *T. leucotreta* larwes geïsoleer en geïdentifiseer as *Meyerozyma caribbica*, *Pichia kluyveri*, *Pichia kudriavzevii* en *Hanseniaspora opuntiae*. 'n Eierleggings voorkeurproef is uitgevoer op *T. leucotreta*-wyfies, wat getoon het dat hulle spesifieke gis-isolate vir eierlegging verkies het, naamlik *Pichia kudriavzevii* en *Hanseniaspora opuntiae*. Biototse is ook op vrugte met *Pichia kudriavzevii* op vrugte uitgevoer om te bepaal of 'n kombinasie van gis met CrleGV-SA larwe mortaliteit verhoog het. 'n Toename in larwe mortaliteit is waargeneem, maar hierdie resultaat is vasgestel om nie statisties betekenisvol te wees nie. Nog biototse op vrugte is uitgevoer om die optimale gis tot viruskonsentrasie (*P. kudriavzevii* met CrleGV-SA) vir effektiwiteit te bepaal. Die verlaging van die gis konsentrasie van 10^8 selle/ml tot 10^6 selle/ml het larwe mortaliteit verhoog en die verskil was statisties betekenisvol. Onlangs is die ondersoek vir nuwe gis spesies uitgebrei na ander geografies duidelike sitrusproduserende streke in Suid-Afrika, om nuwe en unieke gis-isolate te isoleer wat sterker response in *T. leucotreta* kan ontlok as dié wat tot dusver geïsoleer is. Dit het gelei tot die isolasie en identifikasie van drie addisionele gis spesies, naamlik *Pichia fermentans*, *Kluyveromyces marxianus* en *Candida lusitanae*. Tans word proewe met die nuwe isolate gedoen: eierlegging, Y-buis en pasuitgebroeide larwe beweging. Daarbenewens word gis-isolate onderworpe aan gas chromatografie massaspektrometrie (GCMS) analise om die vlugtige bestanddele wat verantwoordelik is vir aantrekkingskrag te identifiseer. Laastens word biototse met Break-THRU® S240 en melasse op vrugte uitgevoer om mortaliteit verder te probeer verhoog.

3.2.17 PROGRESS REPORT: Improvement of the quality of sterile moths for FCM SIT

Project 1221 (2018/4 – 2020/3) by Marelize de Villiers, Sean Moore, Vaughan Hattingh (CRI), Craig Chambers, Nevill Boersma, Clarissa Mouton (RBX)

Summary

Since 2007, the sterile insect technique (SIT) for FCM has been implemented commercially in citrus in South Africa. Despite relatively good success, some opportunities for improvement of moth quality have been identified. This includes the negative effect of cold immobilization on moth fitness, a higher temperature activity threshold of laboratory-reared irradiated moths compared to wild moths, and a marginally reduced level of sterility of irradiated moths than originally recorded. Due to the negative effect of cold immobilization during transport on fitness and performance of moths in the field, immobilization by anoxia (an absence of oxygen) was investigated. Depending on the time that moths can be exposed to anoxic conditions, cold immobilization can either be avoided (if moths can survive periods of anoxia long enough to be transported under anoxia), or the negative effect of cold immobilisation can possibly be counteracted by the anoxia treatment. Results have shown that anoxic periods of more than 5 h cause poor survival of the moths. Longevity of moths exposed to anoxia for periods ranging from 1 to 5 h was tested, with males being treated and kept in the absence of females, as well as with males being grouped with females during and after treatment. Longevity of moths for all anoxia durations for both sexes was

superior compared to untreated moths. Flight ability of moths after exposure to anoxia was tested after a 48 h period in the laboratory. Treatments included: normoxia (untreated moths) with no irradiation, normoxia with irradiation, and anoxia (1½, 3 and 5 h) with irradiation. On average, flight ability was best for untreated moths with no irradiation, followed by untreated moths with irradiation, and finally the anoxia treated moths with irradiation, the latter showing a decrease in flight ability with an increase in the duration period. Due to the negative effect of anoxia on flight ability, no further anoxia experiments will be conducted. To address the higher temperature activity threshold of laboratory moths, the addition of trehalose to the FCM diet is being tested to increase moth activity at low temperatures, thereby increasing the competitiveness of laboratory-reared moths with wild moths (Project 1064). Sterility trials have been initiated, but results are not yet available.

Opsomming

Sedert 2007, is die steriele insektegniek (SIT) vir VKM kommersieël in sitrus in Suid-Afrika geïmplementeer. Ten spyte van relatiewe goeie sukses, is sekere tekortkominge in motkwaliteit geïdentifiseer, wat verbeter moet word. Dit sluit in die negatiewe effek van koue-immobilisering op fiksheid van die motte, 'n hoër temperatuur aktiwiteitsdrempel van laboratorium-geteelde bestraalde motte in vergelyking met wilde motte, en 'n laer vlak van steriliteit van die bestraalde motte as oorspronklik waargeneem. Weens die negatiewe impak van koue-immobilisering gedurende vervoer op fiksheid en gedrag van motte in die veld, is immobilisering deur anoksie ('n afwesigheid van suurstof) ondersoek. Afhangende van die tyd wat die motte aan anoksiese toestande blootgestel kan word, kan koue-immobilisering óf vermy word (as motte periodes van anoksie kan oorleef wat lank genoeg is dat die motte onder anoksie vervoer kan word), óf die negatiewe effek van koue-immobilisering kan moontlik deur die anoksie behandeling teengewerk word. Resultate het gewys dat anoksie periodes van meer as 5 ure swak oorlewing van die motte tot gevolg het. Langlewendheid van motte wat aan anoksie periodes vanaf 1 tot 5 ure blootgestel is, is getoets, met mannetjies wat in die afwesigheid van wyfies behandel en gehou is, sowel as mannetjies wat met wyfies gegroepeer is tydens en na behandeling. Langlewendheid van motte vir alle anoksie periodes vir beide geslagte was beter in vergelyking met die onbehandelde motte. Vliegvermoë van motte na blootstelling aan anoksie is getoets na 'n 48 uur periode in die laboratorium. Behandelings het ingesluit: normoksie (onbehandelde motte) sonder bestraling, normoksie met bestraling, en anoksie (1½, 3 en 5 ure) met bestraling. Gemiddeld was vlugvermoë beste vir onbehandelde motte sonder bestraling, gevolg deur onbehandelde motte met bestraling, en eindelijk anoksie behandelde motte met bestraling, met laasgenoemde wat 'n afname in vlugvermoë toon met 'n toename in die duur van die periode. Weens die negatiewe effek van anoksie op vlugvermoë, sal geen verdere anoksie eksperimente gedoen word nie. Ten einde die hoër temperatuur aktiwiteitsdrempel van die laboratorium motte aan te spreek, word die toevoeging van trehalose tot die VKM dieet om motaktiwiteit by lae temperature te verhoog getoets, om sodoende die mededingendheid van laboratorium motte met wilde motte te verbeter (Projek 1064). Steriliteitsproewe is begin, maar resultate is nog nie beskikbaar nie.

3.3 PROGRAMME: FRUIT FLY

Programme coordinator: Aruna Manrakhan (CRI)

3.3.1 Programme summary

Fruit flies are pests of phytosanitary concern on export citrus. The fruit flies listed currently on citrus in South Africa are *Ceratitis capitata* (Mediterranean fruit fly or Medfly), *Ceratitis rosa* (Natal fly) and *Bactrocera dorsalis* (Oriental fruit fly). The Natal fly was split in two species: *C. rosa* and *Ceratitis quilicii* (Cape fly). The status of citrus as a host for the Cape fly has not been established. There is a zero tolerance of fruit flies in citrus destined for export markets. Moreover, recently, the European Commission amended their phytosanitary measures for imports of citrus from non-EU countries to prevent introduction of non-European fruit flies. With these amendments, control measures for fruit flies both before and after harvest would have to be effectively implemented to ensure freedom of southern African citrus consignments from non-European fruit flies.

There were nine projects carried out under the fruit fly programme between April 2018 and March 2019. Four of these projects were on baits used for pre-harvest control of fruit fly pests in citrus. Three projects addressed post-harvest cold treatments for fruit flies. One project was on the ecology and development of the Natal fly and Cape fly in citrus. The long term project on fruit fly rearing project (3.3.4) continued to ensure supply of fruit fly materials for various research projects.

In Project 1177 (3.3.7) where the timing of baiting in Western Cape Province is being investigated it was found that an earlier application of fruit fly baits in October in soft citrus orchards prevented high catches of the species in February in areas that were not under the Medfly Sterile Insect Technique (SIT). In Project 1211 (3.3.9) on attract and kill methods for fruit flies, the two registered bait stations - M3 bait stations and Magnet Med were found to be equally effective for controlling fruit flies in Valencia orchards. In Project 1248 (3.3.11) on new bait stations, one bait station developed by AVIMA was found to be more effective than M3 bait stations against both Medfly and oriental fruit fly in laboratory bioassays. Project 1147 (3.3.3) on incompatibility of copper and fruit fly baits applied as sprays was terminated in 2019 with assessment on fruit finalised in July 2018. In this project, incompatibility between copper and fruit fly baits was found. Stippling marks were found on fruit treated with copper and fruit fly baits. The timing of baiting reduced incidence of stippling with a lower incidence of these stippling marks when baits were applied in April. In projects addressing post-harvest control treatments for fruit flies, Projects 1171 (3.3.6) and 1213 (3.3.10), Medfly was found to be more cold tolerant than the other fruit fly pests on citrus. The impact of interruptions on Medfly cold treatment efficacy will be investigated in trials conducted in 2019. In Project 1170 (3.3.5) investigating ecology of Natal fly and Cape fly, Cape fly was found to be more widely distributed than Natal fly in citrus orchards in the northern areas. Neither Cape fly nor Natal fly was reared from any citrus sampled from commercial orchards.

Programopsomming

Vrugtevlieë is plaë van fitosanitêre belang op uitvoersitrus. Die vrugtevlieë wat tans op sitrus in Suid-Afrika gelys is, is *Ceratitis capitata* (Mediterreense vrugtevlieë of “Medfly”), *Ceratitis rosa* (Natalese vlieg) en *Bactrocera dorsalis* (Oosterse vrugtevlieë). Die Natalese vlieg is in twee spesies verdeel: *C. rosa* en *Ceratitis quilicii* (Kaapse vlieg). Die status van sitrus as ‘n gasheer vir die Kaapse vlieg is nog nie vasgestel nie. Daar is ‘n nul toleransie vir vrugtevlieë in sitrus wat vir uitvoermarkte bestem is. Verder het die Europese Kommissie onlangs hul fitosanitêre maatreëls vir invoer van sitrus vanaf nie-EU lande aangepas ten einde die binnekoms van nie-Europese vrugtevlieë te voorkom. Met hierdie wysigings, moet die beheermaatreëls vir vrugtevlieë beide vóór en ná oes effektief geïmplimenteer word ten einde te verseker dat suidelike Afrika sitrusbesendings vry van nie-Europese vrugtevlieë is.

Daar is nege projekte onder die vrugtevlieë program tussen April 2018 en Maart 2019 uitgevoer. Vier van hierdie projekte het gehandel oor lokaasmiddels wat gebruik word vir voor-oes beheer van vrugtevlieë plaë in sitrus. Drie projekte het na-oes koue-behandelings vir vrugtevlieë aangespreek. Een projek het oor die ekologie en ontwikkeling van die Natalese en Kaapse vlieg in sitrus gehandel. Die langtermyn projek oor vrugtevlieë teling (3.3.4) het voortgegaan ten einde die verskaffing van vrugtevlieë materiaal vir verskeie navorsingsprojekte te verseker.

In Projek 1177 (3.3.7), waar die tydsberekening van lokaas toediening in die Wes-Kaapprovinsie ondersoek is, is gevind dat vroeër toediening van vrugtevlieë lokaasmiddels in Oktober in sagte sitrus boorde, hoë vangste van die spesies in Februarie in areas wat nie onder die “Medfly Sterile Insect Technique” (SIT) geval het nie, voorkom het. In Projek 1211 (3.3.9) oor die lok- en uitwissingsmetodes vir vrugtevlieë, is gevind dat die twee geregistreerde lokaasstasies, M3 lokaasstasies en Magnet Med, ewe effektief vir die beheer van vrugtevlieë in Valencia boorde was. In Projek 1248 (3.3.11) oor nuwe lokaasstasies, is gevind dat een lokaasstasie wat deur AVIMA ontwikkel is, meer effektief in laboratorium bio-toetse was as M3 lokaasstasies, teen beide die Mediterreense en Oosterse vrugtevlieë. Projek 1147 (3.3.3) oor onverenigbaarheid van koper en vrugtevlieë lokaasmiddels wat as spuite toegedien word, is in 2019 gestop, met die evaluering op vrugte wat in Julie 2018 gefinaliseer is. In hierdie projek

is onverenigbaarheid tussen koper en vrugtevlug lokaasmiddels gevind. Stippelmerke is op vrugte gevind wat met koper en vrugtevlug lokaasmiddels behandel is. Die tydsberekening van lokaas toediening het die voorkoms van stippeling verminder, met 'n laer voorkoms van hierdie stippelmerke wanneer lokaasmiddels in April toegedien is. In projekte wat die na-oes beheer behandelings van vrugtevlug aangespreek het, Projekte 1171 (3.3.6) en 1213 (3.3.10), is gevind dat die Mediterreense vrugtevlug meer koue-tolerant as die ander vrugtevlug plaes op sitrus is. Die impak van onderbrekings op Mediterreense vrugtevlug koue-behandeling effektiwiteit sal in proewe wat in 2019 uitgevoer word, ondersoek word. In Projek 1170 (3.3.5) wat die ekologie van die Natalse en Kaapse vlug ondersoek het, is gevind dat die Kaapse vlug meer wydverspreid as die Natalse vlug in sitrusboorde in die noordelike areas voorkom. Nie die Kaapse vlug óf die Natalse vlug is vanaf enige sitrus wat vanaf kommersiële boorde versamel is, geteel nie.

3.3.2 FINAL REPORT: A new bait for more effective control of all *Ceratitis* fruit flies

Project 915 (April 2008 – March 2018) by Aruna Manrakhan, John-Henry Daneel, Peter Stephen, Sean Moore, Wayne Kirkman, Martin Gilbert, Claire Love, Rooikie Beck and Glorious Shongwe (CRI)

Summary

Protein bait application is one of the main control tools in a fruit fly management programme in a commercial citrus orchard in southern Africa. Fruit fly control with protein baits work on an “attract and kill” principle whereby adult flies are attracted to proteinaceous odours in a bait and are subsequently killed by an insecticide within the bait environment. Protein baits are applied as sprays or stations. This project had three main objectives: (1) Evaluation of alternative fruit fly attractants, (2) Investigation of alternative toxicants in baits and (3) Investigation of a new dispensing technology for baits. Responses of three *Ceratitis* pest species: *C. capitata*, *C. rosa* and *C. cosyra* to currently used baits- HymLure, GF-120 and M3 bait were first evaluated in field cages. *Ceratitis capitata* was the most responsive species to all baits tested. *Ceratitis capitata* and *C. rosa* responded well to all baits tested. *Ceratitis cosyra*, on the other hand, had a poor response to HymLure. In a second series of field cage and field assays, one new promising attractant- ProLure was found which was attractive to all three *Ceratitis* species. Efficacy of different insecticides in combination with different concentrations of HymLure was evaluated in laboratory assays on *C. capitata* females. Spinosad was found to be the most effective alternative toxicant to malathion for use with HymLure at 2% and 10% (dilution with water). A paper-based station containing a malathion-based bait (now referred to as Tephri pyramid) was developed. In field tests conducted over two years (2016 and 2017) in Midnight Valencia orchards in Mpumalanga, Eastern Cape and Western Cape, the paper-based bait station containing malathion at 200 units per ha was as effective as M3 bait station at 300 units per ha in controlling fruit fly pest populations.

Opsomming

Proteïen lokaas-toediening is een van die belangrikste beheerhulpmiddels in 'n vrugtevlug bestuursprogram in 'n kommersiële sitrusboord in suidelike Afrika. Vrugtevlugbeheer met proteïen lokaasmiddels werk op 'n “ aantrek en doodmaak ” beginsel, waar volwasse vlieg na proteïenagtige reuke in 'n lokaasmiddel aangetrek word, en daaropvolgend deur 'n insekdoder binne die lokaasmiddel-omgewing gedood word. Proteïen lokaasmiddels word as spuite of stasies toegedien. Hierdie projek het drie hoof doelwitte gehad: (1) Evaluasie van alternatiewe vrugtevlug lokmiddels, (2) ondersoek van alternatiewe gifstowwe in lokaasmiddels en (3) ondersoek van 'n nuwe vrystellingstegnologie vir lokaasmiddels. Reaksies van drie *Ceratitis* plaagspesies: *C. capitata*, *C. rosa* en *C. cosyra* op huidig gebruikte lokaasmiddels, HymLure, GF-120 en M3 lokaasmiddel, is eerstens in veldhokke geëvalueer. *Ceratitis capitata* was die spesie wat die meeste op al die lokaasmiddels wat getoets is, gereageer het. *Ceratitis capitata* en *C. rosa* het goed gereageer op al die lokaasmiddels wat getoets is. *Ceratitis cosyra*, aan die ander kant, het swak op HymLure gereageer. In 'n tweede reeks veldhok- en veldproewe, is een nuwe belowende lokmiddel gevind, ProLure, wat aanloklik vir al drie *Ceratitis* spesies was. Effektiwiteit van verskillende insekdoders, in kombinasies met verskillende konsentrasies HymLure, is in laboratoriumproewe op *C. capitata* wyfies geëvalueer. Daar is gevind dat Spinosad die effektiwiefte alternatiewe gifstof vir malathion was vir gebruik

saam met HymLure teen 2% en 10% (verdunding met water). 'n Papier-gebaseerde stasie, bevattende 'n malathion-gebaseerde lokaasmiddel (nou verwys na as *Tephri pyramid*) is ontwikkel. In veldproewe wat oor twee jaar (2016 en 2017) in Midnight Valencia boorde in Mpumalanga, Oos-Kaap en Wes-Kaap uitgevoer is, was die papier-gebaseerde lokaasstasie, bevattende malathion teen 200 dele per ha, net so effektief in die beheer van vrugtevliegplaagpopulasies, as M3 lokaasstasie teen 300 dele per ha.

Introduction

Worldwide, the use of protein baits mixed with insecticide, known as the bait application technique, is one of the main methods of fruit fly control (Roessler, 1989). The technique works on an attract and kill principle, whereby adult flies (in particular females) in search of food (protein) to mature sexually are attracted to the bait and are killed by an insecticide mixed with the bait either upon contact or following ingestion of the mixture. Such poisoned bait mixtures limit the use of insecticide and at the same time increase efficacy of control. Baits that have been found to be effective against fruit flies are hydrolysed yeast or vegetable proteins (McPhail, 1939). Poisoned baits can be applied either as foliar sprays (aerial or ground) or in discrete containers known as bait stations. Bait stations are increasingly being used in some fruit fly management programmes. The use of bait stations further limits the release of insecticide in the environment as well as limiting insecticide residues on fruits.

In citrus production areas in South Africa, mixtures of HymLure or LokLure and organophosphates (malathion or trichlorfon) and the ready to use spinosad based GF 120 have been traditionally used as baits which are applied from the ground and from the air for control of fruit fly pests. The M3 bait station was also registered in the early 2000s for control of fruit fly pests in citrus orchards. The M3 bait station consists of protein hydrolysate, proprietary mixture of plant extracts and a toxicant housed in a plastic device. M3 bait stations have been found to be effective in controlling fruit flies in citrus orchards (Ware et al., 2003) and are set at densities varying from 300 to 400 units per hectare. According to the label of M3 bait stations, they are effective for up to 12 weeks in the field. Recently Magnet-Med, a paper based attract and kill device containing ammonium acetate and trimethylamine as attractants and impregnated with deltamethrin, was registered for control of fruit fly pests in different fruit crops including citrus.

Two fruit fly pests in the genus *Ceratitis* are listed on citrus in southern Africa: The Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wiedemann) and the Natal fly, *Ceratitis rosa* Karsch (Grout and Moore, 2015). The oriental fruit fly, *Bactrocera dorsalis* (Hendel), which recently invaded the north and north eastern areas of South Africa is also listed as a pest of citrus in southern Africa (Grout and Moore, 2015). There have been two cases of interception (2006 and 2018) of another *Ceratitis* species, the marula fly- *C. cosyra* (Walker) on oranges exported to EU from South Africa. The control of the latter fruit fly species may become necessary in citrus growing areas.

There are two main challenges that come with the use of baits for control of fruit fly pests. First, since efficacy of a bait depends to a large extent on its attractiveness to the targeted pests, it would be important that a bait be attractive to multiple fruit fly species. It has been shown in previous studies that fruit fly species tend to differ in their responses to protein baits. For instance, Barry et al. (2006) reported a stronger response of the melon fly, *Zeugodacus cucurbitae* (Coquillett) to protein baits compared to *B. dorsalis*. Protein deprived Medfly was found to respond higher to food odours compared to protein deprived *Ceratitis fasciventris* (Bezzi) and Marula fruit fly, *C. cosyra* (Hardman and Hattingh, 2008). The second challenge in the use of baits for fruit fly control is that there are constant changes in insecticide residues allowed on fruit especially for baits that are used as foliar sprays. In January 2009, the maximum residue level (MRL) of malathion on citrus fruit for export was lowered to 0.02 mg/kg and the pre-harvest interval for South African citrus fruits destined to the European markets was set at 28 days (Hardman and Hattingh, 2008). Subsequently the MRL of malathion was increased but for some time, this left growers with limited options for baits. Bait stations which leave no residues are ideal solutions but the deployment of these bait station units can be problematic especially in large farms. The M3 bait station which has been used since the 2000 is also plastic based and bait stations made up of biodegradable materials would be preferred.

This project which ran over 10 years from 2008 to 2018 set out three objectives as listed below to tackle with the two main challenges highlighted above.

Stated objectives

- A. To evaluate alternative fruit fly attractants
- B. To investigate alternative toxicants that can be incorporated into baits
- C. To investigate new dispensing technology for baits through the use of cheaper and biodegradable substrates

Materials and methods

Seven separate trials are described below, four falling under baits for sprays and three under baits for stations. The trials under baits for sprays were carried out under objectives A & B stated above. The trials under baits for stations addressed objective C.

A1. Attraction of Medfly, Natal fly and marula fly to currently used proteinaceous baits

The study was conducted between October 2008 and June 2009 in a screened cage (4 m long, 3.2 m wide and 2.5 m high), located in the grounds of Citrus Research International (CRI) at Nelspruit, South Africa. The cage was made up entirely of high density polyethylene screen with a 1 mm x 2 mm mesh size. A plastic cover was fitted on top of the cage to protect against rain and direct sunlight. Eight potted Star Ruby Grapefruit (*Citrus paradisi* Macfad.) trees were placed inside the cage. A 1.96 m high rotating washing line (rotating pole fitted with 4 arms and nylon rope strung between the arms) was fixed in the middle of the field cage.

Ceratitis capitata, *C. rosa* & *C. cosyra* flies used in all tests were obtained from colonies maintained for approximately 200 generations in the rearing facilities at CRI, Nelspruit. Upon eclosion, flies were fed only with sugar and water and were therefore completely protein deprived. Two-hundred mature, 10-14-day-old males and 200 females of the same age of each species were used for each test. Species were evaluated separately.

Three fruit fly baits registered for fruit fly control in South Africa were evaluated: (1) HymLure (Savoury Food Industries [Pty] Limited, South Africa) which is a protein hydrolysate (2) GF-120 Naturalyte (Dow AgroSciences, South Africa), and (3) M3 bait (also known as Questlure) (River Bioscience [Pty] Ltd., South Africa) which is used in the M3 bait station. Each bait tested was absorbed onto a 2.5 cm x 2.5 cm filter paper. The filter paper containing 2 ml of bait was then placed inside a 4 cm diameter white plastic cup covered with netting to allow dispersion of odour from the bait whilst preventing direct access of flies to the bait.

Flies were released into the field cage 1 hour before start of the test between 14:00 and 16:00. A water dispenser was placed inside the cage to provide a water source for flies. Each bait container was suspended in the middle of an open ended and transparent cylinder (8 cm diameter x 8 cm length) lined with a transparent sticky insert. Cylinders used during tests were hung at equal distances around the horizontally-rotating wheel in the middle of the cage. In each test, there were four cylinders, three containing baits and one control. The control consisted of a blank filter paper placed inside a similar plastic cup as used for the baits. Positions of baits on the wheel were allocated at random. The baits were exposed for 23 hours inside the cages after which they were removed. Exposure of baits for almost a whole day allowed for a full expression of foraging behaviour of each species. Baits were rotated 90° clockwise every 2 hours between 8:00 and 17:00. There were 4 replicates for each test and species tested. The starting position of the baits tested was moved one place at the start of each replicate. At the end of the test, baits were removed from the cage. Male and female flies captured on the sticky insert of each cylinder were counted.

Relative attractiveness of commercial baits

In this experiment, HymLure, GF-120 and M3 bait were evaluated concurrently. HymLure and GF-120 were evaluated at concentrations of 1% and 5% respectively representing the rates at which they are used for fruit fly control in South Africa. In all tests, HymLure and GF-120 were freshly prepared. M3 bait was undiluted as is the case when it is used in the station. Three different ages of M3 bait were evaluated separately against the two

other baits: (1) Fresh, (2) 4 weeks old and (3) 8 weeks old. M3 baits were aged by placing the cup containing the bait outdoors in the shade, but protected from rain.

Influence of concentration on attractiveness of liquid protein baits

Hym-Lure and GF-120 were tested separately at different concentrations. Hym-Lure was tested at 0.4%, 1% and 2% and GF-120 was tested at 1%, 5% and 10%.

Influence of age on attractiveness of M3 bait

Three ages of M3 bait were evaluated concurrently: (1) Fresh, (2) 4 weeks old and (3) 8 weeks old. Aging of the bait was carried out as described previously.

Statistical analysis

For each species tested, data were analysed by a three-way analysis of variance with first order interactions for all experiments. The two common categorical factors in all experiments were sex and replicate. The third categorical factor in the first, second and third experiment was bait type, bait concentration and bait age, respectively. For all experiments, data were log transformed ($\log x+1$) to stabilize variances before analysis. A significance level of 0.05 was set for all tests and means were separated by the Fisher's Least Significant Difference.

A2. Attraction and feeding responses of Medfly to new protein attractants

These studies were carried out between July and August 2009.

Ceratitis capitata flies used in all tests were obtained from colonies maintained for approximately 200 generations in the rearing facilities at CRI, Nelspruit. Upon eclosion, flies were fed only with sugar and water and were therefore completely protein deprived. Two-hundred mature, 10-14-day-old males and 200 females of the same age were used for each attraction test. For the feeding tests, 10 females were used per test.

Attraction and feeding responses of *C. capitata* to six protein based attractants: HymLure (Savoury Food Industries [Pty] Limited, South Africa), Solbait (Prepared by Mangan, USDA-ARS, Texas, in 2009), Corn steep liquor (Merck, Modderfontein, South Africa), Mazoferm (Corn Products International, Eldoret, Kenya), Brewer's yeast (South African Breweries, Sandton, South Africa) and Prolure (Green Trading, Brits, South Africa) were tested. All attractants were evaluated at 2% dilution with water.

Attraction tests

The attraction tests were carried out as described in A1 except that there were six replicates instead of four.

Feeding tests

In the feeding tests, a green food dye was mixed with the test attractant at the rate of 1%. A control consisting of water and green dye at the same rate as above was used. Tests were carried out in aerated plastic containers (26 cm x 25 cm x 14 cm) fitted with a removable glass top. Flies were released in the cages one hour before each test and supplied with sugar and water offered separately. Ten 10 μ l droplets of each attractant were placed on a petri dish which was weighed before bait placement. The droplets were then dried in the oven at 30 degrees for 30 minutes and weighed again before being exposed to flies in the container. Feeding trials ended after 24 hours after which the number of flies containing the green dye for each bait was counted and the glass slide containing the bait was weighed. For each bait, there were two glass slides, one of which was left unexposed to flies and weighed after 24 hours. There were four replicates with two replicates conducted per week. A new batch of fly was used per week.

Statistical analysis

In order to measure relative effectiveness of the baits, the total number of flies captured in one cylinder was divided by the total number of flies captured during the day of the test. Data were arcsine square root transformed to stabilize variances. An ANOVA was carried out on the transformed data to determine differences in responses of flies to different baits.

For analysis of the feeding responses, the percentages of flies showing the green dye for each bait were determined. Percentage data were arcsine square root transformed to stabilize variances. An ANOVA was carried out on the transformed data to compare mean feeding responses of flies to different baits. The consumption of each bait was calculated as follows:

Gross Bait consumption (GC)= (Weight of glass slide before fly exposure – Weight of glass slide after fly exposure) - (Weight of control glass slide - Weight of control glass slide after 24 hours)

Gross bait consumption per fly (GCF) = GC * (Number of fed flies/Total number of flies)

Net bait consumption = GCF_{BAIT} – GCF_{WATER}

The net bait consumption was square root transformed. An ANOVA was carried out to determine differences between baits.

A3. Field evaluation of a promising new attractant- Prolure

Different concentrations of Prolure (Green Trading, Brits, South Africa) were evaluated in traps and compared to 0.4% HymLure in a mango orchard and a citrus orchard in Mpumalanga Province.

Studies were conducted in a mango orchard in February 2009 during mango fruiting season at Oeversig Farm and in a Satsuma citrus orchard in April and May 2009 during the citrus fruiting season at Brackenhill Farm.

Four dilutions of Prolure were tested and compared with the recommended HymLure (Savoury Food Industries [Pty] Limited, South Africa) concentration for use in sprays. The treatments were: (1) 0.4 % Prolure, (2) 1% Prolure, (3) 5% Prolure, (4) 10% Prolure, (5) 0.4% HymLure (Standard Bait), (6) water (negative control).

Treatments were evaluated in Chempac Bucket traps (Chempac [Pty] Limited) which are McPhail type traps with yellow base and a transparent lid.

In both study sites, the same set-up was applied. Two hundred ml of each bait were placed inside each trap (without insecticide). Traps were placed across six selected rows. Each row contained the six treatments placed at random. The rows represented replicates for each treatment. The layout of the treatments followed a Latin Square Design. There were therefore 36 traps in total. All traps were placed at a height of 1.5 m in the shaded side of the tree. Traps within a row were at a distance of about 10-30 m. Traps were emptied, checked and rebaited every 2-7 days for seven weeks in the mango orchard and six weeks in citrus orchard. The traps were rotated within a row at each check. All specimens caught in traps were collected in vials and kept in 70% alcohol. Species were identified and sexed.

Statistical analysis

Catches in each trap were calculated as catches per trap per day for each treatment and replicate were calculated by dividing the catches in a trap containing a particular treatment over the number of days that the trap was exposed. Data were then log transformed (log (x+1)) and subjected to an ANOVA with interactions calculated between treatments and each of three factors: species, replicate and sex.

B. Efficacy of toxicants other than malathion in combination with baits

Laboratory assays

Laboratory bioassays were conducted to monitor the mortality of Mediterranean fruit fly females over 72 hours following exposure to six insecticides, evaluated at five different concentrations, in combination with a protein hydrolysate - HymLure. The second part of the laboratory assays compared the toxicity of the best attractant-insecticide mixture (obtained in part 1) to *C. capitata* and *C. rosa* with two other standard treatments.

Adult flies used were obtained from colonies maintained for 200 generations in the rearing facilities at Citrus Research International (CRI), Nelspruit. Flies were given free access to sugar and water only and were completely deprived of protein following adult emergence. Flies used were between 10 and 14 days old.

Insecticides tested were spinosad (Class: Spinosyn; Tracer 480 SC, Dow AgroSciences SA (Pty) Ltd., South Africa (SA)), abamectin (Class: Avermectin; Biomectin EC [18g/L], Villa Crop Protection (Pty) Ltd, SA), imidacloprid (Class: Neonicotinoid; Confidor WG [700g/kg], Bayer (Pty) Ltd., SA), alpha-cypermethrin (Class: Pyrethroid; Avalanche SC [100g/L], Klub M5, SA), fipronil (Class: phenylpyrazole; Regent SC [200g/L], BASF (SA) (Pty) Ltd., SA) and tartar emetic (antimony based compound; Tartox SP [995g/kg], Brenn-O-Kem, SA). Each insecticide was tested at five different concentrations in a bait solution of HymLure (Savoury Food Industries (Pty) Limited, SA). The highest concentration for each insecticide tested was 2 times the recommended field concentration for any citrus pest in South Africa. This way, maximum mortality could be reached with each insecticide tested. Three concentrations of HymLure (vol:vol water): 0.8%, 2% and 10% were tested in combination with each of the insecticides listed above. For each insecticide and HymLure concentration tested, there was a control consisting of HymLure solution only at the corresponding concentration.

Bioassays were carried out in aerated plastic containers (30 cm x 30 cm x 14 cm). Approximately 20 female flies were placed inside the container 1 hour before the test and provided with sugar and water *ad libitum*. Twenty, 10- μ l droplets of each of the bait solutions were placed onto the outside of the lid of a plastic Petri dish (5.5 cm diameter) which was in turn placed inside the aerated container. The platforms with the bait droplets were removed after 24 hours.

Mortality of flies was recorded after the first 4 hours following introduction of the bait on day 1 and then after 24, 48 and 72 hours. Dead flies were removed and counted at each check. The number of surviving individuals was determined at the end of the tests to determine the exact number of females tested. Tests were replicated three times using different generations of flies.

In a second set of laboratory assays, one attractant-insecticide mixture was selected based on the results obtained from the first assay and was compared with two standard baits recommended for field control of fruit flies in citrus production areas of South Africa. The new attractant- insecticide mixture selected was a 2% HymLure solution plus spinosad at 48 ppm. This new mixture was compared with the standard baits: (1) 5% GF-120 Naturalyte with spinosad at 240 ppm in the undiluted formulation (Dow AgroSciences, South Africa) and (2) a combination of 0.8% HymLure solution plus malathion at 875 ppm. All treatments were diluted with water. A control of water and sugar only was included for each treatment tested. Assays were conducted as described above with treatments exposed for 24 hours and mortality recorded at 4, 24, 48 and 72 hours. This time, the treatments were tested on both *C. capitata* and *C. rosa* adult females. Flies used were of the same age and nutritional status as above. Tests with each fruit fly species were run separately. Tests were replicated 6 times using 2 different generations of flies, with 3 replicates per generation.

Field assay

Trials were conducted in a commercial citrus orchard in Schoemanskloof (S 25° 22' 45" E 30° 31' 56.2"; 946 m altitude) in the years 2010 and 2011 to compare the efficacy of a HymLure-spinosad combination with standard HymLure-malathion and GF-120 treatments for fruit fly control. In both years, the field trials were conducted over a period of 6 weeks. In the first year, the field trial was conducted between March and April whilst in the second year the trial was conducted between April and May, periods which coincided with fruit ripening. The cultivated species in the citrus orchard was mainly mandarin, *Citrus reticulata* Blanco (cv. Nova).

Each year, the trial area was divided into two adjacent blocks with an interval of 10 m between blocks. Each block was subjected to a treatment: (1) 2% HymLure mixed with spinosad at 48 ppm over an area of 3 ha (approximately 2011 trees) for 6 weeks; (2) 0.8% HymLure mixed with malathion at 875 ppm for the first week or first two weeks followed by a 5% GF-120 Naturalyte fruit fly bait spray during the remaining weeks of the field test before harvest. The latter treatment was carried out over an area of 2 ha (approximately 1235 trees) and is a recommended fruit fly treatment program for citrus in South Africa destined for European Union export markets with a 28-day pre-harvest interval for malathion bait sprays and a 1-day pre-harvest interval when using GF-120. Treatments were assigned to the same block every year. All dilutions were carried out in water. The volume of HymLure-spinosad treatments applied per ha per week was 100 L. The volume of HymLure-malathion treatment applied per ha per week was 78 L whilst for the 5% GF-120 treatment, a volume of 45 L per ha was used per week. Treatments were

applied with a vehicle-mounted spray machine with spray guns. The nozzle of each of two spray guns used was 1.5 mm in diameter. The pressure of the spray pump was adjusted to 1000 kPa and the vehicle speed was adjusted to deliver a required volume of bait spray mixture onto the trees, specific to the bait used. A volume of about 150 ml of HymLure/spinosad mixture and HymLure/malathion mixture was targeted per tree while for GF-120 the targeted volume was about 40 ml. The recommended volume of a protein and toxicant mixture on citrus trees for fruit fly control in South Africa varies between 100 ml and 800 ml depending on age of trees and canopy size. For GF-120, the current recommended dosage rate for fruit trees is 1 L in 10-30 L of water per ha (Dow AgroSciences Southern Africa (Pty) Ltd).

Adult fruit fly population dynamics were monitored by trapping to determine the efficacy of the different treatments. Monitoring was initiated 1 week before treatments were applied. Monitoring ended 1 week after the treatments were stopped. Male and female *C. capitata* and *C. rosa* populations were monitored in each treatment block using two types of attractants: Capilure (River Bioscience, Port Elizabeth, South Africa) which contains the male attractant Trimedlure – tert-butyl 4 (and 5) - chloro-2-methylcyclohexane-1-carboxylate - (1.8 g active) and Questlure (River Bioscience, Port Elizabeth, South Africa) which contains the food attractant protein hydrolysate (2.0 g active). Each attractant was housed separately in a Sensus trap (River Bioscience, Port Elizabeth, South Africa) which is a bucket-type trap with a transparent plastic bottom and a blue overhanging lid with 12 square openings (0.7 mm x 0.7 mm) just underneath the lid. A 3 g tablet with 19.5% dichlorvos was placed in the lid above the attractant capsule to kill attracted flies. Traps were inspected weekly and attractants and insecticides were replaced every 6 weeks. In each treatment block, there were 3 Questlure and 3 Capilure baited traps. Distances between traps were between 20 and 30 m. The type of attractant used was alternated between and within rows.

Additionally, to evaluate efficacy of control, a fruit damage assessment was carried out in each treatment block. At harvest, 500 fruits were selected at random in each block and inspected for fruit fly damage (punctures, exit holes). Suspected damaged fruits were brought to the lab and weighed and incubated at ambient room temperature over a layer of sterile sand for a period of 6 weeks to confirm fruit fly infestation and determine the fruit fly species.

Statistical analysis

In the first laboratory assay, average corrected percentage mortality (over 3 replicates) for the different treatments (including control) was calculated for 4, 24, 48 and 72 hours after exposure. Data was missing for the 4-hour mortality in the second replicate of insecticides tested with 2.0% HymLure and therefore for this time point and HymLure concentration, mortality was averaged over 2 replicates. To correct for death in the control group, Abbott's formula was used. The percentage corrected mortality was calculated as 1 minus the percentage of survivors in the treatment divided by the percentage of survivors in the control, the answer multiplied by 100. If the value returned by Abbott's formula was negative, suggesting that the number of deaths were more in the control group than in the treatment, the value was re-set to 0. Thus, corrected mortality did not exceed 0 percent. Corrected percentage fly mortality was arcsine square root transformed to stabilize variances. A repeated measures ANOVA was used to analyse the transformed corrected mortality data.

In the second part of the laboratory bioassays, percentage female *C. capitata* and *C. rosa* mortality for different bait treatments were determined at various time intervals. Abbott's formula was used to correct for control mortality. Corrected percentage fly mortality was arcsine square root transformed to stabilize variances. A repeated measures ANOVA was used to analyse the transformed corrected mortality data.

In the field assays, male and female populations for each species were presented as number of flies per trap per week. The mean fly catch per trap per week was calculated for each treatment during the period of the year when treatments were carried out. Fly catches in each treatment were log (x+1) transformed to stabilize variances and compared using Student's t-test.

A significance level of 0.05 was set for all statistical tests and means were separated by the Fisher's Least Significant Difference.

C. Development of a paper based bait station- Tephri bait station

Following a series of laboratory and semi field experiments conducted between 2011 and 2013, a new pulp paper based bait station for control of fruit fly pests named the Tephri bait station was developed (Fig. 3.3.2.1). The developmental work, not presented in this report, entailed (1) testing a suitable substrate for containing the bait and toxicant, (2) testing efficacy of bait mixtures as a suitable attractant and (3) testing toxicants which can be combined with the bait mixture. The attractant component of the Tephri bait station had to be modified after an early version of the Tephri bait station failed to control fruit flies in field tests in a mango orchard and subsequently in a citrus orchard in 2012. The failure of the bait station was attributed to the high alkalinity of the attractant component which decomposed toxicants combined with it. The highly alkaline chemical which was in the attractant component was then removed. There was also an interruption in the production of local pulp paper in 2014 which halted the development and evaluation of the bait station for a few months. Import pulp paper subsequently became available and work on the Tephri bait station resumed. The attractant and toxicant mixture will not be disclosed in this final report in case of commercialisation of the bait station. The toxicity and attraction of the modified Tephri bait station were determined in laboratory and field cages respectively before further field tests. These are reported below.



Figure 3.3.2.1 Picture of a Tephri bait station

C1 Toxicity and attraction of the Tephri bait station

Adult flies used were obtained from colonies maintained in the rearing facilities at Citrus Research International (CRI), Nelspruit. Protein deprived, 7– 10 days old, adult *C. capitata*, *C. rosa*, *C. cosyra* and *B. dorsalis* flies were used. Flies were fed only with sugar and water since emergence. For mortality tests, 10 females of each species were used for each replicate of each treatment. For attraction tests on the *Ceratitidis* species, 100 males and 100 females of each species were used concurrently per test. For attraction tests on *B. dorsalis*, numbers of males and females of the species varied between the tests (from 60 to 100 adults in total, males and females in equal numbers).

The toxicity and attractiveness of a spinosad based Tephri bait station and malathion based Tephri bait station were compared with M3 bait station, GF-120 (at 20% dilution with water), a mixture of HymLure and malathion mixture at 0.04% of HymLure and 0.0175% of malathion (dilution with water).

GF-120 and the mixture of HymLure and malathion were prepared fresh on each test day. All bait stations (both Tephri bait stations and M3 bait stations) were tested at six ages: fresh, two weeks, four weeks, six weeks, eight weeks and twelve weeks. The stations were weathered in an open shed in the CRI Nelspruit compound. Tests on bait stations aged over four weeks were not carried out on *B. dorsalis* due to limited fly availability at the time of the tests.

Toxicity test

Tests were carried out in aerated plastic cages (30 cm x 30 cm x 14 cm) in a temperature controlled room. Female flies of each species were placed separately inside the plastic cage two hours before the test and provided with sugar and water *ad libitum*. Treatments were evaluated in separate cages. Each treatment was placed in the middle of a cage. There was one control cage which had no treatment and only sugar and water *ad libitum*. Treatments were removed from the cage after 24 hours. Mortality of flies was recorded at 24 and 48 hours after exposure. Dead flies were removed and counted at each check. The number of survivors was counted in each cage to determine the exact number of females tested in each cage. There were 4 replicates for each age of stations over two weeks tested using two generations of flies.

Attraction test

Tests on attractiveness of the treatments were conducted in two nylon screen field cages. Ten potted Ruby Grapefruit trees were placed inside the cage. Flies were released into the cage one hour before start of the test. Two water dispensers were placed inside each cage to provide a water source for flies. Each treatment was placed in the middle of Moroccan trap. GF-120 and the mixture of malathion and HymLure were placed in aerated plastic containers (sauce tubs with nets fitted onto lids). Each sauce tub contained 20 ml of the liquid bait. A control trap with no treatment was included. There was therefore a total of six traps which were hung at equal distances in the middle of each cage. The positions of baits were allocated at random. The stations were exposed for 24 hours inside the cages after which they were removed. Treatments were rotated every hour between 8:00 – 17:00. There were four replicates for each age of stations tested over two weeks using 2 generations of flies.

Statistical analysis

In toxicity assays, average percentage mortality for the different treatments (including control) was calculated for 24 and 48 hours after exposure. To correct for death in the control group, Abbott's formula was used. The percentage corrected mortality was calculated as 1 minus the percentage of survivors in the treatment divided by the percentage of survivors in the control, the answer multiplied by 100. If the value returned by Abbott's formula was negative, suggesting that the number of deaths were more in the control group than in the treatment, the value was re-set to 0. Thus, corrected mortality did not exceed 0 percent. For each age of bait station tested, corrected percentage fly mortality was arcsine square root transformed to stabilize variances. A repeated measures ANOVA was used to analyse the transformed corrected mortality data.

In the attraction assays, average percentage response of males and females of each species to the different treatments (including control) was calculated. The percentage response was arcsine square root transformed to stabilize variances. For each age of bait station tested, a multi way ANOVA was used to determine the effects of treatment, species, sex and interactions of treatment and species on fly responses.

C2 Field evaluation of a malathion based Tephri and spinosad based Tephri bait station

The field trial was conducted in Midnight Valencia orchards (total area of 13.9 ha) at Siyalima Boerdery, Low's creek, Mpumalanga Province between June and August 2013. The orchards were divided in 10 blocks.

Four treatments were tested, with each treatment tested in two blocks:

- (1) Malathion based Tephri bait station at 200 stations per ha
- (2) Spinosad based Tephri bait station at 200 stations per ha
- (3) M3 bait station set at 400 stations per ha
- (4) Weekly GF-120 sprays (at 10% dilution with water)

Two control plots were included where no fruit fly control were implemented over 10 weeks.

Fruit fly monitoring

Population of adult fruit flies were monitored using two attractants – Capilure (Trimedlure) and Questlure. Each type of attractant were housed separately in a Sensus trap. A dichlorvos strip was placed inside the trap to kill attracted flies. Traps were hung about 1.5 m above ground. The distance between each trap in a block was approximately 30 m. There were 3 Capilure and 3 Questlure baited traps in each treated block. 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. Catches were identified to species and sex.

A fruit damage assessment was carried out at harvest where 500 fruit in each block were selected at random on the trees and visually examined for fruit fly stings. Any damaged fruit found were brought to the lab, weighed and reared individually in aerated containers to determine percentage infestation and degree of infestation.

Statistical analysis

For each treatment, male and female populations of the dominant fruit fly species were presented as catches per week per Capilure baited trap and per Questlure baited trap respectively. Data was square root transformed to equalise variances. An analysis of variance was used to determine differences in male catches and female catches between treatments during the treatment period. Fruit infestation was calculated as percentage of infested fruits which was the total number of infested fruits over the total number fruits sampled. The degree of infestation was calculated as total number of fruit fly pupae and adults per kilogram of sampled and infested fruits.

C3 Field evaluation of a modified malathion based Tephri bait station in different citrus production regions in South Africa

After the first field trial, a second set of modifications were made to the malathion based Tephri bait station. The amount of malathion in the Tephri bait station was reduced by 5 fold and a polymer was added to keep the bait station moist and to enable a more sustained release of odour. Further work on the spinosad based bait station discontinued due to high cost of the toxicant.

The performance of the modified malathion-based Tephri cone was evaluated over two seasons in Midnight Valencia orchards in three provinces in South Africa: Mpumalanga, Eastern Cape and Western Cape between 2016 and 2017. In Mpumalanga, the trial was conducted in two farms. The trial was carried out in one farm in each of the other provinces. In each season, trials were conducted at the peak of fruit fly activity between April and June. Trials were conducted over seven to 13 weeks and included a week prior to treatment.

In the two farms in Mpumalanga, three treatments were evaluated over the two seasons:

1. Malathion-based Tephri cone stations at 200 units per ha
2. M3 bait stations at 300 units per ha
3. Weekly GF-120 bait sprays at 10% dilution with water

In the farms in Eastern Cape and Western Cape, two treatments were evaluated over the two years:

1. Malathion-based Tephri cone stations at 200 units per ha
2. M3 bait stations at 300 units per ha

In each season, there were three replicate blocks of each treatment in each farm, each of about 0.5-1 ha. In the second season, the Tephri bait station was redesigned and changed from a cone shape to a pyramid shape (Fig. 3.3.2.2).

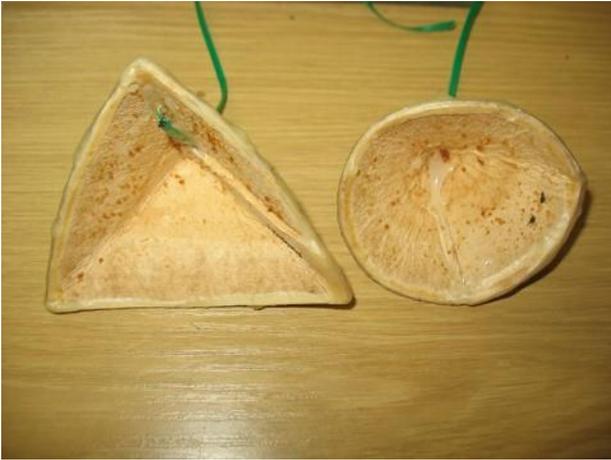


Figure 3.3.2.2 The pyramid shape of the Tephri bait station (on the left) and the cone shape of the Tephri bait station (on the right).

The efficacy of the different treatments was assessed by monitoring adult fruit flies using trapping and by conducting a fruit damage assessment in each replicate block.

Fruit fly monitoring was carried out using 3-component Biolure baited Chempac Bucket traps, Capilure baited Sensus traps and Questlure baited Sensus traps. Methyl Eugenol baited Lynfield traps were only used in farms in Mpumalanga. There were two traps of each type in each replicate block of each treatment. Traps were checked and emptied weekly. Attractants and insecticides inside traps were replaced after five weeks. Flies from each trap were identified according to species and sex.

Fruit were assessed for fruit fly damage either close to or at harvest. In each replicate block, 500 fruit were selected at random from trees within the replicate and checked carefully to determine presence of fruit fly punctures and discolouration of peel resulting from fruit fly punctures. Fruit showing the above damage symptoms were brought to the lab, weighed and incubated individually at ambient room temperature over a layer of sterile sand for a period of 8 weeks to confirm fruit fly infestation, determine the fruit fly species and degree of fruit fly infestation (number of flies per kg of infested fruit). Additionally, ~3 kg of fruit were collected from the ground from each replicate block, brought back to the lab, weighed and incubated in bulk in aerated plastic containers over a sand layer at ambient room temperature for 8 weeks to determine fruit fly infestation and degree of infestation.

Statistical analysis

For each trap type in each replicate block of each treatment on each farm in each year, male and female numbers of the dominant fruit fly pest in specified traps were processed as number of flies per trap per week. Data was square root transformed to equalise variances. The effects of treatments and sites on catches of males and females of the dominant fruit fly pest were determined using ANOVA. Interactions between sites and treatments were also determined. The percentage fruit fly damage was calculated for each replicate block of each treatment for fruit assessed on the trees at or near harvest. In case of fruit fly damage on trees, the degree of infestation was processed as total number of flies over total weight of damaged fruit within the replicate block. The degree of fruit fly infestation was determined for fruit collected from the ground in each replicate block and was presented as number of fruit flies per kg of collected fruit within each replicate block of each treatment.

Results and discussion

A1. Attraction of Medfly, Natal fly and marula fly to proteinaceous baits

The three fruit fly species had different profiles of response to commercial proteinaceous baits. Medfly and Natal fly responded well to the three types of commercial baits. Marula fly, on the other hand, had a generally low response towards HymLure. Female flies were significantly more attracted to baits than were males, irrespective of species tested. Generally higher numbers of Medfly responded to baits compared to Natal fly and marula fly.

The generally higher response of Medfly to protein baits compared to Natal fly and marula fly could, as discussed by Aluja et al. (1989), possibly be due to differences protein requirements between fruit fly species. Medfly could have a higher protein requirement compared to the two other species.

A numerical increase in fly response to HymLure for all species was observed following an increase in concentration of the bait, although this was only significant for Medfly, particularly the males of this species. With GF-120, a significant increase in fly response was observed for males and females of Medfly and females of Natal fly as the concentration of the bait increased up to 5%, beyond which fly response decreased. Fabre et al. (2003) also found that an increase in concentration of protein hydrolysates was associated with an increase in response of the melon fly, *Zeugodacus cucurbitae* (Coquillett). Attraction of fruit flies to protein bait is mainly mediated by ammonia (Bateman and Morton, 1981) and the more concentrated baits possibly have more active compounds, including ammonia. Mazor et al. (1987) found a positive correlation between ammonia release of baits and their attractiveness to Medfly, although the authors discussed that volatiles other than ammonia might also play a role in attraction to baits.

Medfly females and marula fly females and males had a higher response to fresh M3 bait than to the aged M3 baits, although differences were not significant between bait ages. M3 bait attracted significantly more females than males for Medfly and marula fly but not for Natal fly. The attractiveness of the M3 bait even after 8 weeks of aging suits its use as a bait station.

More detailed results can be obtained in the following paper: Manrakhani A & Kotze C (2011) Attraction of *Ceratitidis capitata*, *C. rosa* and *C. cosyra* (Diptera: Tephritidae) to proteinaceous baits. Journal of Applied Entomology 135: 98-105

A2. Attraction and feeding responses of Medfly to new protein attractants

Among the new protein attractants tested on Medfly, Prolure was found to be a promising one (Fig. 3.3.2.3). Prolure and HymLure at 2% dilution with water were the most attractive baits for Medfly females ($F_{6,35}=6.45$, $P=0.00$). Prolure at 2% dilution with water was the most attractive bait for Medfly males ($F_{6,35}=4.92$, $P=0.00$).

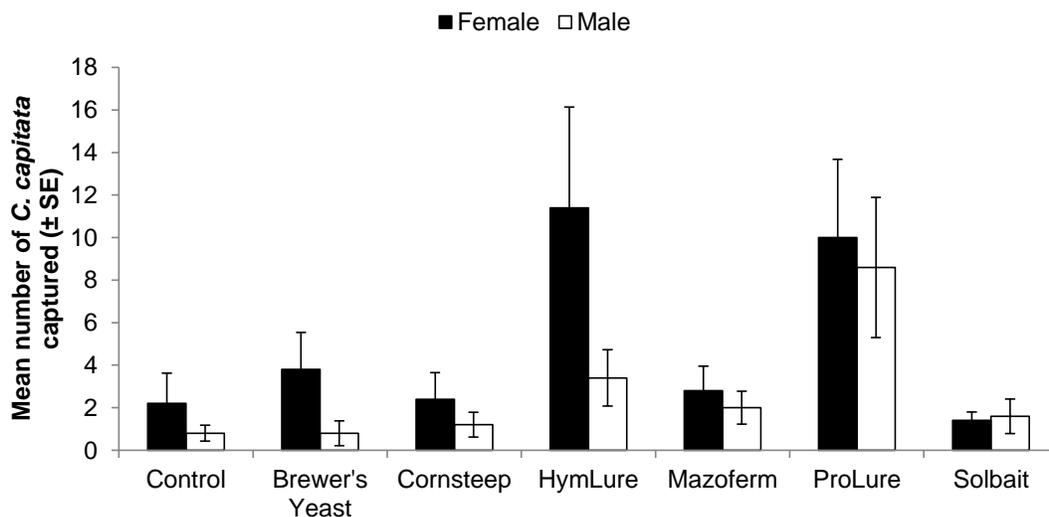


Figure 3.3.2.3. Responses of *C. capitata* females and males to odours from different protein baits in a field cage at CRI Nelspruit. All baits were diluted at 2% with water. A control (no bait) was also included.

Medfly females consumed more Prolure and HymLure compared to the other protein hydrolysates (Fig. 3.3.2.4). Differences in net consumption of protein solutions were however not statistically significant ($F_{5,23}=2.65$, $P=0.06$).

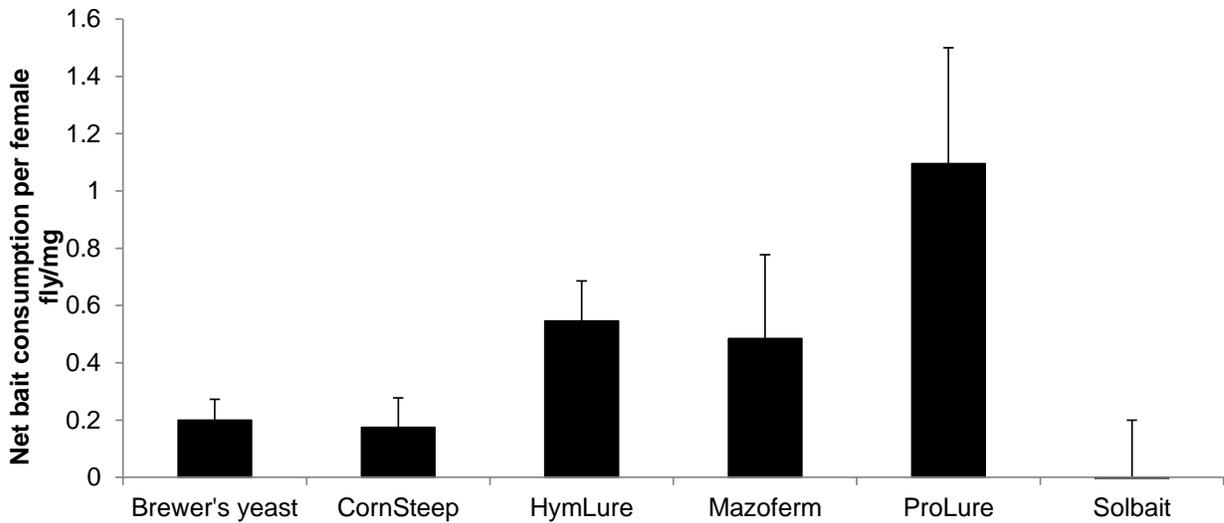


Figure 3.3.2.4. Consumption of different protein baits by *C. capitata* females over 24 hours. All baits were diluted at 2% with water.

The type of protein attractant had an effect on the percentage of flies feeding (confirmed by the presence of green dye) ($F_{6,27}=20.70$, $P<0.0001$). Flies fed preferably on Prolure compared to the other baits, although differences between Prolure and HymLure were not statistically significant (Fig. 3.3.2.5).

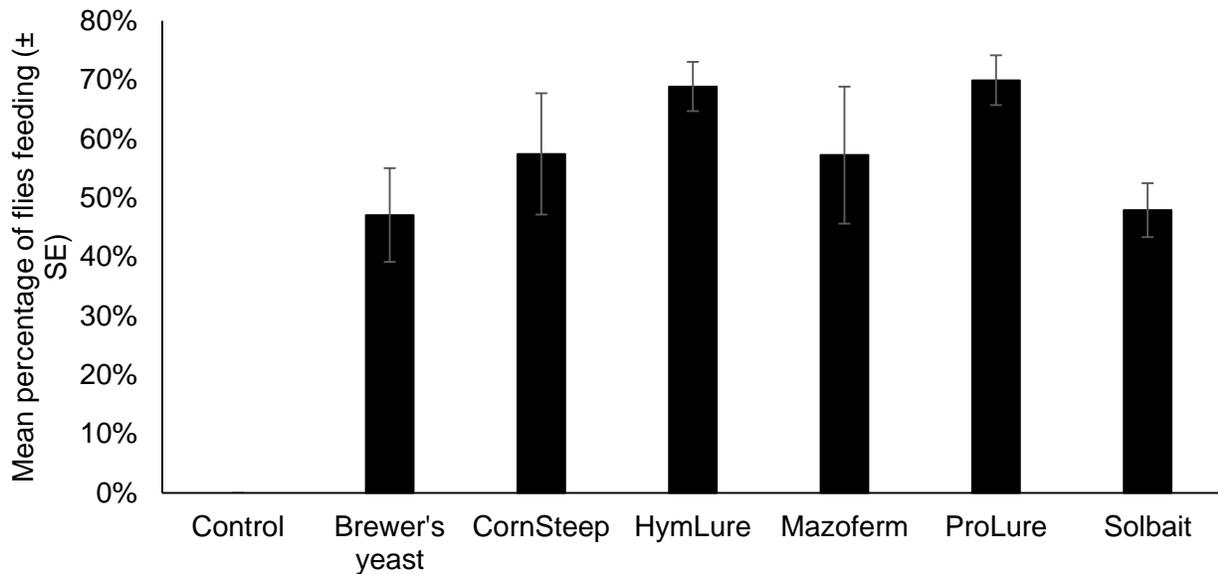


Figure 3.3.2.5. Feeding responses of *C. capitata* females to different protein baits over 24 hours. All baits were diluted at 2% with water.

A3. Field evaluation of a promising new attractant - Prolure

There were significant Natal fly and marula fly populations in both study sites whilst Medfly population was higher in the citrus orchard in Brackenhill and practically non-existent in the mango orchard in Oeversig.

There were significant differences among the different concentrations of Prolure tested, 0.4% HymLure and the control (water) in both study sites (Fig 3.3.2.6). There were significant effects of species and sex on responses to

baits in both study sites (Table 3.3.2.1). The interactions between treatment and species were also significant indicating that the species reacted differently to the treatments offered. There was no significant interaction between treatment and sex, indicating that both males and females reacted the same way to the treatments. As such, data was presented separately for each species and male and female catches were pooled together.

In both sites, the highest number of Natal flies was caught in the 1% Prolure concentration and for marula fly catches were highest for the 10% concentration of Prolure. Medfly catches were highest at the 10% Prolure concentration in the citrus orchard at Brackenhill. A 0.4% Prolure concentration was significantly more attractive to Natal fly and marula fly compared to a 0.4% HymLure.

ProLure seems to be a promising candidate that could be an alternative to HymLure and could be more effective against marula fruit fly.

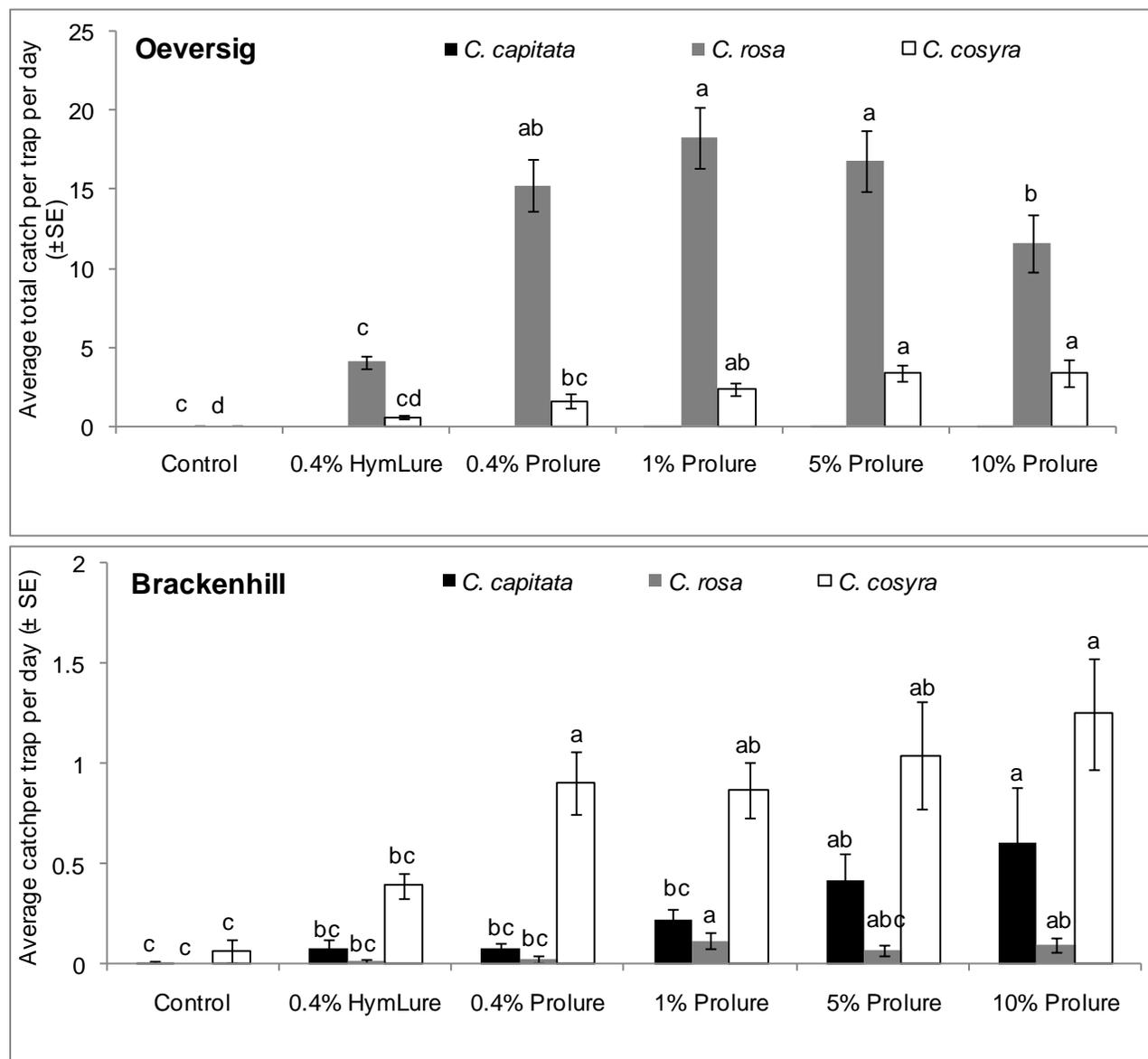


Figure 3.3.2.6. Average catch of *C. capitata*, *C. rosa* and *C. cosyra* per trap per day for four different Prolure concentrations, 0.4% HymLure (Standard) and water (negative control) in the mango orchard in Oeversig and citrus orchard in Brackenhill. For each species, treatments with different letters were significantly different (P<0.05).

Table 3.3.2.1. ANOVA for comparisons of catches in different concentrations of Prolure and 0.4% HymLure in Oeversig and Brackenhill.

Factors	Oeversig			Brackenhill		
	F	df _(corrected=1535)	P	F	df _(corrected=1295)	P
Treatment	187.50	5	<0.01	17.72	5	<0.01
Species	1431.57	2	<0.01	101.30	2	<0.01
Sex	22.89	1	<0.01	23.33	1	<0.01
Replicate	2.96	5	0.01	2.93	5	0.01
Treatment x Species	83.15	10	<0.01	5	10	<0.01
Treatment x Sex	1.42	5	0.22	1.10	5	0.36
Treatment x Replicate	1.06	25	0.39	1.36	25	0.11

B. Efficacy of toxicants other than malathion in combination with baits

There was a significant effect of type of insecticide, HymLure concentration and time on mortality of Medfly females (Insecticide: $F_{5,1049}= 159.96$, $P<0.01$; HymLure concentration: $F_{2, 1049}= 19.40$, $P<0.01$; Time: $F_{3, 1049}= 223.27$, $P<0.01$). Among the toxicants tested in this study, spinosad was the most effective in combination with HymLure in achieving a higher and faster kill of Medfly females. Mortality of Medfly females after 4 hours of toxic bait exposure was below 50% for all baited insecticides except when the two highest spinosad concentrations were combined with 10% HymLure.

When spinosad was combined with 0.8% HymLure, average cumulative mortalities reached above 80% after 72 hours for the whole range of concentrations tested. However, when spinosad was combined with 2% HymLure, mortalities above 80% were attained already after 24 hours for all spinosad concentrations tested. With a mixture of spinosad and 10% HymLure, cumulative mortalities were above 90% for all concentrations of spinosad after 24 hours of exposure.

Fipronil, tartar emetic and imidacloprid were effective only at the higher concentrations tested when combined with 2% and 10% HymLure solutions. All three toxicants, however, were generally slower acting compared to spinosad. When fipronil was combined with 0.8 % HymLure, an average fly kill of above 80% was achieved after 72 hours with the highest concentration of the toxicant. With increasing HymLure concentration in a HymLure-fipronil solution, mortality values of above 80% were attained earlier, after 48 hours, with again the highest concentration of the toxicant. With tartar emetic, all mortality values were below 80% even after 72 hours when the toxicant was combined with 0.8% HymLure. However, when tested in combination with 2% HymLure, up to 100% fly mortality was attained after 72 hours with the highest concentration of the toxicant (7 978.5 ppm). With imidacloprid, cumulative mortality values above 70% were only achieved after 48 hours when the second highest concentration of the toxicant was combined with a 10% HymLure solution.

Alpha-cypermethrin and abamectin were not as effective as other toxicants in killing Medfly females at the rates tested. The highest average cumulative mortality (after 72 hours) achieved when the highest concentrations of alpha-cypermethrin (194.4 ppm) and abamectin (36.5 ppm) were combined with 10% HymLure were 72% ± 6% and 55% ± 9%, respectively.

In laboratory assays, mortality of Medfly females was significantly higher with a mixture of 2% HymLure and spinosad (48 ppm) compared to the standard treatments of GF-120 and HymLure-malathion after 24 hours ($F_{2,17}=6.76$, $P=0.01$). After 48 hours, mortality of Medfly females was significantly higher with both spinosad based

treatments: mixture of 2% HymLure and spinosad and GF-120, than with the HymLure-malathion combination ($F_{2,17}=6.25$, $P=0.01$). For Natal fly after 24 hours, mortality was significantly higher with mixtures of HymLure with either spinosad or malathion compared to GF-120 ($F_{2,17}=16.82$, $P<0.01$). After 48 hours, there were no significant differences in mortality of Natal fly females between treatments ($F_{2,17}=3.06$, $P=0.08$). For both species at the first interval of 4 hours and at the last interval of 72 hours, there were no significant differences between the treatments.

In the first year of field evaluation, male Medfly catches were significantly lower in the HymLure-spinosad treated block compared to the block with a standard treatment ($P=0.01$) whilst for Natal fly there were no significant differences between treatments. In the second year of evaluation, there were no significant differences in male Medfly catches between treatments but Natal fly male catches were lower in the block with a standard treatment compared to the block with the HymLure-spinosad treatment ($P=0.04$). There were however no significant differences in female Medfly and Natal fly catches between treatments in the two years of field trial. No fruit fly damage was recorded in either of the two treated blocks in the first year. However, in the second year, in each treatment there was one fruit fly infested fruit recorded out of 500 fruit examined on the tree at harvest time. Only Natal fly adults were reared from each infested fruit. The infested fruit in the HymLure-spinosad treatment had a higher degree infestation (10 flies per 100 g of fruit) compared to the standard treatment (1 fly per 100 g of fruit).

Since the commercial availability of GF-120, the use of spinosad mixed with other protein attractants for fruit fly control was not much explored. Recently a mixture of 0.1% Spintor 480® (spinosad) and a protein hydrolysate, Nu-lure, (at 0.5%) was found to be as effective as the standard treatments of GF-120 and HymLure and malathion in reducing Medfly adult populations in the field in Spain (Chueca et al., 2007) which concurs with our field results. For all toxicants tested, efficacy of the attractant-toxicant mixture was improved following an increase in the concentration of HymLure. An increase in the concentration of HymLure would most likely increase the attractiveness of the mixture to flies as well as lead to an increased ingestion of the mixture.

More detailed results of this study can be found in Manrakhan A, Kotze C, Daneel J-H, Stephen P-R & Beck R-R (2013) Investigating a replacement for malathion in bait sprays for fruit fly control in South African citrus orchards. *Crop Protection* 43: 45-53.

C1 Toxicity and attraction of the Tephri bait station

The malathion based Tephri bait station and the spinosad bait station (fresh and aged stations for up to 12 weeks) were both as or more toxic than the standard baits for all fruit fly species (Fig 3.3.2.7 and Table 3.3.2.2). Medfly was the most susceptible fruit fly species to all baits including the Tephri bait stations. There was a higher mortality of all fruit fly species on all baits tested after 48 hours. For Medfly, the toxicity of both the malathion based and the spinosad based Tephri bait stations remain unchanged for 12 weeks reaching up to 100 % corrected mean mortality (Figure 3.3.2.7) after 48 hours. For the six and eight weeks weathered stations, the spinosad based Tephri bait station was more effective than the malathion based Tephri bait station for Natal fly. The spinosad based Tephri bait station was also more effective than the malathion based Tephri bait station for marula fly at eight weeks.

The spinosad based Tephri bait station was more attractive than the other baits at all ages except when tested fresh (Fig 3.3.2.8 and Table 3.3.2.3). When bait stations were fresh, the M3 bait station was the most attractive of all the baits. Attractiveness of the M3 bait station decreased after two weeks of aging. Again, like in the toxicity assays, Medfly was the most responsive flies to all baits. The malathion based Tephri bait station was as attractive as fresh solutions of either GF-120 or mixture of HymLure and malathion.

C2 Field evaluation of a malathion based Tephri and spinosad based Tephri bait station

Medfly was the dominant fruit fly species in the study orchards. There were differences in catches of males and females of Medfly between the treatments (males: $F_{4,77}= 24.05$, $P<0.0001$; females: $F_{4,77}=22.00$, $P<0.0001$). Among the treatments tested, GF 120 was the most effective in reducing catches of both males and females of Medfly (Fig. 3.3.2.9). Catches of Medfly males in Capilure baited traps in blocks treated with all bait stations (Tephri bait stations and M3 bait stations) remained mostly above threshold (4 males per trap per week) during treatment

(Figure 3.3.2.9). The spinosad based Tephri bait station was more effective than the M3 fruit fly bait station in reducing catches of Medfly males. Catches of Medfly females in Questlure baited traps were however lower in GF-120 and M3 treated blocks compared to blocks treated with the Tephri bait stations and the untreated control. Catches of Medfly females in Questlure baited traps in blocks treated with Spinosad based Tephri bait station remained at or below threshold (1 female fly per trap per week) three weeks after the stations were set out (Fig. 3.3.2.9). In contrast, catches of Medfly females in Questlure baited traps in blocks treated with malathion based Tephri bait stations failed to consistently stay at or below threshold during treatment (Figure 3.3.2.9). No fruit fly infestation was detected in any of the fruit examined on the trees in all the blocks including control.

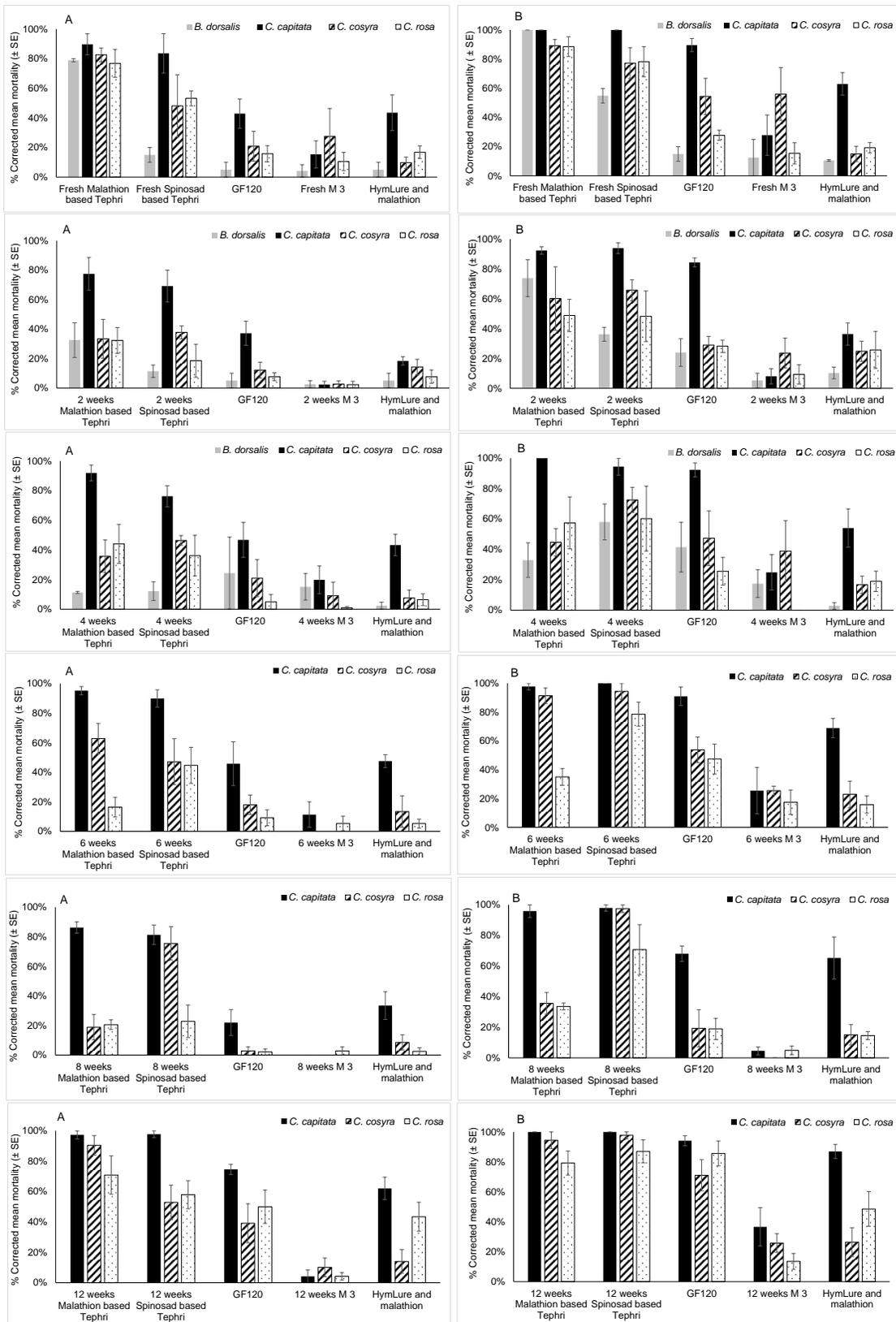


Figure 3.3.2.7. Corrected mean mortality of *B. dorsalis*, *C. capitata*, *C. cosyra* and *C. rosa* at 24 h (A) and 48 h (B) following exposure to Malathion based Tephri bait station, Spinosad based Tephri bait station, GF-120, M3 fruit fly bait station and mixture of HymLure and malathion. Bait stations were evaluated at different ages and compared with fresh GF-120 and fresh HymLure and malathion mixture.

Table 3.3.2.2. Repeated measures ANOVA results showing the effects of species, treatment (baits), time and interactions between species and treatment on fly mortality. Results shown are degrees of freedom (df [numerator, denominator]), F value, p value on a significance level of 5% for each age of bait station tested.

Age of bait station	Effects	df	F	p
Fresh	Species	3,50	9.88	<0.0001
	Treatment	4,50	31.32	<0.0001
	Time	1,69	86.64	<0.0001
	Species x Treatment	12,50	1.55	0.14
Two weeks	Species	3, 60	15.03	<0.0001
	Treatment	4,60	25.89	<0.0001
	Time	1,79	114.76	<0.0001
	Species x Treatment	12,60	1.92	0.05
Four weeks	Species	3,55	18.91	<0.0001
	Treatment	4,55	15.14	<0.0001
	Time	1,74	78.11	<0.0001
	Species x Treatment	12,55	1.35	0.22
Six weeks	Species	2,45	34.82	<0.0001
	Treatment	4,45	35.26	<0.0001
	Time	1,59	106.42	<0.0001
	Species x Treatment	8,45	2.92	0.01
Eight weeks	Species	2,45	37.16	<0.0001
	Treatment	4,45	55.86	<0.0001
	Time	1,59	73.05	<0.0001
	Species x Treatment	8,45	5.38	<0.0001
Twelve weeks	Species	2,45	13.38	<0.0001
	Treatment	4,45	44.99	<0.0001
	Time	1,59	75.45	<0.0001
	Species x Treatment	8,45	2.20	0.05

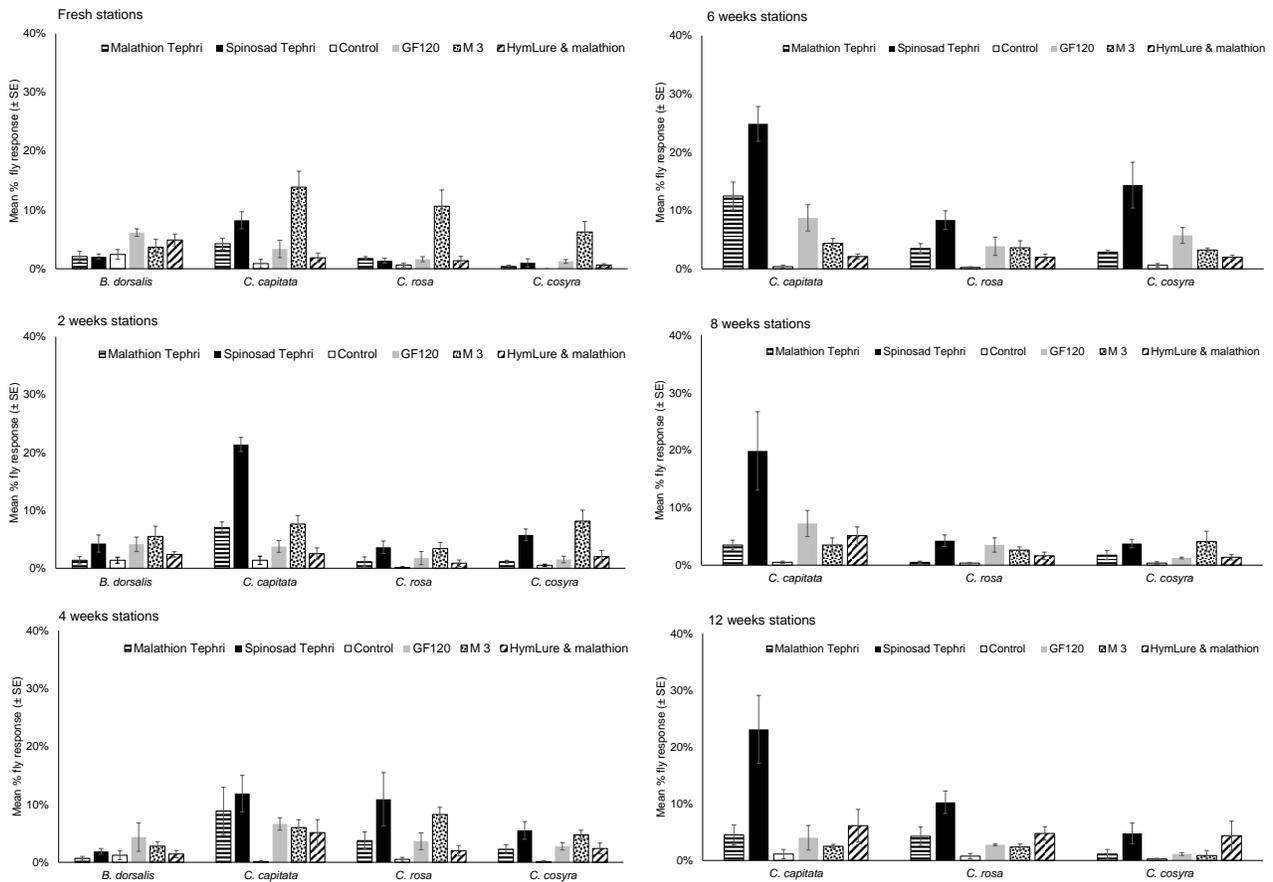


Figure 3.3.2.8. Mean percentage adult fly (males and females combined) responses of different fruit fly species to different bait treatments and control (no bait) in a semi field cage at CRI Nelspruit. The bait stations were evaluated at six ages presented in separate charts. GF120 and HymLure and malathion mixtures were evaluated fresh.

Table 3.3.2.3. ANOVA results showing the effects of treatment (baits), replicate, species, sex and interactions between species and treatment on fly responses for each age of bait station. Results shown are degrees of freedom (df [numerator, denominator]), F value, p value on a significance level of 5% for each age of bait station tested.

Age of bait stations	Effect	df	F	p
Fresh	Treatment	5,191	28.78	<0.0001
	Replicate	3,191	1.86	0.14
	Species	3,191	15.18	<0.0001
	Sex	1,191	0.18	0.67
	Treatment x Species	15,191	5.78	<0.0001
2 weeks	Treatment	5,191	40.58	<0.0001
	Replicate	3,191	2.69	0.05
	Species	3,191	38.19	<0.0001
	Sex	1,191	0.00	1.00
	Treatment x Species	15,191	12.28	<0.0001
4 weeks	Treatment	5,191	13.60	<0.0001
	Replicate	3,191	5.98	0.001
	Species	3,191	13.69	<0.0001
	Sex	1,191	0.24	0.63
	Treatment x Species	15,191	2.70	0.001
6 weeks	Treatment	5,143	54.52	<0.0001
	Replicate	3,143	1.95	0.13
	Species	3,143	27.40	<0.0001
	Sex	1,143	0.61	0.44
	Treatment x Species	15,143	7.34	<0.0001
8 weeks	Treatment	5,143	15.21	<0.0001
	Replicate	3,143	0.87	0.46
	Species	3,143	22.15	<0.0001
	Sex	1,143	1.94	0.17
	Treatment x Species	15,143	6.56	<0.0001
12 weeks	Treatment	5,143	22.79	<0.0001
	Replicate	3,143	0.39	0.76
	Species	3,143	14.16	<0.0001
	Sex	1,143	2.60	0.11
	Treatment x Species	15,143	4.99	<0.0001

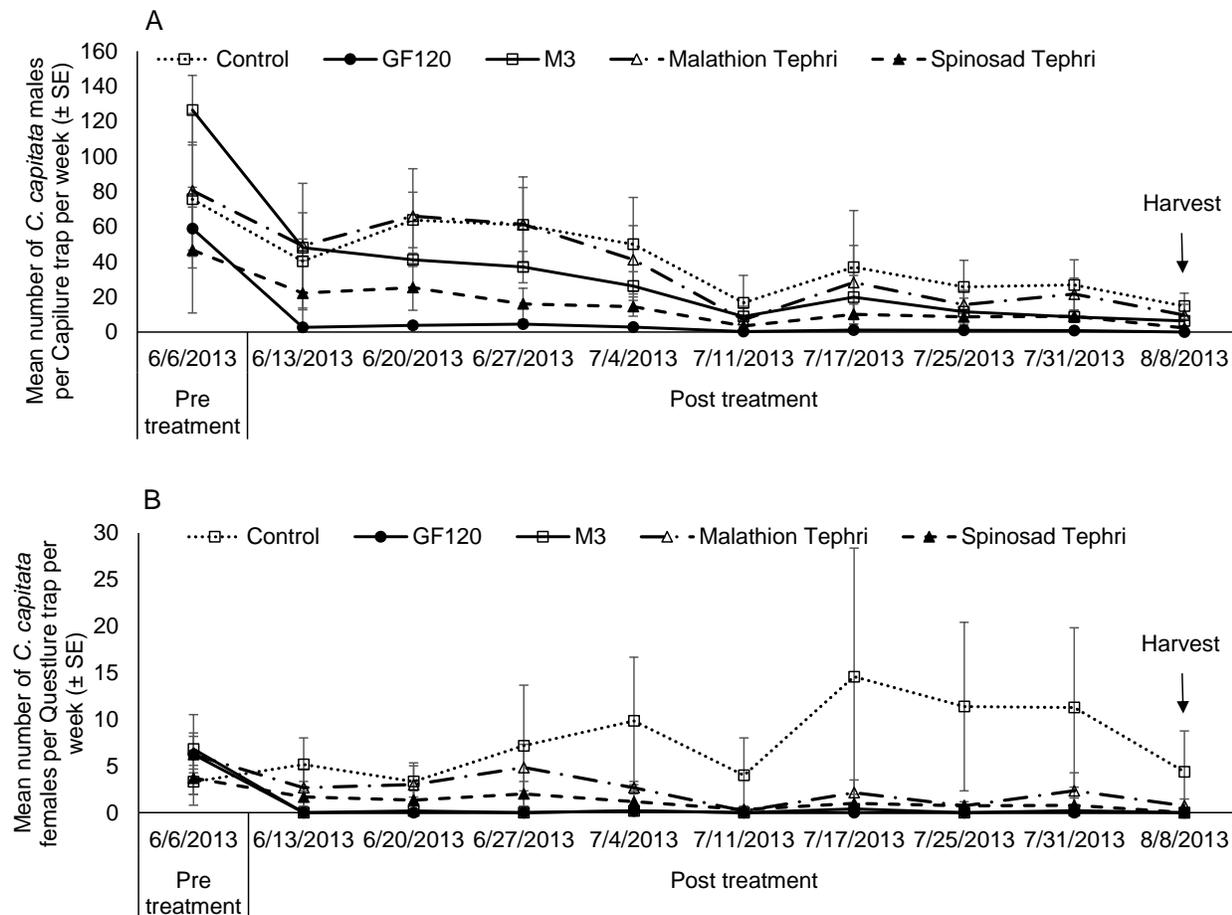


Figure 3.3.2.9. Catches of *C. capitata* males (A) and females (B) in Capilure and Questlure baited traps respectively in blocks prior to and after treatment with different fruit fly baits in MidKnight Valencia orchards in Low's Creek, Mpumalanga Province between June and August 2013.

C3 Field evaluation of a modified malathion based Tephri bait station in different citrus production regions in South Africa

Medfly was the dominant fruit fly species in the citrus orchards selected for the trials on the modified malathion based Tephri bait stations in all three provinces. All comparisons between treatments were therefore carried out on Medfly adult populations.

In trials conducted in Mpumalanga, there were significant differences in catches of Medfly males between treatments in both years (2016: $F_{2,199}=16.39$, $P<0.0001$; 2017: $F_{2,131}=5.20$, $P=0.01$). In 2016 and 2017, catches were significantly lower in GF-120 treated blocks compared to blocks treated with the two types of bait stations (Fig. 3.3.2.10A). In 2016, mean catches of Medfly males in blocks treated with the two types of bait stations were at or above threshold (4 males per trap per week) whilst catches stayed below threshold in blocks treated with GF-120 (Fig. 3.3.2.10A). In 2017, mean catches of Medfly males remained below threshold in all treatments (Fig. 3.3.2.10A). At high fruit fly populations, bait application would be more effective as sprays than as stations since there would be more bait spots per surface area in the former than in the latter. In both trial years, differences in catches of Medfly males between blocks treated with Tephri bait stations and those with M3 were not statistically significant (Fig. xx). There were significant effects of sites in catches of Medfly males in 2016 ($F_{1,199}=14.99$, $P=0.00$). In 2017, there were no significant effects of sites in catches of Medfly ($F_{1,131}=1.12$, $P=0.29$). In both years, there were no significant interactions between sites and treatments (2016: $F_{2,199}=1.48$, $P=0.23$; 2017: $F_{2,131}=1.60$, $P=0.21$) indicating that effects of treatments were the same across the two sites in Mpumalanga.

For female Medfly in trials conducted in 2016 in Mpumalanga, catches in Questlure baited traps were significantly higher in blocks treated with Tephri bait station compared to the other treatments ($F_{2,199}=7.13$, $P=0.001$) (Fig. 3.3.2.10B). In contrast in 2017, there were no significant differences in catches of Medfly females in Questlure baited traps between treatments ($F_{2,131}=2.33$, $P=0.10$). There was an effect of site on catches of Medfly females in Questlure baited traps in 2016 ($F_{1,199}=27.18$, $P<0.001$) and in that same year, there was a significant interaction between site and treatment ($F_{2,199}=8.18$, $P=0.00$). Catches of Medfly females in Questlure baited traps were higher in blocks treated with Tephri bait station compared to the other treatments in only one of the site in Mpumalanga in 2016. In the other site, there were no significant differences in catches of Medfly females between the treatments. In 2017, there were no effects of sites on catches of Medfly females in Questlure baited traps ($F_{1,131}=0.13$, $P=0.72$) and no significant interactions between sites and treatments ($F_{2,131}=1.93$, $P=0.15$).

In contrast to what was depicted of the Medfly populations in Questlure baited traps in trials conducted in Mpumalanga, there was no significant effect of treatment on Medfly female catches in Biolure baited traps in both 2016 and 2017 (2016: $F_{2,199}=1.95$, $P=0.15$; 2017: $F_{2,131}=1.03$, $P=0.36$). It is highly likely that the Questlure baited traps do not reflect female populations in orchards treated with M3 bait stations. Questlure and M3 bait stations have similar attractants and shutdown of Questlure baited traps can occur. Although there were differences in catches of Medfly females in Biolure baited traps between the sites in both trial year (2016: $F_{1,199}=27.17$, $P<0.0001$; 2017: $F_{1,131}=10.80$, $P=0.001$), there were no significant interactions between sites and treatments (2016: $F_{2,199}=1.34$, $P=0.26$; 2017: $F_{2,131}=0.40$, $P=0.67$).

Trials conducted in Eastern Cape Province over the two years showed numerically higher catches of *C. capitata* males and females in blocks treated with Tephri bait stations compared to those treated with M3 bait stations (Fig. 3.3.2.11 A). Differences between the two bait stations were however only statistically significant for the *C. capitata* male catches in 2016 ($F_{1,29}=5.36$, $P=0.03$).

In Western Cape Province, lower catches of *C. capitata* males were found in blocks treated with Tephri bait station compared to those treated with M3 bait stations in 2016 ($F_{1,54}=7.64$, $P=0.01$) (Fig. 3.3.2.12). In 2017, there were no significant differences in *C. capitata* male numbers between blocks treated with the two types of bait stations ($F_{1,29}=0.26$, $P=0.62$). There were no significant differences in catches of *C. capitata* females between blocks treated with the two types of bait stations in both trial years (Questlure: 2016: $F_{1,54}=0.43$, $P=0.51$, 2017: $F_{1,29}=1.00$, $P=0.33$; Biolure: 2016: $F_{1,54}=1.79$, $P=0.19$, 2017: $F_{1,29}=0.26$, $P=0.62$).

No fruit fly damage was recorded on fruit on trees at harvest in blocks treated with the different bait treatments in all trial sites in both years. For fruit collected from the ground in the second year, however, there was one positive fruit fly infestation from one replicate block of the M3 fruit fly bait station in one orchard in Mpumalanga. The fruit fly infestation rate in that particular replicate block of the M3 fruit fly bait station was 0.65 Medfly adults per kg of fruit (2 Medfly females reared from 3.09 kg of fruit).

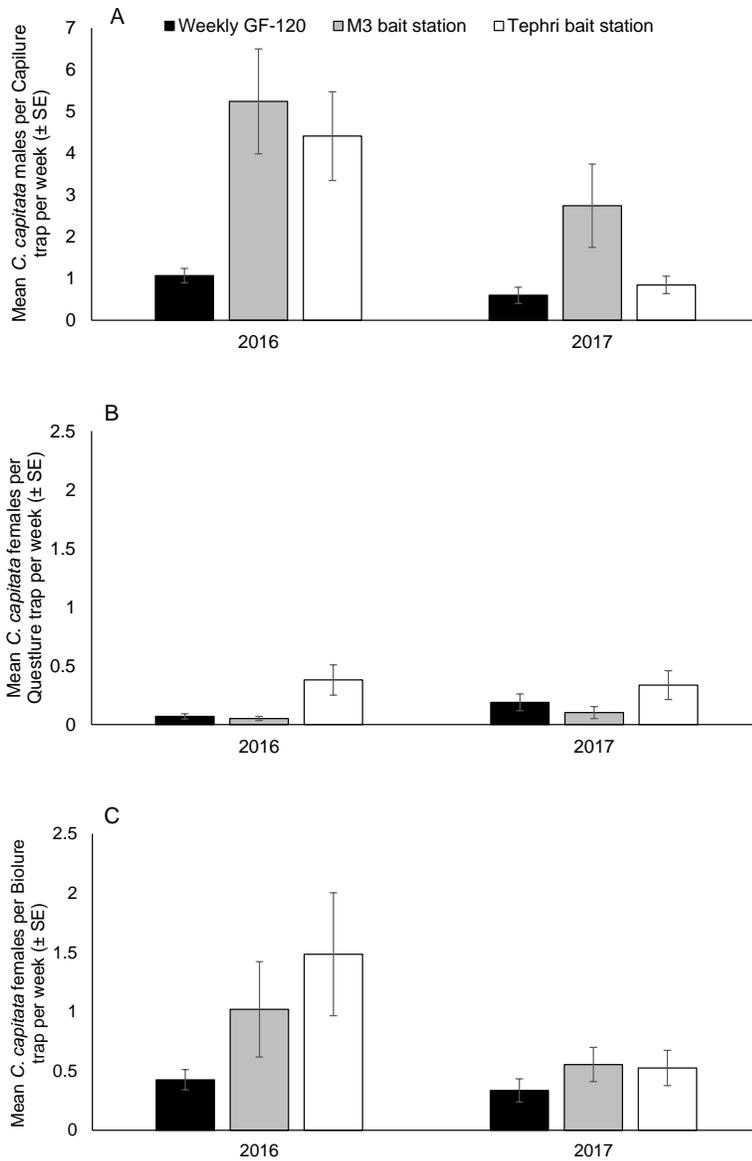


Figure 3.3.2.10. Catches of *C. capitata* males in Capilure baited traps (A), *C. capitata* females in Questlure baited traps (B) and *C. capitata* females in Biolure baited traps in trials conducted on three bait treatments (Weekly GF-120 sprays, M3 bait station at 300 units per ha and Tephri bait stations at 200 units per ha) in 2016 and 2017 in Midnight Valencia orchards in Mpumalanga Province.

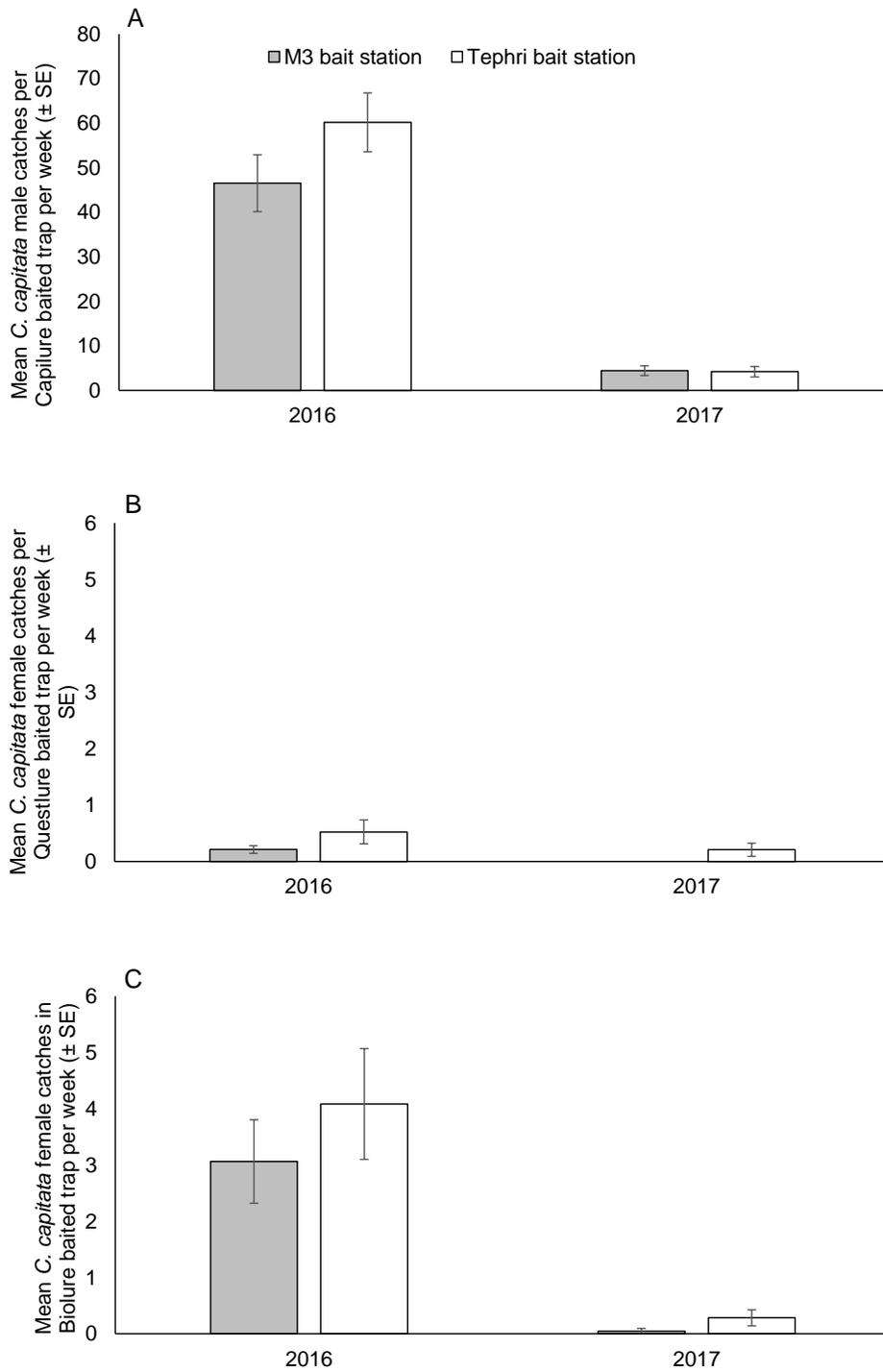


Figure 3.3.2.11. Catches of *C. capitata* males in Capilure baited traps (A), *C. capitata* females in Questlure baited traps (B) and *C. capitata* females in Biolure baited traps in trials conducted on three bait treatments (Weekly GF-120 sprays, M3 bait station at 300 units per ha and Tephri bait stations at 200 units per ha) in 2016 and 2017 in Midnight Valencia orchards in Eastern Cape Province.

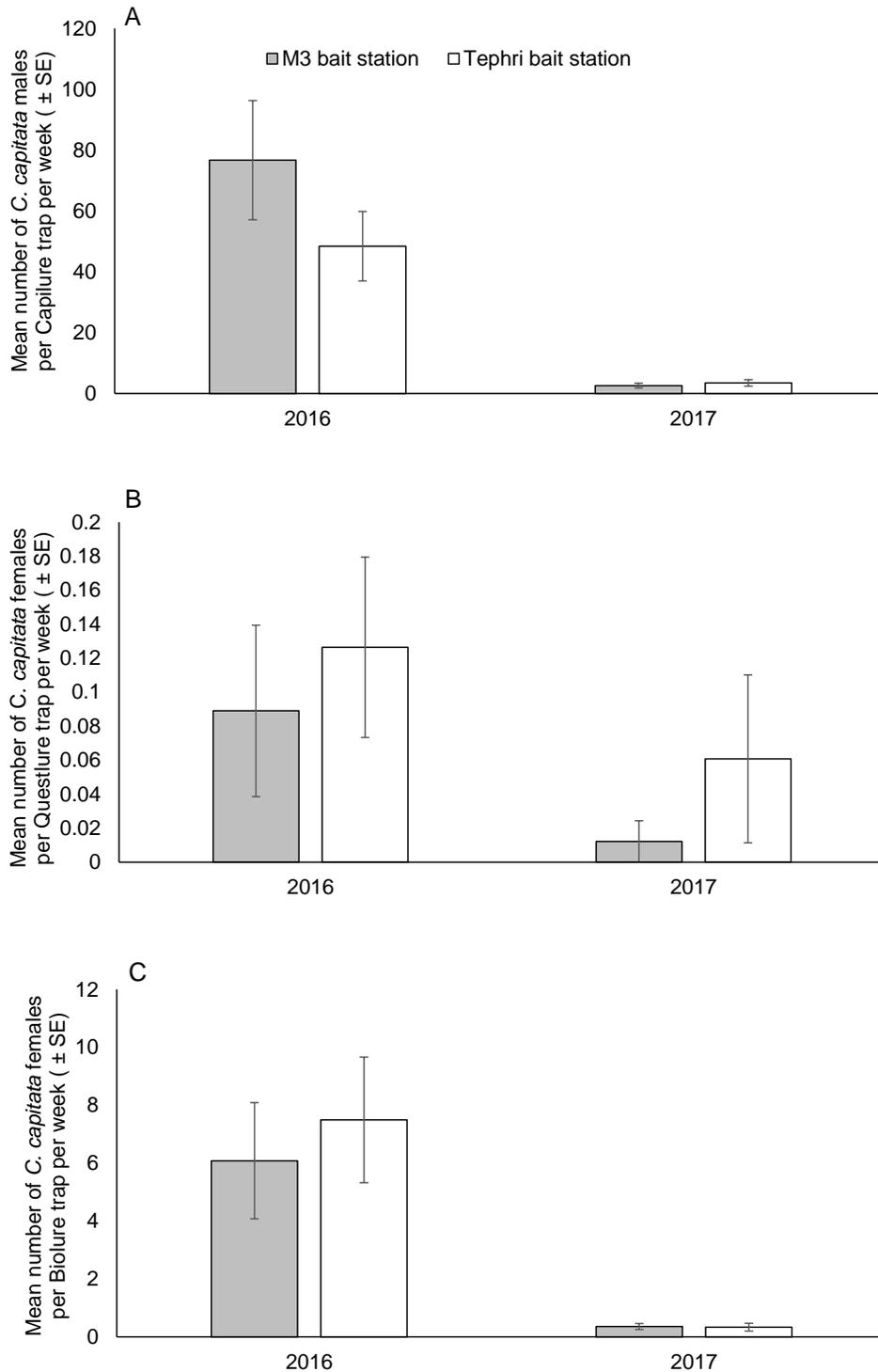


Figure 3.3.2.12. Catches of *C. capitata* males in Capilure baited traps (A), *C. capitata* females in Questlure baited traps (B) and *C. capitata* females in Biolure baited traps in trials conducted on three bait treatments (Weekly GF-120 sprays, M3 bait station at 300 units per ha and Tephri bait stations at 200 units per ha) in 2016 and 2017 in Midnight Valencia orchards in Western Cape Province.

Conclusion

1. ProLure is a promising new bait that is attractive and palatable to three fruit fly pest species: Medfly, Natal fly and marula fly. This bait should be registered for use in fruit fly bait sprays.
2. Spinosad was found to be the most effective alternative toxicant to malathion. A combination of 0.01% spinosad and 2% HymLure was found to be as or more effective than the standard treatments of GF-120 and HymLure and malathion, both in laboratory and field assays.
3. The paper based Tephri bait station at the rate of 200 units per ha was found to be as effective as the M3 fruit fly bait station at the rate of 300 units per ha in field trials in different citrus production regions in South Africa. Efforts towards commercialisation of this bait should continue as it would be an added option in the control package for fruit flies.

Future research

Further work on optimal baits for *B. dorsalis* should be carried out. This pest was found to have a lower response to fruit fly baits, even the currently registered ones.

Technology transfer

- Results on the efficacy of the HymLure and spinosad mixture were presented during the 7th Citrus Research Symposium in Drakensberg 19-22 August 2012.
- Results on baits presented at Fruit fly, CRI Pest and Disease Management Workshops, 3-13 September 2013.
- Results on baits presented at Fruit fly pests of citrus, CRI Pest and Disease Workshops, 15 September – 6 October 2015.
- Results on baits presented at Fruit fly, Pest and Disease workshop, September 2017
- Results on baits presented at Fruit fly, Pest and Disease workshop, September 2018

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

Summary

There have been claims of phytotoxicity when baits for control of fruit flies are applied on copper treated fruit. The aim of this project was to determine whether there is an incompatibility between copper and fruit fly bait on citrus fruit. Trials were conducted in a Valencia orchard in Nelspruit, Mpumalanga Province. Three copper products were tested: cuprous oxide, copper hydroxide and copper oxychloride at different application rates, varying from a single application in January up to four monthly applications between October and January. Two fruit fly baits: GF-120 and a mixture of HymLure and malathion were applied separately on fruit treated with copper and those without copper. This study was carried out over two years between 2016 and 2018. In the first year, baits were applied once as a cover spray on fruit in February. In the second year, baits were applied, again only once, to marked areas on fruit in February, March and April. In the second year, water was also included as a control for the baits. In both years, treated fruit were collected at harvest in July for assessment of phytotoxic symptoms. In the first year, stippling marks were found on fruit treated with both types of bait irrespective of whether copper was applied. In the second year, stippling marks were observed on fruit treated with both types of bait and copper, irrespective of copper products and application rates. There were very few or no stippling marks when water was applied on copper treated fruit and no stippling marks when water was applied on fruit with no copper. The timing of bait application had an influence on incidence of stippling with fewer marks observed when baits were applied in April.

Opsomming

Daar was al bewerings van fitotoksisiteit wanneer lokaasmiddels vir beheer van vrugtevlieë op koper-behandelde vrugte toegedien word. Die doel van hierdie projek was om te bepaal of daar 'n onverenigbaarheid tussen koper en vrugtevlieë lokaasmiddels op sitrusvrugte is. Proewe is in 'n Valencia boord in Nelspruit, Mpumalanga-provinsie, uitgevoer. Drie koper produkte is getoets: koper(I)oksied, koperhidroksied en koperoksichloried teen verskillende toedieningstempo's, wat vanaf 'n enkel toediening in Januarie, tot vier maandelikse toedienings tussen Oktober en Januarie gevarieer het. Twee vrugtevlieë lokaasmiddels: GF-120 en 'n mengsel van HymLure en malation is afsonderlik op vrugte wat met koper behandel is, en sonder koper, toegedien. Hierdie studie is oor twee jaar tussen 2016 en 2018 uitgevoer. In die eerste jaar is lokaasmiddels eenmalig as 'n bedekkingsspuit op vrugte in Februarie toegedien. In die tweede jaar is lokaasmiddels weereens slegs eenmalig op gemerkte areas op vrugte in Februarie, Maart en April toegedien. In die tweede jaar is water ook as 'n kontrole vir die lokaasmiddels ingesluit. In beide jare is behandelde vrugte tydens oes in Julie versamel vir die beraming van fitotoksiese simptome. In die eerste jaar is stippelmerke op vrugte gevind wat met beide tipes lokaasmiddels behandel is, ongeag of koper toegedien is. In die tweede jaar is stippelmerke op vrugte waargeneem wat met beide tipes lokaasmiddels en koper behandel is, ongeag die koperprodukte en toedieningstempo's. Daar was baie min of geen stippelmerke wanneer water toegedien is op koper-behandelde vrugte, en geen stippelmerke wanneer water toegedien is op vrugte met geen koper nie. Die tydsberekening van lokaas-toediening het 'n invloed gehad op die voorkoms van stippeling, met minder merke wat waargeneem is wanneer lokaasmiddels in April toegedien is.

Introduction

Fruit flies and Citrus Black Spot (CBS) are among the key phytosanitary pests in the citrus industry. Control of these two pests is therefore necessary and for both pests control is mainly done using sprays. In the case of fruit flies, bait sprays commence in late summer (around February). For CBS, fungicidal sprays such as copper sprays are usually applied between October and January (Schutte et al., 1997). The phytotoxicity of fruit fly baits on fruit with and without copper residues has been reported by a few authors (Allwood, 1996, Georgala, 1969). In 2009, a preliminary investigation was carried out by CRI to quantify the phytotoxicity of a fruit fly bait mixture (protein hydrolysate - Loklure and malathion UL), used for aerial application, on Valencia orange fruit with and without copper residues (Manrakhan & Kotze, unpublished). As warned by Georgala (1969), an incompatibility was found

between the fruit fly bait mixture and copper residues. The incompatibility was more pronounced on fruit that had 4 spray applications of copper oxychloride than fruit that had 1 copper oxychloride spray application. Phytotoxicity of the fruit fly bait GF-120 was recently investigated on Nadorcott mandarin. GF-120 was found to be phytotoxic to Nadorcott fruit when applied at the green and colour break stages. With increasing use of copper sprays in citrus orchards to control CBS in order to comply with current European Union regulations, it will be important to determine and quantify the compatibility of fruit fly baits and copper. Various factors such as types of bait sprays, copper formulations and number of copper spray applications on fruit damage would need to be investigated.

Stated objectives

- A. To determine the phytotoxicity of fruit fly baits on fruit with previous exposure to copper sprays.
- B. To determine the influence of copper formulation, number of copper applications and bait type on fruit damage.

Materials and methods

Study site

The study was conducted over two years in a Valencia orange orchard at Crocodile Valley Estates, Nelspruit, Mpumalanga, South Africa. In the first year, a total of 18 trees were selected for the trial. In the second year, a different methodology was used and a total of 40 trees were selected. All treatments were applied to fruit distributed in the middle to lower canopy (between 0.5- 1.0 m above ground) of each tree.

Copper formulation and application rates

Three copper formulations were tested and there was one control (no copper):

1. Cuprous oxide
2. Copper hydroxide
3. Copper oxychloride

In the first year, there were two application rates of each copper formulation: (1) Two consecutive monthly spray applications of copper (December– January) and (2) only one application of copper in January. In the second year, a third application rate of each copper formulation was included which was four consecutive monthly spray applications of copper (October– January). For ease of application, different copper formulations were applied on separate trees.

In the first year, the two application rates of the same copper formulation and the no copper treatment were tested on the same tree. Fruit on a tree which received no copper treatment or only one copper treatment was bagged using a transparent plastic bag before a copper spray. Plastic bags were removed just after the copper spray. There were six trees per copper formulation (with control included on each tree (18 control fruit batches). In the second year, separate trees were used for each copper formulation and application rate and there were separate trees for the no copper treatment. There were 36 trees with copper treatments (4 replicate trees per copper formulation and application rate) and four trees with no copper treatment.

Fruit fly baits

Two fruit fly baits were tested:

1. Hymlure at 0.8% mixed with malathion at 0.175%, diluted with water
2. GF-120 at 10%

In the first year, there was no control for the fruit fly bait. In the second year, water was used as a control for the bait.

In the first year, the two types of baits were applied only once in February. In the second year, the two baits were applied only once in February, March and April.

In the first year, two ml of each bait type was applied on each fruit using a hand held sprayer. In the second year, two μ l of each bait type or water were applied on fruit using an auto pipette on each of 4 marked circles on the stylar end of fruit.

Copper and bait treatment layout

In the first year, on each tree the same treatment was applied to 10 individual fruit. On each tree there were therefore six treatments with 4 copper and bait treatments and 2 bait treatments without copper.

In the second year, the same treatment (bait type including control and bait application period) was applied on 10 fruit on each of 40 trees.

Phytotoxic evaluation

Fruit with treatments were allowed to ripen naturally on the tree. Fruit were picked at full colour and brought back to the laboratory for examination of visible damage symptoms (stippling symptoms). In the first year, the entire fruit was checked for visible damage symptoms whilst in the second year, only the marked areas where bait or no bait was applied were checked. The presence or absence of damage symptoms will be recorded as 1 (present) and 0 (absent) during examination.

Residue analysis

In 2018, on the day of the bait application six fruit were collected from each of the four trees under each copper treatment and for each of the four trees under control in order to quantify copper residues on the fruit. Copper residues were analysed from the washed peels of the sampled fruit at the SGS laboratory in Somerset West, Western Cape Province.

Data analysis

Data was summarised as percentage of fruit showing visible stippling symptoms for each treatment. Data was arcsine square root transformed to equalise variances. A multi factor ANOVA was used on the transformed data to determine effect of copper formulation, copper application rates and bait type on fruit damage. The copper and copper application rates factors were nested.

Results and discussion

In 2017 when the two fruit fly baits: GF-120 and a mixture of HymLure and malathion were applied once in February as a cover spray on fruit treated with and without copper, visible stippling marks (Fig. 3.3.3.1) were found on fruit when the latter were in full colour in July (Fig. 3.3.3.2). The stippling marks were seen as localised green areas with small black necrotic spots which on a closer look were found around the oil glands (Fig. 3.3.1). The incidence of stippling varied between 17% and 60%. There was a significant effect of bait on stippling with a lower incidence of stippling when HymLure and malathion was applied particularly on fruit with no copper (Bait: $F_{1,92} = 4.55$, $P = 0.04$). There was a significantly lower incidence of stippling marks on fruit treated with copper oxychloride and baits (Copper: $F_{3,92} = 3.92$, $P = 0.01$).

In 2018 when the two fruit fly baits: GF-120 and a mixture of HymLure and malathion were applied in February as droplets in marked areas on fruit treated with and without copper, there were again visible stippling marks when the fruit were in full colour in July (Fig. 3.3.3.3 and 3.3.3.4). The incidence of stippling marks when baits were applied in February in the second year of the study varied between 3% and 68%. With water as a control in the second year, the stippling marks could be clearly attributed to baits since there were no or very few stippling marks when water was applied on fruit treated with copper and those left untreated (Fig. 3.3.3.4). This then demonstrated that Valencia fruit were susceptible to phytotoxicity from fruit fly baits applied in February, when fruit are still immature. It was shown before that Nadorcott mandarin fruit were susceptible to one of the fruit fly baits tested in

this study: GF-120 when the fruit were green and at colour break (Manrakhan et al., 2015). This could then mean that other citrus cultivar might also be susceptible to fruit fly baits, particularly at the green immature stage.

It was clear in the second year of the trial that the timing of bait application had a significant effect on incidence of stippling marks with lower incidence of stippling when baits were applied in March and April (Fig. 3.3.3.4). The copper residues in fruit including in the control were also much lower in the fruit after February (Table 3.3.3.1). In March and April however, there was a higher incidence of stippling when fruit fly baits, particularly the mixture of Hymlure and malathion, were applied on fruit which were previously treated with copper.

In the second year of the study, all factors (bait, copper product, time of bait application, copper application rate,) investigated had significant effects on incidence of stippling marks. There was a significantly higher incidence of stippling with GF-120 compared to the mixture of HymLure and malathion and water ($F_{2,343}=224.31$, $P<0.0001$). Incidence of stippling was lower on fruit with no copper ($F_{3,343}=17.73$, $P<0.0001$). Among the fruit treated with copper, incidence of stippling was lower on fruit treated with copper oxychloride. There were differences in incidence of stippling with application rates of copper product ($F_{6,343}=2.34$, $P=0.03$), although there were no specific trends observed. The incidence of stippling was significantly lower when baits were applied in April ($F_{2,343}=9.1$, $P=0.00$).

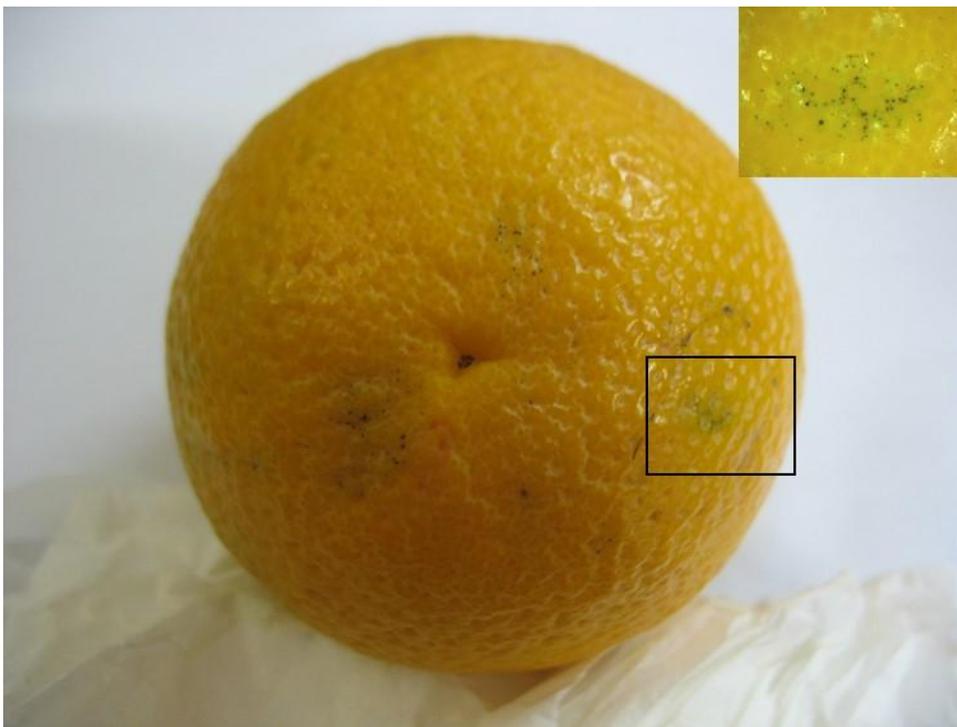


Figure 3.3.3.1 Stippling marks visible to the naked eye on Valencia fruit (treated with fruit fly bait in February 2017). The marks on the fruit are seen on a section of the fruit marked with an open black rectangle. The inset on the top right corner of the picture is a close up view of the stippling marks.

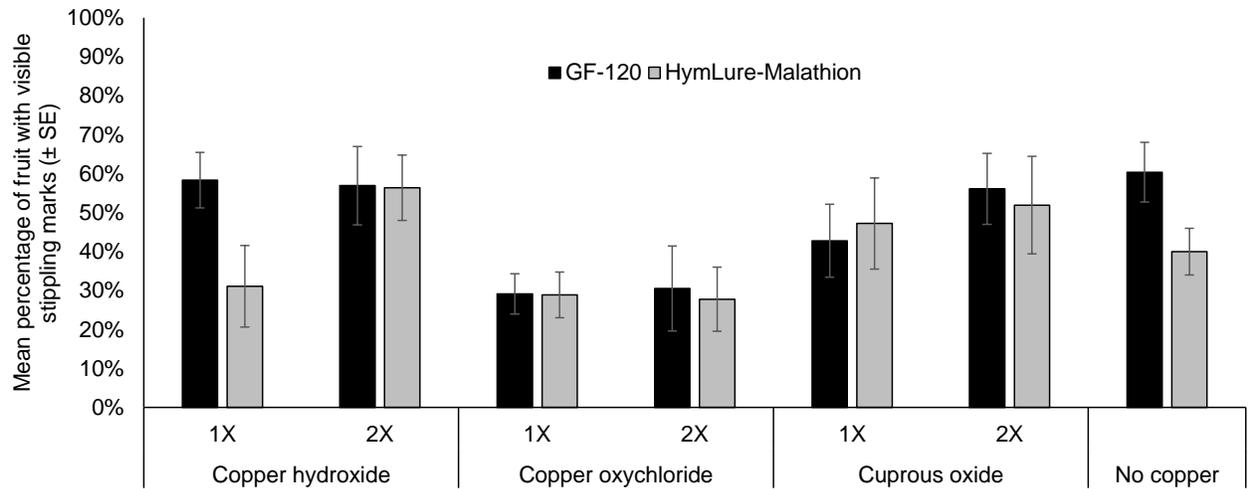


Figure 3.3.3.2 Mean (\pm SE) percentage of fruit with visible stippling marks from combinations of three copper products, two copper application rates (1X: one application, 2X: 2 consecutive monthly applications) and fruit fly baits (GF-120 and mixture of HymLure and malathion) in a Valencia orchard at Crocodile Valley Estates in a trial conducted between 2016 and 2017. Fruit fly baits were applied once in February. No copper was used as control treatment for copper application.

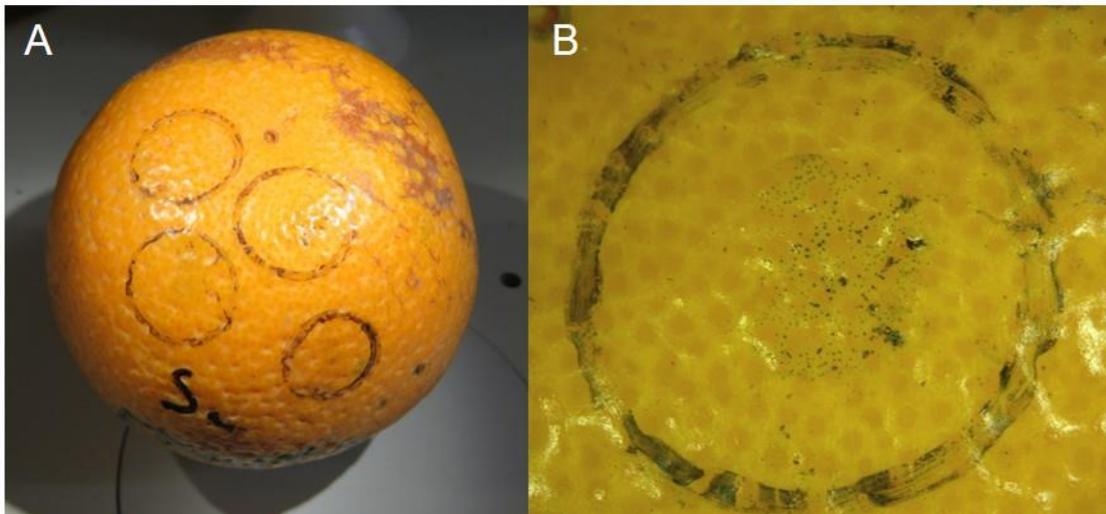


Figure 3.3.3.3 Stippling marks visible to the naked eye in top right marked circle on Valencia fruit (A). A closer view of the stippling marks visible under the microscope (B).

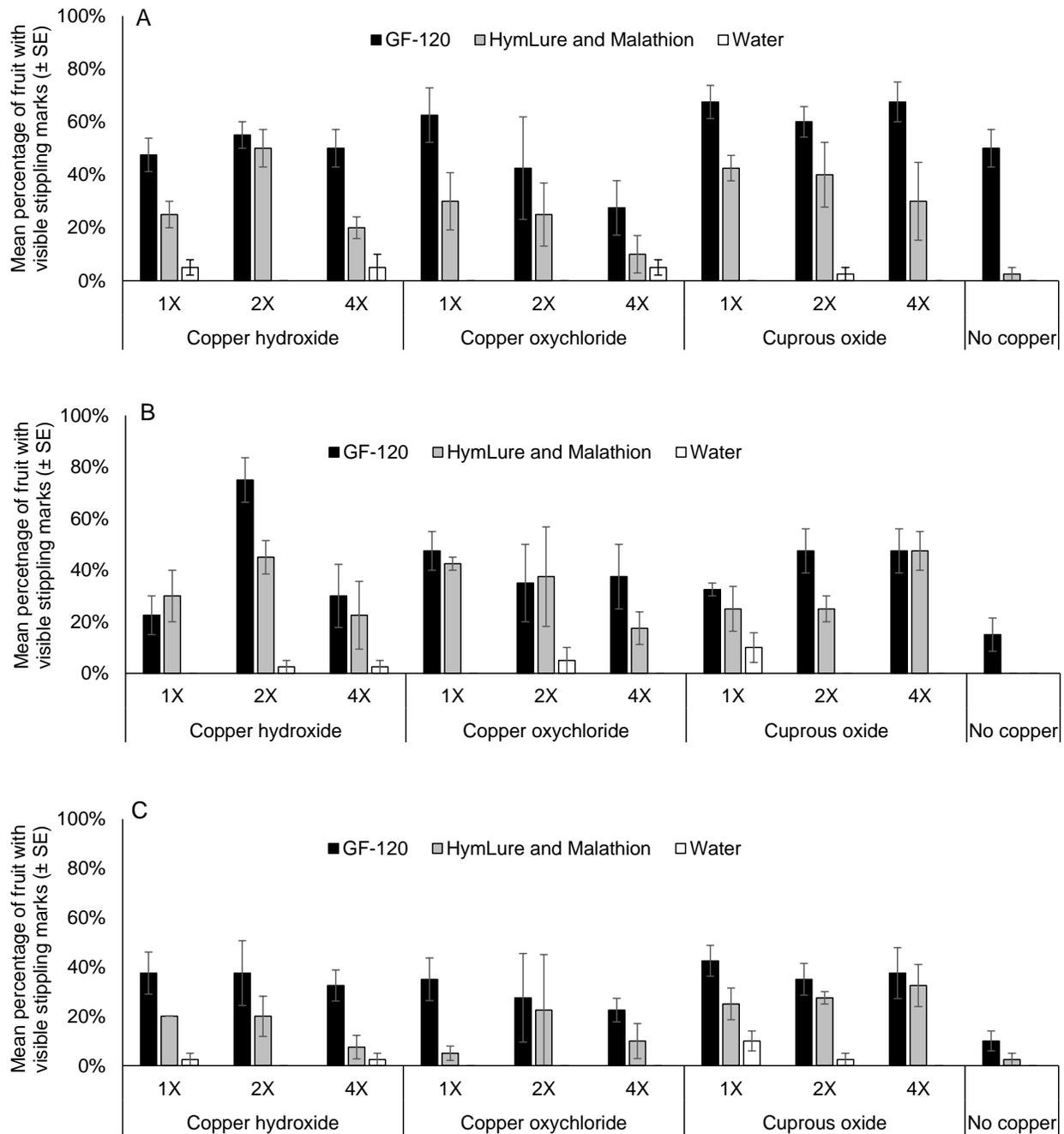


Figure 3.3.3.4 Mean (\pm SE) percentage of fruit with visible stippling marks from combinations of three copper products, three copper application rates (1X: one application, 2X: 2 consecutive monthly applications and 4X: 4 consecutive monthly applications) and fruit fly baits (GF-120, mixture of HymLure and malathion) applied once in (A) February, (B) March and (C) April. No copper and water were used as control treatments for copper application and bait application respectively.

Table 3.3.3.1 Copper residues washed from sampled Valencia fruit surface in 2018 at the three fruit fly bait application times. The last copper application was carried out in January 2018 in all treatments except the control (no copper).

Time	Copper product	Copper application rate	Copper residues washed from peel (mg/kg)
February	Control (no copper)		11.50 ± 0.97
	Copper hydroxide	1x	51.75 ± 3.30
	Copper hydroxide	2x	76.25 ± 2.25
	Copper hydroxide	4x	202.75 ± 9.84
	Copper oxychloride	1x	31.25 ± 2.17
	Copper oxychloride	2x	71.50 ± 8.49
	Copper oxychloride	4x	167.75 ± 11.21
	Cuprous oxide	1x	7.90 ± 0.41
	Cuprous oxide	2x	58.23 ± 4.82
	Cuprous oxide	4x	224.25 ± 4.61
March	Control (no copper)		2.24 ± 0.23
	Copper hydroxide	1x	3.81 ± 0.33
	Copper hydroxide	2x	5.96 ± 0.37
	Copper hydroxide	4x	6.97 ± 0.84
	Copper oxychloride	1x	4.35 ± 0.28
	Copper oxychloride	2x	5.43 ± 0.61
	Copper oxychloride	4x	6.27 ± 0.87
	Cuprous oxide	1x	3.91 ± 0.23
	Cuprous oxide	2x	4.14 ± 0.23
	Cuprous oxide	4x	5.91 ± 0.39
April	Control (no copper)		2.44 ± 0.15
	Copper hydroxide	1x	2.93 ± 0.12
	Copper hydroxide	2x	3.44 ± 0.32
	Copper hydroxide	4x	4.57 ± 1.11
	Copper oxychloride	1x	3.92 ± 0.97
	Copper oxychloride	2x	3.02 ± 0.11
	Copper oxychloride	4x	2.86 ± 0.20
	Cuprous oxide	1x	2.87 ± 0.10
	Cuprous oxide	2x	3.75 ± 0.73
	Cuprous oxide	4x	5.68 ± 0.37

Conclusion

1. Fruit fly baits applied on Valencia fruit with and without previous copper exposure caused stippling marks on the rind. Incidence of stippling was higher in February.
2. An incompatibility between copper and fruit fly bait was found when baits were applied after March.

Future research

Alternative methods of bait spray application should be sought for citrus orchards in order to avoid damage on the fruit before colour break. Application of baits on panels suspended on trees can be investigated.

Technology transfer

Results were presented at the CRI pest and disease workshops in September 2017 and September 2018.

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3.3.4 PROGRESS REPORT: Fruit fly rearing

Project 407 (April 1999 – March 2019) by Aruna Manrakhan, John-Henry Daneel, Glorious Shongwe and Rooikie Beck (CRI)

Summary

Colonies of 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), are being maintained at Citrus Research International (CRI), Nelspruit. Fruit flies from these colonies were used in CRI Projects 1213, 1211, 1171 and 1248. Medfly pupae were provided to Green Trading and River Bioscience for tests on fruit fly baits. Fruit fly eggs were supplied to Agribiotech Research Consultancies for post-harvest disinfestation trials on non-citrus fruit crops. In order to ensure supply of good quality fly materials for studies, colonies have to be either refreshed (wild males crossed with laboratory reared females) or re-established (founded using males and females reared from wild fruit). In August and October 2018, the Medfly and Natal fly colonies were refreshed respectively from fruit collected in Mpumalanga Province. Medfly males were reared from coffee collected in Burgershall while Natal fly males were reared from loquat collected in Nelspruit. In July 2018, a Cape fly colony was established from flies reared from guavas collected near Nelspruit. This Cape fly colony was subsequently boosted by addition of flies reared from mangoes, milkplum, peaches and Jambos collected in different areas in Mpumalanga Province between December 2018 and February 2019. A new oriental fruit fly colony was started using flies reared from mango, marula, milkplum and Jambos collected from Nelspruit in January and February 2019. A new marula fly colony was started using flies reared from mango, marula and wild mango collected in Nelspruit in January 2019. In February and March 2019, two additional Cape fly colonies were started using flies reared from different fruit types collected in Pretoria and Stellenbosch.

Opsomming

Kolonies van vyf vrugtevlieg spesies: *Ceratitis capitata* (Mediterreense vrugtevlieg of "Medfly"), *Ceratitis rosa* (Natale vlieg), *Ceratitis quilicii* (Kaapse vlieg), *Ceratitis cosyra* (Maroela vlieg) en *Bactrocera dorsalis* (Oosterse vrugtevlieg), word by Citrus Research International (CRI), Nelspruit, in stand gehou. Vrugtevlieë vanaf hierdie kolonies is in CRI Projekte 1213, 1211, 1171 en 1248 gebruik. Mediterreense vrugtevlieg papies is aan Green Trading en River Bioscience verskaf vir toetse op vrugtevlieg lokaasmiddels. Vrugtevlieg eiers is aan Agribiotech Research Consultancies verskaf vir na-oes disinfestasië proewe op nie-sitrus vrugtegewasse. Ten einde die verskaffing van goeie kwaliteit vlieg materiaal vir studies te verseker, moet kolonies óf versterk word (wilde mannetjies moet met laboratorium-geteelde wyfies gekruis word), óf hervestig word (gevestig deur die gebruik van mannetjies en wyfies wat vanaf wilde vrugte geteel is). In Augustus en Oktober 2018, is onderskeidelik die Mediterreense vrugtevlieg en Natale vlieg kolonies vanaf vrugte wat in Mpumalanga-provinsie versamel is, versterk. Mediterreense vrugtevlieg mannetjies is vanaf koffie geteel wat in Burgershall versamel is, terwyl die Natale vlieg mannetjies geteel is vanaf lukwart wat in Nelspruit versamel is. In Julie 2018, is 'n Kaapse vlieg

kolonie vanaf vlieë wat vanaf koejawels geteel is wat naby Nelspruit versamel is, gevestig. Hierdie Kaapse vlieg kolonie is daarna versterk deur die byvoeg van vlieë wat vanaf mango's, stamvrug, perskes en Jambos geteel is en in verskillende areas in Mpumalanga-provinsie tussen Desember 2018 en Februarie 2019 versamel is. 'n Nuwe Oosterse vrugtevlieg kolonie is begin deur gebruik te maak van vlieë wat vanaf mango, maroela, stamvrug, en Jambos geteel is en in Nelspruit in Januarie en Februarie 2019 versamel is. 'n Nuwe maroela vlieg kolonie is begin deur vlieë te gebruik wat vanaf mango, maroela en wilde mango geteel is en in Nelspruit in Januarie 2019 versamel is. In Februarie en Maart 2019, is twee addisionele Kaapse vlieg kolonies begin deur vlieë te gebruik wat vanaf verskillende vrugtetipes geteel is en in Pretoria en Stellenbosch versamel is.

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

Project 1170 (April 2016 – March 2019) by J-H Daneel, J van den Berg and A Manrakhan

Summary

The Natal fly, *Ceratitis rosa* Karsch, was split into two species: *C. rosa* and *Ceratitis quilicii* De Meyer, Mwatawala and Virgilio. The aim of this project was to quantify similarities and differences between *C. rosa* and *C. quilicii* in their ecology in citrus orchards and in their development in citrus. The distribution and seasonal fluctuations of the two species were determined using attractant based traps in nine citrus farms in the Limpopo and Mpumalanga provinces. The larval development of the two species was determined in four citrus types (grapefruit, mandarin, lemon and orange) by artificial inoculation of eggs into the pulp and dissection of fruit daily thereafter for 15 days. *Ceratitis quilicii* was found to be the most widely distributed of the two species among the study sites. *Ceratitis quilicii* was also the most abundant of the two species except in the Hot-Humid regions. The peaks of adult populations of the two species depicted by trapping were different. *Ceratitis rosa* populations generally peaked in May while *C. quilicii* populations generally peaked in February. For both species, the male lure, EGO Pherolure (*C. quilicii* = 566 males, *C. rosa* = 49 males), was more attractive than Capilure (*C. quilicii* = 108 males, *C. rosa* = 19 males) traditionally used in citrus orchards for monitoring of *Ceratitis* pest species. Neither *C. rosa* nor *C. quilicii* were reared from citrus (Clementine, grapefruit, lemon and oranges) collected from the trees and from the ground in commercial orchards. *Ceratitis rosa* was reared from the families Myrtaceae (*Syzygium cordatum*, *Syzygium jambos*), Rosaceae (*Eriobotrya japonica*, *Prunus persica*), Rutaceae (*Casimiroa edulis*) and Sapotaceae (*Englerophytum magalismontanum*). *Ceratitis quilicii* was reared from Anacardiaceae (*Mangifera indica*), Myrtaceae (*Acca sellowiana*, *P. guajava* and *S. jambos*), Rosaceae (*P. persica*), Salicaceae (*Dovyalis caffra*) and Sapotaceae (*E. magalismontanum*). In artificially inoculated citrus, development of both species was faster in oranges (cv. Valencia) compared to grapefruit (cv. Star Ruby) and lemon (cv. Eureka). Larval survival was lower in mandarin compared to the other three citrus types. The peaks in the development of each larval stage were the same for both species in all citrus types.

Opsomming

Die Natalse vrugtevlieg, *Ceratitis rosa*, is verdeel in twee spesies: *C. rosa* en *Ceratitis quilicii* De Meyer, Mwatawala and Virgilio. Die doel van hierdie projek was om die ooreenkomste en verskille tussen *C. rosa* en *C. quilicii* in sitrus te kwantifiseer ten opsigte van hulle ekologie in sitrusboorde en hulle ontwikkeling in sitrus. Die verspreiding en seisoenale skommelings van die twee spesies was bepaal deur gebruik te maak van lokaas gelaaide lokvalle op nege sitrus plase in die Limpopo en Mpumalanga provinsies. Die larwes van die twee spesies se ontwikkeling was in vier sitrussoorte (pomelos, mandaryne, suurlemoene en lemoene) bepaal, deur die pulp van die vrugte kunsmatig met eiers te inokkuleer en daarna vrugte vir 15 dae lank, daaglik te dissekteer. Daar is bevind dat *C. quilicii* die wydste verspreid van die twee spesies tussen die studieterreine was. *Ceratitis quilicii* is ook die volopste van die twee spesies behalwe in die Warm-Klam streek. Die volwasse populasie se pieke wat deur lokvalvangste tussen die twee spesies bepaal was, was verskillend. Die *C. rosa* populasies se hoogste pieke was oor die algemeen gedurende Mei terwyl die *C. quilicii* populasies, oor die algemeen die hoogste was in Februarie. Vir beide spesies was die manlike lokaas: EGO Pherolure (*C. quilicii* = 566 mannetjies, *C. rosa* = 49 mannetjies) meer

geskik as *Capilure* (*C. quilicii* = 108 mannetjies, *C. rosa* = 19 mannetjies), wat tradisioneel in sitrusboorde aangewend word vir die monitering van *Ceratitis* plaagspesies. Nie *C. rosa* of *C. quilicii* was geteel uit die sitrus (Clementine, pomelo, suurlemoene en lemoene) wat van bome versamel of van die grond opgetel was in kommersiele boorde nie. *Ceratitis rosa* was geteel uit die families Myrtaceae (*Syzygium cordatum*, *Syzygium jambos*), Rosaceae (*Eriobotrya japonica*, *Prunus persica*), Rutaceae (*Casimiroa edulis*) en Sapotaceae (*Englerophytum magalismontanum*). *Ceratitis quilicii* was geteel vanuit Anacardiaceae (*Mangifera indica*), Myrtaceae (*Acca sellowiana*, *P. guajava* en *S. jambos*), Rosaceae (*P. persica*), Salicaceae (*Dovyalis caffra*) en Sapotaceae (*E. magalismontanum*). In die kunsmatige geinokkuleerde sitrus, was die ontwikkeling van beide spesies vinniger in lemoene (cv. Valencia) in vergelyking met pomelos (cv. Star Ruby) en suurlemoene (cv. Eureka). Die larwe oorlewing was laer in die mandaryne in vergelyking met die ander drie sitrustipes. Die ontwikkelingspieke van elke larwe stadium was dieselfde vir beide spesies vir al die sitrustipes.

3.3.6 PROGRESS REPORT: Efficacy of FCM partial cold treatments for fruit fly pests of citrus

Project 1171 (April 2017 - March 2020) by Aruna Manrakhan, John-Henry Daneel, Rooikie Beck, Glorious Shongwe, Sean Moore and Vaughan Hattingh (CRI)

Summary

In light of the recent changes in the EU regulations on non-European Tephritidae (fruit flies), it is becoming increasingly important to determine whether the cold shipping options in the citrus false codling moth systems approach (Citrus FCM SA) would also mitigate risk of fruit flies in citrus. The shipping options in the citrus FCM SA are partial treatments which along with other FCM control measures, prior to shipment, would mitigate the risk of FCM in citrus. The fruit fly pests affecting citrus in South Africa are *Ceratitis capitata* (Mediterranean fruit fly or Medfly), *Ceratitis rosa* (Natal fly) and *Bactrocera dorsalis* (oriental fruit fly). *Ceratitis rosa* was recently split in two species: *Ceratitis rosa* and *Ceratitis quilicii*. The status of citrus as a host for the latter species has not been established. There are internationally recognised cold treatments for Medfly at pulp temperatures of 3°C and below. While these treatments would be sufficient to justify the use of some shipping options under the Citrus FCM SA in a proposed fruit fly systems approach, the efficacy of shipping options above 2°C (with resulting pulp temperatures of above 3°C) on fruit flies is still unknown. The cold tolerance of the third larval stage of Medfly, Natal fly and oriental fruit fly on a carrot based diet were compared concurrently at 3.52°C ± 0.00°C at ten exposure periods over 18 consecutive days. Medfly was more cold tolerant than Natal fly and Oriental fruit fly. For the most cold tolerant species - Medfly, there were no survivors beyond 12 days of cold treatment at the above mentioned temperature. The estimated exposure periods at 3.5°C required for 99% mortality (95% Confidence Intervals) of the third larval stage of Medfly, Natal fly and oriental fruit fly were 13.22 (12.84-13.63), 6.19 (5.96-6.44), 8.39 (8.03-8.79) respectively.

Opsomming

In die lig van die onlangse veranderinge in die EU-regulasies rakende nie-Europese Tephritidae (vrugtevlieë), word dit toenemend belangrik om vas te stel of die koue verskeppings-opsies in die sitrus vals kodlingmot sisteembenadering ("Citrus FCM SA") ook die risiko van vrugtevlieë in sitrus sal verminder. Die verskeppings-opsies in die "Citrus FCM SA" is gedeeltelike behandelings wat tesame met ander VKM beheermaatreëls, vóór verskeping, die risiko van VKM in sitrus sal verminder. Die vrugtevlieg plaë wat sitrus in Suid-Afrika affekteer, is *Ceratitis capitata* (Mediterreense vrugtevlieg of "Medfly"), *Ceratitis rosa* (Natalse vlieg) en *Bactrocera dorsalis* (Oosterse vrugtevlieg). *Ceratitis rosa* is onlangs in twee spesies verdeel: *Ceratitis rosa* en *Ceratitis quilicii*. Die status van sitrus as 'n gasheer vir laasgenoemde spesie, is nog nie vasgestel nie. Daar is internasionaal erkende koue-behandelings vir Mediterreense vrugtevlieg teen pulptemperature van 3°C en laer. Terwyl hierdie behandelings voldoende sal wees om die gebruik van sommige verskeppings-opsies onder die "Citrus FCM SA" in 'n voorgestelde vrugtevlieg sisteembenadering te regverdig, is die effektiwiteit van verskeppings-opsies bó 2°C (met gevolglike pulptemperature van bó 3°C) op vrugtevlieë steeds onbekend. Die koue-toleransie van die derde larvale stadium van die Mediterreense vrugtevlieg, Natalse vlieg en Oosterse vrugtevlieg, op 'n wortel-gebaseerde dieet,

is samelopend teen $3.52^{\circ}\text{C} \pm 0.00^{\circ}\text{C}$ by tien blootstellingsperiodes oor 18 opeenvolgende dae vergelyk. Die Mediterreense vrugtevlieg was meer koue-tolerant as die Natalse vlieg en Oosterse vrugtevlieg. Vir die mees koue-tolerante spesie, Mediterreense vrugtevlieg, was daar geen oorlewendes ná 12 dae van koue-behandeling teen die bogenoemde temperatuur nie. Die geskatte blootstellingsperiodes teen 3.5°C benodig vir 99% mortaliteit (95% Vertrauensintervalle) van die derde larvale stadium van die Mediterreense vrugtevlieg, Natalse vlieg en Oosterse vrugtevlieg, was onderskeidelik 13.22 (12.84-13.63), 6.19 (5.96-6.44), 8.39 (8.03-8.79).

3.3.7 **PROGRESS REPORT: The assessment of control and monitoring for fruit fly in the Western Cape**

Project 1177 (2017-2020) by Martin Gilbert and Claire Love (CRI)

Summary

Fruit fly control during the citrus season is important to prevent fruit damage and subsequent rejection from sensitive export markets. The current recommendations advise that fruit fly control should be implemented from approximately January or February in citrus orchards until harvest. This study aims to determine whether the use of M3 bait stations (both with and without SIT) earlier in the season would result in decreased fruit fly populations, as well as a reduction in fruit damage. The start of this project for the 2017/18 season was delayed as there was some difficulty in finding appropriate sites. Due to the delay, the project was restructured for the first season and the M3 bait stations were tested in lemon orchards. Only trap monitoring was conducted as lemons are not a suitable host for fruit flies. The M3 treated blocks were laid out in May 2018. Mean trap catches in the control blocks remained consistently higher than in the M3 treated blocks for the Capilure traps and were also slightly higher in the Biolure traps from May to August 2018. Monitoring was discontinued in the lemons from the end of August to begin running the full experiment in soft citrus orchards at two sites.

The first site was in the Porterville area, outside of the Fruit Fly Africa SIT release programme. The second site was in the De Doorns area where the farm falls under the fruit fly SIT release programme. The timing of M3 bait station application was done by comparing early (October/early November), late (January/February) and control (no bait stations) treatments at both the SIT and non-SIT sites. The M3 bait stations were hung out for the early treatment in October for the Porterville site and end of October for the De Doorns site. Weekly monitoring of the fruit fly populations also began at this time, with both Capilure and Biolure traps being used in each treatment block. The late treatment was hung out in January at the Porterville site and in mid-February for the De Doorns site. Monitoring results comparing the early and control treatments at Porterville show that the fruit flies per trap per day (FTD) was very similar for the two treatments, with the fruit fly population being very low until mid-December, which was shown by both male captures in Capilure traps and female captures in Biolure traps. After the late treatment bait stations were placed, there was a spike in average trap catches for this treatment when compared to the control and early treatment, but numbers began to decrease about seven weeks later. At the De Doorns (SIT) site, the FTD was once again similar for the early and control treatments although at times, the early treatment population would be higher. The application of the late treatment did not result in an increase in fly catches as had been recorded at Porterville. Fruit inspections were done on fruit on the tree and on the ground, but to date no flies have been reared from laboratory incubated fruit. Fruit inspections and monitoring will continue throughout the season.

Opsomming

Vrugtevliegbeheer gedurende die sitrusseisoen is van groot belang om vrugskade en afkeurings van sensitiewe uitvoermarkte te voorkom. Die huidige aanbevelings stel voor dat vrugtevliegbeheer vanaf ongeveer Januarie of Februarie in sitrusboorde geïmplementeer moet word. Die doel van hierdie studie was om vas te stel of die gebruik van M3 lokmiddelstasies (met en sonder SIT) vroeër in die seisoen tot verminderde vrugtevlieg populasies, asook 'n vermindering van vrugskade, kan lei. Die aanvang van hierdie projek vir die 2017/18 seisoen was uitgestel, aangesien daar probleme was met die bevinding van gepaste persele. As gevolg van die uitstelling, die projek vir die eerste seisoen is herstruktureer en die M3 lokmiddelstasies in suurlemoenboorde getoets. Slegs

lokvalmonitering is uitgevoer aangesien suurlemoene nie 'n gepaste gasheer vir vrugtevlieë is nie. Die M3 behandelde blokke is in Mei 2018 uitgelê. Die gemiddelde lokvaal vangste in die kontrole blokke het konsekwent hoër gebly as in die M3 behandelde blokke vir die Capilure lokval en was ook effens hoër in die Biolure lokvaal van Mei tot Augustus 2018. Monitoring het in die suurlemoene vanaf die einde van Augustus opgehou om die volle eksperiment in sagte sitrusboorde op twee persele te begin.

Die eerste perseel was in die Porterville gebied, wat buite die SIT vrylatingprogram is. Die tweede plek was in die De Doorns omgewing waar die plaas deel is van die vrugtevlieg SIT vrylatingprogram. Die tydsberekening van M3 lokvaalstasie toediening is gedoen deur vroeg (Oktober / vroeg November), laat (Januarie / Februarie) en kontrole (geen lokvalle) behandelings by SIT en nie-SIT persele te vergelyk. Die M3 lokvalstasies is opgehang vir die vroeg behandeling in Oktober vir die Porterville perseel en einde Oktober vir die De Doorns perseel. Weeklikse monitering van die vrugtevlieg populasies het ook begin, met albei Capilure en Biolure lokvalle wat in elke behandelingsblok gebruik word. Die laat behandeling is in Januarie by die Porterville perseel en in die middel van Februarie vir die De Doorns perseel uitgehang. Moniteringsresultate wat die vroeg en kontrole behandelings by Porterville vergelyk, toon dat die vlieë per lokval per dag (FTD) baie dieselfde was vir die twee behandelings. Die vrugtevlieg populasie was baie laag tot middel Desember, wat deur die mannetjie opnames getoon is in die Capilure lokval en vangste van wyfies in die Biolure lokval. Na die laat behandeling was daar 'n toename in die gemiddelde vangste vir hierdie behandeling in vergelyking met die kontrole en vroeg behandelings, maar getalle begin sowat sewe weke later afneem. Op die De Doorns perseel (SIT) was die FTD weereens soortgelyk aan die vroeg en kontrole behandelings, hoewel die vroeë behandelingspopulasie soms hoër sou wees. Die toepassing van die laat behandeling het nie gelei tot 'n toename in vliegvangste soos by Porterville aangeteken is nie. Vrugbesmeting inspeksies is op vrugte op die boom en op die grond gedoen, maar tot op hede het geen vlieë van uit laboratorium geïnkubeerde vrugte na vore gekom nie. Vrugbesmeting inspeksies en monitering sal deur die seisoen aanhou.

3.3.8 **PROGRESS REPORT: The impact of interruptions on Medfly cold treatment efficacy**

Project 1204 (2018/9 – 2019/20) by T G Grout, P R Stephen, K C Stoltz and V Hattingh (CRI)

Summary

Due to *ad hoc* cold treatment research for market access and other capacity issues, no research was possible during the report period. Research will start with the first oranges in May 2019 and will probably have to continue into 2020/21.

Opsomming

Weens *ad hoc* koue-behandeling navorsing vir marktoegang en ander kapasiteitskwessies, was geen navorsing gedurende die verslagperiode moontlik nie. Navorsing sal met die eerste lemoene in Mei 2019 begin en sal moontlik tot in 2020/21 voortgaan.

3.3.9 **PROGRESS REPORT: Attract and kill methods for fruit flies: efficacy and application of new and registered products**

Project 1211 (April 2018 - March 2020) by Aruna Manrakhan, John-Henry Daneel, Rooikie Beck, Glorious Shongwe, Sean Moore, Wayne Kirkman, Melissa Peyper, Tamryn Marsberg and Sonica Albertyn (CRI)

Summary

One of the objectives of this project was to determine the efficacy of attract and kill products for fruit flies. The efficacy of two densities of M3 bait station (300 and 400 units per ha) and Magnet Med (50 and 75 units per ha) were determined in Valencia orchards in Mpumalanga and Eastern Cape over 6-9 weeks between June and August 2018. Field cage trials were conducted at the same time to determine the effects of weathering on

attractiveness of the Magnet Med and M3 bait stations to females of the Mediterranean fruit fly (Medfly), *Ceratitidis capitata*. Both types of bait stations were weathered for up to 24 weeks in a Valencia orchard in Nelspruit. The responses of a new bait from Arysta LifeScience referred to as 'Arysta bait' and GF-120 to Medfly and oriental fruit fly, *Bactrocera dorsalis*, were determined in choice tests in field cages. Both M3 and Magnet Med at the two densities tested kept Medfly male and female catches under threshold until the start of harvest in both study areas. No fruit fly infestation of fruit was recorded in any of the treatments in both study areas. The weathering trials on M3 bait stations and Magnet Med showed that M3 bait stations lost their attractiveness to Medfly females after eight weeks while Magnet Med retained their attractiveness for up to 24 weeks. Responses of Medfly to the Arysta bait were higher than those to GF-120, although differences between the two baits were not statistically significant. For the oriental fruit fly, there was no significant difference between responses to GF-120 and those to Arysta bait. The oriental fruit fly had a much lower response to the Arysta bait than Medfly.

Opsomming

Een van die doelwitte van hierdie projek was om die effektiwiteit van lok- en uitwissingsprodukte vir vrugtevlieë te bepaal. Die effektiwiteit van twee digthede van die M3 lokaasstasie (300 en 400 eenhede per ha) en Magnet Med (50 en 75 eenhede per ha) is in Valencia boorde in Mpumalanga en die Oos-Kaap oor 6-9 weke tussen Junie en Augustus 2018 bepaal. Veldhokproewe is dieselfde tyd uitgevoer om die effek van verwerking op die aanloklikheid van die Magnet Med en M3 lokaasstasies op wyfies van die Mediterreense vrugtevlieg ("Medfly"), *Ceratitidis capitata*, vas te stel. Beide tipes lokaasstasies is vir tot 24 weke in 'n Valencia boord in Nelspruit verweer. Die reaksies van 'n nuwe lokaasmiddel vanaf Arysta LifeScience, waarna verwys word as 'Arysta lokaas', en GF-120, teenoor Mediterreense vrugtevlieg en Oosterse vrugtevlieg, *Bactrocera dorsalis*, is in keuse toetse in veldhokke bepaal. Beide M3 en Magnet Med, teen die twee digthede wat getoets is, het die Mediterreense vrugtevlieg mannetjie en wyfie vangste tot die begin van oes onder die drempelwaarde gehou, in beide studie-areas. Geen vrugtevlieg infestasië van vrugte is in enige van die behandelings in beide studie-areas aangeteken nie. Die verweringsproewe op M3 lokaasstasies en Magnet Med het getoon dat M3 lokaasstasies hul aanloklikheid vir Mediterreense vrugtevlieg wyfies na ag weke verloor, terwyl Magnet Med hul aanloklikheid vir tot 24 weke behou. Reaksies van Mediterreense vrugtevlieg op Arysta lokaas was hoër as dié teenoor GF-120, hoewel verskille tussen die twee lokaasmiddels nie statisties betekenisvol was nie. Vir die Oosterse vrugtevlieg was daar geen betekenisvolle verskil tussen reaksies op GF-120 en dié op Arysta lokaas nie. Die Oosterse vrugtevlieg het 'n veel laer reaksie op Arysta lokaas gehad as die Mediterreense vrugtevlieg.

3.3.10 PROGRESS REPORT: Cold tolerance of immature stages of *Ceratitidis capitata* (Wiedemann) and *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae)

Project 1213 (January 2018 – March 2020) by Aruna Manrakhan, John-Henry Daneel, Peter Stephen and Vaughan Hattingh (CRI)

Summary

In November 2017, the Japanese Ministry of Agriculture, Forestry and Fisheries (MAFF) questioned whether the current cold treatment schedule for citrus fruit exports from South Africa to Japan of $-0.6^{\circ}\text{C} \pm 0.6^{\circ}\text{C}$ for 12 to 14 days for disinfestation of Mediterranean fruit fly (Medfly), *Ceratitidis capitata*, would be efficacious against the oriental fruit fly, *Bactrocera dorsalis*. MAFF requested that a study be conducted to determine the cold sensitivity of Medfly and the oriental fruit fly. Based on a trial design stipulated by MAFF, cold tolerances of the two species were compared at four immature stages on a carrot-based medium at $-0.59^{\circ}\text{C} \pm 0.00^{\circ}\text{C}$ at six exposure periods over 11 consecutive days. Medfly was found to be more cold tolerant than the oriental fruit fly for all life stages tested. The results therefore demonstrated that the current cold treatment schedule for citrus fruit exports from South Africa to Japan would be efficacious against both Medfly and oriental fruit fly. In the cold tolerance study on artificial medium, older larvae of Medfly were found to be more cold tolerant than younger larvae. Since this result was different to outcomes of previous research conducted at CRI where young Medfly larvae were found to be the most cold tolerant in citrus, a follow up study was initiated to compare the cold tolerance between different life

stages of Medfly reared *in vivo* (in citrus) and *in vitro* (in the carrot based medium). In *in vivo* trials, there were no survivors of any of the immature stages beyond 7 days of cold exposure at 1°C. In *in vitro* trials, there were survivors of the young and mature larvae until 9 days of cold exposure at 1°C but no survivors of the egg stage beyond 7 days of cold exposure at 1°C.

Opsomming

In November 2017 het die Japannese Ministerie van Landbou, Bosbou en Visserye (“MAFF”) dit bevaagteken of die huidige koue-behandelingskedule vir sitrusvrug uitvoere vanaf Suid-Afrika na Japan van $-0.6^{\circ}\text{C} \pm 0.6^{\circ}\text{C}$ vir 12 tot 14 dae vir disinfestasië van die Mediterreense vrugtevlug (“Medfly”), *Ceratitidis capitata*, effektief sal wees teen die Oosterse vrugtevlug, *Bactrocera dorsalis*. “MAFF” het versoek dat ‘n studie uitgevoer word ten einde die koue-sensitiwiteit van die Mediterreense vrugtevlug en die Oosterse vrugtevlug te bepaal. Gebaseer op ‘n proefontwerp soos bepaal deur “MAFF”, is koue-toleransies van die twee spesies by vier onvolwasse stadia op ‘n wortel-gebaseerde medium teen $-0.59^{\circ}\text{C} \pm 0.00^{\circ}\text{C}$ by ses blootstellingsperiodes oor 11 opeenvolgende dae vergelyk. Daar is gevind dat die Mediterreense vrugtevlug, vir al die lewensstadia wat getoets is, meer kouetolerant as die Oosterse vrugtevlug was. Die resultate dui dus aan dat die huidige koue-behandelingskedule vir sitrusvrug uitvoere vanaf Suid-Afrika na Japan effektief sal wees teen beide die Mediterreense en Oosterse vrugtevlug. In die koue-toleransie studie op kunsmatige medium, is gevind dat ouer larwes van die Mediterreense vrugtevlug meer koue-tolerant as jonger larwes is. Aangesien hierdie resultaat verskil het van uitkomst van vorige navorsing wat by CRI uitgevoer is, waar gevind is dat jong Mediterreense vrugtevlug larwes die meeste kouetolerant in sitrus is, is ‘n opvolgstudie begin ten einde die koue-toleransie tussen verskillende lewensstadia van Mediterreense vrugtevlug te vergelyk wat *in vivo* (in sitrus) en *in vitro* (in die wortel-gebaseerde medium) geteel is. In *in vivo* proewe was daar geen lewendiges van enige van die onvolwasse stadia ná 7 dae van koueblootstelling by 1°C nie. In *in vitro* proewe, was daar lewendiges van die jong en volwasse larwes tot 9 dae van koueblootstelling by 1°C, maar geen lewendiges van die eierstadium ná 7 dae van koueblootstelling by 1°C nie.

3.3.11 PROGRESS REPORT: Laboratory bioassays on AVIMA’s bait stations for control of Medfly and Oriental fruit fly

Project 1248 (January - August 2019) by Aruna Manrakhan, John-Henry Daneel, Phindile Rikhotso, Rooikie Beck and Glorious Shongwe (CRI)

Summary

AVIMA developed three bait stations and contracted CRI for determination of the efficacy of kill of these bait stations. The test bait stations were compared to the standard M3 fruit fly bait station in laboratory trials. Two fruit fly species were tested: *Ceratitidis capitata* (Mediterranean fruit fly or Medfly) and *Bactrocera dorsalis*, oriental fruit fly. Mortality of these two fruit fly species when exposed to fresh and aged bait stations were determined in aerated plastic cages. By March 2019, trials were completed on the fresh and one-month old bait stations. Two of the AVIMA bait stations (coded orange and yellow) were about two times more effective than M3 bait stations when fresh for Medfly. With bait stations aged outdoors for one month, the same two bait stations were three times more effective than M3 bait stations for Medfly. For oriental fruit fly, the yellow bait station was five times more effective than M3 bait stations when both stations were fresh. With bait stations aged outdoors for one month, the yellow bait station was 12 times more effective than M3 bait stations for the oriental fruit fly. The yellow bait station seems a promising bait station for both Medfly and oriental fruit fly with up to 100% fly mortality after 72 hours.

Opsomming

AVIMA het drie lokaasstasies ontwikkel en CRI gekontrakteer om die effektiwiteit van hierdie lokaasstasies se uitwissingsaksie te bepaal. Die toets lokaasstasies is met die standaard M3 vrugtevlug lokaasstasie in laboratoriumtoets vergelyk. Twee vrugtevlug spesies is getoets: *Ceratitidis capitata* (Mediterreense vrugtevlug of “Medfly”) en *Bactrocera dorsalis*, Oosterse vrugtevlug. Die mortaliteit van hierdie twee vrugtevlug spesies,

wanneer blootgestel aan vars en verouderde lokaasstasies, is in deurlugte plastiekhokke bepaal. Teen Maart 2019, was proewe op die vars en een-maand-oue lokaasstasies voltooi. Twee van die AVIMA lokaasstasies (oranje en geel gemerk) was omtrent twee keer meer effektief as die M3 lokaasstasies vir die Mediterreense vrugtevlieg wanneer hulle vars is. Met lokaasstasies wat buite vir een maand verouder het, was dieselfde twee lokaasstasies drie keer meer effektief as die M3 lokaasstasies vir Mediterreense vrugtevlieg. Vir die Oosterse vrugtevlieg, was die geel lokaasstasie vyf keer meer effektief as die M3 lokaasstasies wanneer beide stasies vars was. Met lokaasstasies wat buite vir een maand verouder het, was die geel lokaasstasies 12 keer meer effektief as die M3 lokaasstasies vir die Oosterse vrugtevlieg. Die geel lokaasstasie blyk 'n belowende lokaasstasie te wees vir beide die Mediterreense en Oosterse vrugtevlieg, met tot 100% vlieg mortaliteit ná 72 uur.

3.4 PROGRAMME: OTHER PESTS

Programme coordinator: Tim G Grout (CRI)

3.4.1 Programme summary

This programme is now an amalgamation of the previous programmes Key non-phytosanitary pests, Mealybug and other phytosanitary pests and Minor pests and mites. For several years we have been losing plant protection products that can be used to control psylla, late thrips and woolly whitefly in February or March and we had no products registered for leafhoppers that we could use at that time. Research conducted over five years (3.4.2) has resulted in an emergency registration for green citrus leafhopper and gave some indication in bioassays of the efficacy of various products against woolly whitefly, but field trials against the other pests could not be conducted. An unusual case where a grower was getting most of his crop rejected for export due to high numbers of beetle mites resulted in an investigation of the source of the mites and a means of controlling them (3.4.3). The mites were not found on the framework branches and did not seem to enter the tree from the ground but they increased to large numbers in late summer and moved onto the fruit. Profenofos, chlorfenapyr and full cover oil sprays reduced their numbers significantly but their origin could not be resolved. Due to the importance of citrus thrips as a key pest in citrus IPM and the availability of two natural enemies of thrips from BioBee, research was conducted to determine whether these natural enemies could suppress citrus thrips (3.4.4). Even though supplemental food in the form of *Artemia* cysts was applied when releases of *Orius thripoborus* and *Amblyseius swirskii* were made several times on potted citrus plants, neither natural enemy established and the project was terminated. Collaborative research in Mauritius to gain experience on the control of *Diaphorina citri* on citrus trees was conducted over three seasons (3.4.5). Numbers of *D. citri* recovered on yellow sticky traps remained low throughout this research but treatments of acetamiprid, imidacloprid and acephate did suppress the pest in treated blocks. The addition of ACP Pherolure to the Alpha Scents yellow sticky trap did not improve catches of *D. citri*. In the quest for efficacious entomopathogenic fungal treatments, seven isolates and two commercial mycoinsecticides were tested for their susceptibility to temperature extremes, relative humidity and UV radiation (3.4.6). Temperature and relative humidity had little effect but all isolates with the exception of the mycoinsecticides were extremely susceptible to UV radiation. This showed the importance of formulating these products with adjuvants that reduce susceptibility to UV radiation. Further research to evaluate the efficacy of commercial mycoinsecticides was conducted where a preventative programme using both *Beauveria bassiana* and *Metarhizium anisopliae* based products showed no significant efficacy against red scale, mealybug or thrips (3.4.7). Corrective treatments against red scale had no effect but two products reduced mealybug numbers by 23 and 32% relative to an untreated control. Entomopathogenic fungi are also being investigated in combinations with various entomopathogenic nematodes to determine efficacy against false codling moth and mealybug (3.4.8). The growing trend of using 20% shade netting over citrus orchards is changing the status of some citrus pests and this is receiving attention in several research projects. In the Mpumalanga lowveld, nets placed over an existing grapefruit orchard caused an obvious reduction in wind scarring (3.4.9). Trap counts of fruit fly were higher in the open orchard but FCM trap counts were higher under net, although fruit infestation levels by FCM were similar. Residue analyses showed higher levels on fruit under nets. In another project where potted Valencia plants are being compared in the open with identical plants under net, citrus red mite infestation under net was 75% higher,

silver mite 14% higher and red scale 33% higher (3.4.10). Measurements of UV-B radiation under commercial nets adjacent to open citrus orchards show that the nets reduce UV-B levels by approximately 26%. However, pesticide residues were only found to be slightly higher for strobilurin fungicides. After releases of the parasitoid *Cales noacki* were made for woolly whitefly in the northern regions, research in the Western Cape has shown that the same parasitoid is now widely distributed in that region and numbers of woolly whitefly have declined (3.4.11). Although this is welcome news for citrus growers it has meant that insufficient numbers of parasitoids have been available to try to establish it in the Eastern Cape. In order to prepare for the arrival of *Diaphorina citri*, the Asian citrus psyllid, in South Africa, some systemic plant protection products were screened for their efficacy against the brown citrus aphid on potted citrus plants (3.4.12). Sulfoxaflor as a soil drench gave similar results to Confidor and two non-registered systemic products also had some promise, although one would probably require an uneconomical dosage. The intention to duplicate this work in SE Kenya where *D. citri* is now present has been stalled by an inability to rear high numbers of *D. citri* for screening purposes. Attempts are being made to use *Bergera koenigii* for this purpose. An attempt is being made to find an alternative to methyl bromide fumigation for budwood entering South Africa because of that fumigant's detrimental effect on bud viability (3.4.13). Only preliminary experiments have been possible due to an inability to find a good source of citrus bud mite, but we hope to find a suitable site in the future.

Programopsomming

Hierdie program is nou 'n samesmelting van die vorige programme "Sleutel nie-fitosanitiere plaë, witluis en ander fitosanitiere plaë" en "Plaë en myte van minder belang". Ons het nou al vir verskeie jare plantbeskermingsprodukte verloor wat vir die beheer van bladvlooi, láát blaaspootjies en wollerige witvlieg in Februarie of Maart gebruik kon word, en ons het geen geregistreerde produkte vir bladspringers gehad wat ons daardie tyd kon gebruik nie. Navorsing wat oor vyf jaar uitgevoer is (3.4.2), het 'n nood-registrasie vir groensitrusbladspringer tot gevolg gehad, en het in biotoetse 'n aanduiding gegee van die effektiwiteit van verskeie produkte teen wollerige witvlieg, maar veldproewe kon nie teen die ander plaë uitgevoer word nie. 'n Buitengewone geval waar 'n produsent se grootste deel van sy oes weens hoë getalle kewermyte vir uitvoer verwerp is, het tot 'n ondersoek van die bron van die myte gelei en 'n manier om hulle te beheer (3.4.3). Die myte is nie op die raamwerktakke gevind nie en dit het ook nie gelyk of hulle die boom vanaf die grond binne gegaan het nie, maar hulle het tot groot getalle in láát somer toegeneem en tot op die vrugte beweeg. Profenofos, chlorfenapyr en vol-bedekking oliespuitte het hulle getalle betekenisvol verlaag, maar hul oorsprong kon nie vasgestel word nie. Weens die belang van sitrusblaaspootjies as 'n sleutel plaag in sitrus "IPM" (geïntegreerde plaagbestuur), en die beskikbaarheid van twee natuurlike vyande van blaaspootjies vanaf BioBee, is navorsing uitgevoer ten einde vas te stel of hierdie natuurlike vyande sitrusblaaspootjies kan onderdruk (3.4.4). Selfs al is aanvullende kos in die vorm van *Artemia* siste toegedien, wanneer vrystelling van *Orius thripoborus* en *Amblyseius swirskii* verskeie kere op sitrusplante in potte gemaak is, het nie een van die natuurlike vyande gevestig nie, en die projek is gestop. Gesamentlike navorsing in Mauritius ten einde ondervinding in die beheer van *Diaphorina citri* op sitrusbome op te doen, is oor drie seisoene uitgevoer (3.4.5). Getalle van *D. citri* wat op geel kleeflokvalle herverkry is, het dwarsdeur hierdie navorsing laag gebly, maar behandelings van acetamiprid, imidacloprid en acephate het die plaag in behandelde blokke onderdruk. Die toevoeging van ACP Pherolure tot die Alpha Scents geel kleeflokvalle het nie vangste van *D. citri* verbeter nie. In die soeke na effektiewe entomopatogeniese swambehandelings, is sewe isolate en twee kommersiële miko-insektisiedes vir hul vatbaarheid vir temperatuur uiterstes, relatiewe humiditeit en UV-bestraling getoets (3.4.6). Temperatuur en relatiewe humiditeit het min effek gehad, maar al die isolate, met die uitsondering van die miko-insektisied, was uiters vatbaar vir UV-bestraling. Dit dui op die belang daarvan om hierdie produkte met bymiddels te formuleer wat hul vatbaarheid vir UV-bestraling sal verminder. Verdere navorsing om die effektiwiteit van kommersiële miko-insektisiedes te evalueer, is uitgevoer, waar 'n voorkomende program deur die gebruik van beide *Beauveria bassiana* en *Metarhizium anisopliae* gebaseerde produkte geen betekenisvolle effektiwiteit teen rooidopluis, witluis of blaaspootjies getoon het nie (3.4.7). Korrektiewe behandelings teen rooidopluis het geen effek gehad nie, maar twee produkte het witluis getalle met 23 en 32% relatief tot 'n onbehandelde kontrole verminder. Entomopatogeniese swamme word ook in kombinasies met verskeie entomopatogeniese aalwurms ondersoek ten einde effektiwiteit teen vals kodlingmot en witluis te bepaal (3.4.8). Die groeiende neiging om 20%

skudunet oor sitrusboorde te gebruik, verander die status van sommige sitrusplae, en dit ontvang aandag in verskeie navorsingsprojekte. In die Mpumalanga laevel, het die plasing van nette oor 'n bestaande pomeloboord tot 'n duidelike verlaging in letsels weens windskaade gelei (3.4.9). Lokval getalle van vrugtevlieë was hoër in die oop boord, maar vals kodlingmot getalle was hoër onder net, hoewel vrug-infestasië vlakke deur vals kodlingmot dieselfde was. Residu-analises het hoër vlakke op vrugte onder net getoon. In 'n ander projek waar Valencia plante in potte in die oopte met identiese plante onder net vergelyk word, was sitrusrooimyt infestasië onder net 75% hoër, silwermyt was 14% hoër en rooidopluis was 33% hoër (3.4.10). Metings van UV-B-bestraling onder kommersiële nette aangrensend aan oop sitrusboorde, het getoon dat die nette UV-B vlakke met ongeveer 26% verlaag. Plaagdoder residue was egter slegs effens hoër vir strobilurien swamdoders. Nadat vrystelling van die parasitoïed, *Cales noacki*, vir wollerige witvlieg in die noordelike areas gemaak is, het navorsing in die Wes-Kaap getoon dat dieselfde parasitoïed nou wyd-verspreid in daardie area voorkom en dat getalle van wollerige witvlieg afgeneem het (3.4.11). Hoewel dit welkome nuus vir sitrusprodusente is, het dit beteken dat onvoldoende getalle van parasitoïedes beskikbaar was om te probeer om dit in die Oos-Kaap te vestig. Ten einde vir die aankoms van *Diaphorina citri*, die Asiatiese sitrusbladvlooi, in Suid-Afrika voor te berei, is 'n paar sistemiese plantbeskermingsprodukte vir hul effektiwiteit teen die bruinsitrusplantluis op sitrusplante in potte geëvalueer (3.4.12). Sulfoxaflor as 'n grondreukbehandeling het soortgelyke resultate as Confidor gegee, en twee nie-geregistreerde sistemiese produkte het ook belofte getoon, hoewel een moontlik 'n onekonomiese dosis sal vereis. Die voorneme om hierdie werk in SO Kenia te dupliseer waar *D. citri* nou teenwoordig is, is gestaak weens die onvermoë om hoë getalle van *D. citri* vir evalueringdoeleindes te teel. Pogings word aangewend om *Bergera koenigii* vir hierdie doel te gebruik. 'n Poging word aangewend om 'n alternatief vir metielbromied beroking vir okuleerhout wat Suid-Afrika binnekom, te vind, weens die berokingsmiddel se nadelige effek op ogie lewensvatbaarheid (3.4.13). Slegs voorlopige eksperimente was tot dusver moontlik weens die onvermoë om 'n goeie bron van sitrusknopmyt te vind, maar ons hoop om 'n geskikte bron in die toekoms te vind.

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

Project 1061 (2013/4 – 2018/9) by T G Grout and P 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. Although field trials with citrus thrips, citrus psylla and woolly whitefly were not possible, three trials were conducted with leafhoppers in the Mookgopong/Marble Hall area. Two unregistered organic products with short preharvest intervals, Kangroshield100 and Xterminator, were both compared at 500 ml/hl water to a Phosdrin standard at 30 ml/hl. The two products were as effective as Phosdrin against the green citrus leafhopper for the first week after treatment but not effective against the citrus leafhopper. These trials led to the emergency registration of Xterminator for green citrus leafhopper on citrus. Unfortunately, Requiem, that had shown some promise in laboratory trials against woolly whitefly, is no longer available in South Africa.

Opsomming

Daar is 'n 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 ongeregisteerde middels en middels wat onlangs teen ander sitrusplae geregistreer is, met moontlike kort vóóroes intervalle, te evalueer. Hoewel veldproewe met sitrus blaaspootjies, sitrus bladvlooi en wollerige witvlieg nie moontlik was nie, is drie proewe met bladspringers in die Mookgopong/Marble Hall area uitgevoer. Twee nie-geregistreerde organiese produkte met kort voor-oes intervalle, Kangroshield100 en Xterminator, is beide teen 500 ml/hl water met 'n Phosdrin standaard teen 30 ml/hL vergelyk. Die twee produkte was net so effektief as Phosdrin teen die groen sitrus bladspringer vir die eerste week ná behandeling, maar nie effektief teen die sitrus bladspringer nie. Hierdie

proewe het tot die nood registrasie van Xterminator vir groen sitrus bladspringer op sitrus gelei. Requiem, wat belofte in laboratoriumproewe teen wollerige witvlieg getoon het, is ongelukkig nie meer in Suid-Afrika beskikbaar nie.

Introduction

With endosulfan no longer permitted for use on the summer growth flush in February/March, control of psylla is difficult because Phosdrin, which is also registered for psylla, provides almost no residual control. By this time, treatments that had been suppressing populations of psylla, thrips and woolly whitefly earlier in the season no longer have any effect. Leafhoppers are also problematic between February and May when fruit start to mature and products that are known to be effective such as Phosdrin and Lannate may not be available for use much longer. Some products that have been recently registered for other insects can be used late in the season, such as Delegate, Exirel and Coragen, but their efficacy against some of the above-mentioned citrus pests requires investigation. Some short-residual products such as botanical pesticides may provide adequate control at this late stage in the season whereas under high pest densities they are considered ineffective and may have been largely ignored in the past, e.g. Requiem, Bio-Cure and Xterminator. Although neem was previously found to be ineffective against citrus thrips, the combination of neem and natural pyrethrin in the form of Erador had sometimes been effective and various botanical combinations are now available from China that may provide similar efficacy.

Due to the need to find products that can be used against soft bodied insects such as psylla, thrips, woolly whitefly and leafhoppers, shortly before harvest, the first author attended the first international conference on pesticidal plants held at ICIPE in Nairobi in January 2013. Several international speakers were there and good contacts were made with researchers in Canada, United Kingdom, India and Italy. The quality of the research ranged from trial-and-error herbalist experiments to genetic/molecular/biochemical investigations. Several papers were presented on Intellectual Property issues and it is clear that after the court cases involving *Hoodia gordonii* and CSIR/Unilever/Pfizer, few companies are interested in dealing with South Africa on any indigenous plant products. Some plants such as the weed *Tephrosia vogelli*, which contains several rotenoids, featured in many talks but with most countries in the world not having an MRL for rotenone it is unlikely that this will be acceptable on citrus late in the season. Often plants that are being used medicinally with water as a solvent can be insecticidal when a more effective solvent is used or just when a surfactant is added to the water in the extraction process. Different chemotypes sometimes exist where plants that look identical contain different chemicals, so a single source of a plant should not be used in trials. Surprisingly, pyrethrin and neem are the only registered botanicals in India so China is probably the best place to source commercial botanicals. Research should include commercial botanical products as contact insecticides and perhaps locally-manufactured products that could later be commercialised. Botanical products that should be evaluated include Requiem (*Chenopodium ambrosioides*), cevadine (the most active component in sabadilla), oxymatrine or matrine (*Sophora flavescens*) contained in Bio-Cure and perhaps Tophelex from Ecossearch.

Stated objective

Evaluate prospective short residual chemicals for late season control of thrips, psylla, leafhoppers and woolly whitefly.

Materials and methods

Woolly whitefly *Aleurothrixus floccosus* was being used as a screening pest in bioassays because we needed to keep a woolly whitefly culture for parasitoid releases (Project 1082). However, the culture collapsed for an unknown reason and this research was stopped while the remaining parasitoids were released. We were unable to restart the culture but also had little time for this project in 2015/6 because of having to focus on *ad hoc* FCM research. Subsequent to that, field populations of woolly whitefly declined in the northern regions, either due to the release of *Cales noacki* (Grout and Stephen 2019), indigenous natural enemies or drier seasons.

Screening products against woolly whitefly to determine field dosages

A technique for using woolly whitefly in bioassays was developed where leaves infested with nymphs were cleaned of wax wool, dipped in the pesticide solution and then stood vertically with their petioles in water for long enough to determine mortality. Sixty nymphs spread over at least four leaves were used per concentration and mortality determined after 40 h and 90 h based on honeydew production. Effective dosage ranges could then be evaluated in the field against woolly whitefly.

Testing field dosages on woolly whitefly

High populations of woolly whitefly were common in the Brits area where farmers grow fruit for processing but when sites were looked for to evaluate products that looked promising in bioassays, none could be found from 2015 onwards. It is possible that drier seasons and natural enemies reduced the field populations.

Orchard trials against all target pests

Based on the expected results against woolly whitefly, field trials were planned for psylla, thrips and leafhoppers. However, suitable sites for psylla and thrips were not found so the only field trials that could be conducted were on leafhoppers. Field trials with psylla are very difficult to conduct because growers have a zero tolerance for this vector of greening disease. This is why it takes a long time for chemical companies to get sufficient registration data to put citrus psylla on pesticide labels.

Products that have shown most promise and warrant field evaluation are: Requiem 0.5%, KangroShield 100 0.5%, Xterminator 0.5%, and Ecosearch Extract 1%. However, Requiem is no longer being handled by Bayer and does not appear to be available. Ecosearch Extract is being formulated in small quantities for mosquito control so is also not available for field trials. The new products sulfoxaflor, cyazypyr and flupyradifurone should also be included in evaluations where possible.

Leafhopper field trials

The opportunity arose to conduct an orchard trial on green citrus leafhopper *Empoasca distinguenda* near Mookgopong on Rotterdam farm in July 2013. Rustenburg navel orange trees that had just been harvested had high numbers of green leafhopper so the orchard was divided into blocks of approximately 0.5 ha and two sticky yellow traps (Chempac) placed in each for 6 days in order to get a precount of infestation levels. The orchard was divided into two areas and each contained four treatment blocks. Two products that have now been registered on citrus for other pests were compared with Phosdrin (mevinphos 500 SL) at 30 ml/hl water. The new products were Exirel (cyazypyr 100 SE) at 75 ml/hl and Closer (sulfoxaflor 240 SC) at 12 ml/hl water. Trees were sprayed with the grower's automatic machine (Superbird) at just under 4000 L/ha on 3 July 2013. Approximately 2 h after spraying, 4 yellow sticky traps were hung along the centre row of each replicate block and these were left for 5 days before being replaced with fresh traps that were hung for a further 7 days. Numbers of green citrus leafhopper on the traps were counted and these numbers converted to counts per week, then transformed to square root of the number plus 0.5 in order to normalise the data before an analysis of variance was conducted. Means were further compared using Student-Newman-Keuls' *post hoc* test at $\alpha = 0.05$.

A trial was also conducted on citrus leafhopper *Penthimiola bella* and green citrus leafhopper at Copperzone near Marble Hall in 2017. KangroShield 100 and Xterminator, two botanical pesticides containing natural pyrethrins, were compared with Phosdrin SL. Yellow traps were first hung out for a week to determine relative levels of both leafhoppers at the site, then large treatment blocks chosen with two replicates (185 trees per replicate) so that the leafhopper numbers were as similar as possible in the blocks before treatment application. Treatments were applied on 2 May 2017 and four yellow traps hung in the centre of each replicate block the next day. These were replaced on 9 May with fresh traps for another week. There was already damage on the fruit when the trial started

so results had to depend on trap catches. Counts were normalised with the use of a log + 1 transformation before conducting an ANOVA and using Tukey's *post hoc* test at $\alpha = 0.05$ to compare means.

This trial was repeated in 2018 at Copperzone with Phosdrin, KangroShield 100 and Xterminator again being used at the same rates but with the inclusion of Exirel as well at 50 ml/hl, which was a lower dosage than used previously at Mookgopong. Yellow traps were first hung out for a week to determine relative levels of both leafhoppers at the site, then large treatment blocks chosen with two replicates so that the numbers were as similar as possible. Treatments were applied on 10 April 2018 using approximately 3 850 L/ha and there were 340 trees per treatment. Four sticky yellow traps were hung in the centre rows of each replicate block late in the afternoon after sprays were applied in the morning. Numbers of green citrus leafhopper and the citrus leafhopper were counted on the traps after 7 days and the traps replaced. Further counts were made after another 7 days of exposure. Fruit damage was not determined because there were damaged fruit before the trial started. Counts were normalised with the use of a log + 1 transformation before conducting an ANOVA and using Tukey's *post hoc* test at $\alpha = 0.05$ to compare means.

Results and discussion

Woolly whitefly culture

The culture of woolly whitefly collapsed for an unknown reason and several attempts to re-establish it failed. Further bioassays beyond May 2014 were therefore not possible and no field trials with woolly whitefly have been conducted due to the populations being generally low under drought conditions.

Bioassays with woolly whitefly nymphs

Four bioassays were conducted with woolly whitefly nymphs in 2014 (Tables 3.4.2.1-4). BP medium distillation range oil at 0.5% was used as a standard in all the trials and was consistently the best treatment. However, the white wool had largely been removed from these nymphs so efficacy in the field on the underside of leaves would be much lower. Apart from the medium oil 0.5% standard, the best treatments with final mortality of 98% or higher were Requiem 0.5%, Pygar Super (or Kangroshield 100) 0.5%, Dursban 75 ml/hl, Xterminator 0.5% and abamectin 20 ml plus medium oil 250 ml/hl. Results from sulfoxaflor 12 ml/hl (Table 3.4.2.2) were surprisingly poor but this may be because its contact toxicity is not as good as the systemic toxicity. Runner or methoxyfenozide has a growth regulator mode of action so it is not surprising that it had no effect in this type of bioassay (Table 3.4.2.3).

Table 3.4.2.1. Bioassay 1: Treated 31 Mar 2014

Treatments	Mortality after 35 h (%)	Mortality after 84 h (%)
Water control	13.9	11.7
BP medium oil 0.5%	100.0	100.0
Requiem 0.5%	97.4	98.0
Exirel 100 ml/hl	83.2	87.0
Delegate 20 g/hl	64.2	85.3

Table 3.4.2.2. Bioassay 2: Treated 24 Apr 2014

Treatments	Mortality after 45 h (%)	Mortality after 92 h (%)
Water control	15.8	55.3
Sulfoxaflor 12 ml/hl	13.8	31.9
Bio-Cure 150 ml/hl	65.9	92.8
BP Medium oil 500 ml/hl	99.8	100.0
Pygar Super* 0.5%	100.0	100.0

*Later known as KangroShield 100

Table 3.4.2.3. Bioassay 3: Treated 6 May 2014

Treatments	Mortality after 43 h (%)	Mortality after 92 h (%)
Water control	6.5	13.5
Teepol 100 ml/hl	70.5	74.1
Runner 60 ml/hl	12.8	13.6
Dursban EC 75 ml/hl	94.3	99.8
BP Medium oil 500 ml/hl	100.0	100.0

Table 3.4.2.4. Bioassay 4: Treated 22 May 2014

Treatments	Mortality after 48 h (%)	Mortality after 95 h (%)
Water control	8.3	14.3
Ecosearch plant extract 1%	93.7	92.3
Xterminator 0.5%	98.6	98.4
Abamectin 20 ml plus medium oil 250 ml/hl	97.7	99.4
BP Medium oil 0.5%	99.6	99.9

Leafhopper field trials

The results of both new chemicals against green citrus leafhopper were disappointing and were inferior to Phosdrin at the dosages used, but perhaps these were a bit low (Table 3.4.2.5). Closer showed a bit more activity than Exirel after the first week but clearly neither had knockdown action because trap catches shortly after spraying were the same as the control.

Table 3.4.2.5. Results with two new chemicals against the green citrus leafhopper near Mookgopong

Treatments	Mean green citrus leafhoppers per trap per week		
	Pre-treatment over 6 d	First 5 DAT	6-12 DAT
Untreated control	169.2	39.6 a	25.1 a
Phosdrin 500 SL 30 ml/hl	189.3	10.2 b	12.0 b
Exirel 100 SE 75 ml/hl	163.3	32.9 a	20.5 ab
Closer 240 SC 12 ml/hl	202.4	32.2 a	15.4 ab

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

At Marble Hall in 2017, Phosdrin at 30 ml/hl gave good knockdown of both species of leafhoppers and this has been shown in many trials over the years. In this trial both Xterminator and KangroShield 100 gave good knock down of the green citrus leafhopper and control was still significant after 14 days (Table 3.4.2.6), but neither of these products had a significant effect on the citrus leafhopper *Penthimiola bella*.

Table 3.4.2.6. Results against both leafhopper species on citrus near Marble Hall in 2017

Treatments	Mean number of leafhoppers per trap			
	3-9 May 2017 (1-7 DAT)		9-16 May 2017 (7-14 DAT)	
	Green citrus leafhopper	Citrus leafhopper	Green citrus leafhopper	Citrus leafhopper
Control	63.6 a	21.6 a	16.1 a	11.0 a

Kangroshield100 500 ml/hl	2.3	b	11.0	a	3.0	b	9.1	a
Xterminator 500 ml/hl	4.9	b	13.6	a	4.3	b	8.0	a
Phosdrin 30 ml/hl	2.5	b	4.9	b	2.3	b	3.4	b

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

In 2018, numbers of the citrus leafhopper were not as high as in 2017 and Xterminator showed no significant difference from the control in both evaluations (Table 3.4.2.7), although some of the other treatments were significantly better in one or both evaluations. All treatments were significantly effective against the green citrus leafhopper in the first evaluation but by the second evaluation only Phosdrin was significantly different from the control. Numbers of green citrus leafhopper in the Exirel treatment were significantly inferior to Phosdrin in both evaluations but with this active ingredient it is possible that they could fly or jump onto traps soon after treatment but they may not have been capable of feeding and causing damage. This would have to be confirmed by looking at fruit damage in trials where there was no previous damage.

Table 3.4.2.7. Results against both leafhopper species on citrus near Marble Hall in 2018

Treatments	Mean number of leafhoppers per trap							
	17 April 2018 (0-7 DAT)			24 April 2018 (7-14 DAT)				
	Green citrus leafhopper	Citrus leafhopper		Green citrus leafhopper	Citrus leafhopper			
Control	34.4	c	6.1	b	21.8	b	9.1	c
Phosdrin 30 ml/hl	3.8	a	1.1	a	4.1	a	2.6	a
Xterminator 500 ml/hl	7.8	ab	4.0	ab	11.3	b	8.0	bc
Kangroshield 500 ml/hl	6.6	ab	1.8	a	10.0	ab	5.4	abc
Exirel 50 ml/hl	13.6	b	3.3	ab	11.8	b	3.8	ab

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

Conclusion

Some products used in bioassays have been surprisingly effective but this is with perfect coverage as a dip. Most of these products would cause short-residual contact mortality but this may be adequate for late in the season. KangroShield 100 and Xterminator were both effective against green citrus leafhopper and Xterminator has received an emergency registration. These products would most likely have some impact on citrus thrips and citrus psylla but we have been unable to verify this in the field. Results from Closer and Exirel on green citrus leafhopper were disappointing but this may have been because our evaluations were based on traps and these products may not have caused knockdown shortly after spraying.

Future research

At this stage no further research is planned due to a lack of capacity and the need to focus on new systemics for *Diaphorina citri* (Project 1148). If new, promising products become available that have a short preharvest interval they could be investigated under a new project when further levy funding is available.

Technology transfer

IPM workshops September 2018.

Reference cited

Grout, T.G. and P.R. Stephen. 2019. An update on the status of several whiteflies (Hemiptera: Aleyrodidae) found on citrus in South Africa. *African Entomology* 27(1): 254-257.

3.4.3 **FINAL REPORT: Preharvest management of oribatulid mites on citrus in KwaZulu-Natal** Project 1172 (2017/8 – 2018/9) by T G Grout and P R Stephen (CRI)

Summary

A citrus grower in southern KwaZulu-Natal has 30-60% of his fruit infested with a harmless arboreal mite *Siculobata sicula* at harvest and these are resulting in export rejections. We sampled in the trees and under the trees of three orange orchards every two months for a season and found that there was no obvious movement of these mites from the soil into the canopy that could be stopped with a trunk barrier. The mites remain in the outer canopy all year and become more abundant during late summer, resulting in high levels of fruit infestation at harvest. There is no obvious correlation with honeydew-producing pests and these mites are not known to be phytophagous, so there may be sufficient algae, lichen or fungi present to support them. Many of the acaricides registered for use on citrus have had little effect on these mites but profenofos was found to be very effective in addition to full cover horticultural mineral oil sprays, or chlorfenapyr applied as a medium cover spray soon after petal fall. Although we could not establish where the mites are coming from the grower can now use treatments that are registered for other citrus pests to prevent further export rejections.

Opsomming

'n Sitrusprodusent in suidelike KwaZulu-Natal se vrugte is tydens oes 30-60% met 'n skadelose boomlewende myt, *Siculobata sicula*, geïnfesteer, en dit lei tot uitvoer-verwerpings. Ons het monsters in die bome en onder die bome van drie lemoenboorde elke twee maande, vir 'n seisoen lank geneem, en gevind dat daar geen duidelike beweging van hierdie myte vanaf die grond in die boomlower in was, wat met 'n stamversperring gestop kon word nie. Hierdie myte bly in die buitenste boomlower dwarsdeur die jaar en word meer volop gedurende laat somer, wat tot hoë vlakke van vrug-infestasië tydens oes lei. Daar is geen voor-die-hand-liggende korrelasie met heuningdoudoenderende plaë nie, en hierdie myte is nie daarvoor bekend dat hulle plantetend is nie, so daar mag voldoende alge, ligene of swamme teenwoordig wees om hulle te onderhou. Baie van die mytdoders, geregistreer vir gebruik op sitrus, het min effek op hierdie myte gehad, maar daar is gevind dat profenofos baie effektief was, tesame met volbedekking tuinboukundige minerale oliespuit, of chlorfenapyr, toegedien as 'n medium bedekkingsspuit, spoedig ná blomblaarval. Hoewel ons nie kon vasstel waar die myte vandaan kom nie, kan die produsent nou behandelings gebruik wat vir ander sitrusplaë geregistreer is, ten einde verdere uitvoer-verwerpings te voorkom.

Introduction

Donovale Farming Company is situated east of Pietermaritzburg on the Table Mountain road, near the Umgeni River. The farm has 50 ha citrus besides sugarcane and avocados, and the workers own 49% of the farm. They have the option to buy more land and expand the citrus but in 2015 they lost around R1 million from rejected exports due to the presence of the live scavenger beetle-mite *Siculobata sicula* (Oribatulidae) on their fruit under the calyx. In 2016 we assisted them in trying to control the mite in the orchard based on research we had conducted previously (Grout and Stephen 2009) but there were still large numbers of infested fruit going through the packline. We then assisted them with the experimental fumigation of fruit in bins using carbon dioxide (Grout and Stoltz 2016) which controlled most of the mites before the packline and allowed them to export. However, future expansion of this farming enterprise is on hold unless they can prevent the mites from moving under the calyx of large numbers of fruit.

This mite has been recorded on citrus before in the Letsitele area (national collection) and in Egypt (Rasmy et al. 1972) and it is known from Europe and elsewhere. It is known as an arboreal oribatulid and it may be associated with lichens (Seyd and Seaward 1984) but there is no information on what it is doing in citrus trees without obvious

lichen and whether it moves between the orchard floor and the tree during certain times of the year or not. Almost all the mites found under the calyx appear to be adults so it is possible that they are in a dispersal mode, moving upwards and outwards in the tree during autumn and winter. This may mean that younger life stages may live on the orchard floor and move up the trunk of the trees when they become adults. This would give the opportunity of stopping them from climbing the trunk with the use of chemical or physical stem barriers.

A research project was proposed to sample mites on the orchard floor, on the tree framework and in the canopy every two months to better understand the behaviour of the mites, then develop an appropriate control mechanism that will prevent them from infesting the fruit.

Stated objectives

A: Sample mites on orchard floor, tree framework and in the citrus canopy.

B: Evaluate appropriate field control measures to prevent mites from moving under the calyx.

Materials and methods

In the first year, sampling was conducted approximately every two months to determine where the mites are found and also to see whether there was any obvious association with lichen or moss. It was not possible to visit the farm in April so the first samples were taken in June 2017.

Eight data stations, each comprising a single row of about 10 trees were selected to represent the 3 orchards/blocks as follows: Valencia oranges = 3 Data stations, Block F (Navel oranges) = 1 Data station and Navels = 4 Data stations (Fig. 3.4.3.1).



Figure 3.4.3.1. Layout of eight data stations on the farm near Pietermaritzburg.

The following methods of assessing beetle mite infestation were used.

Fruit counts

At least 50 fruit were examined per data station. The calyx sepals of each fruit were gently lifted to ascertain beetle mite and mealybug infestation (Fig. 3.4.3.2). Red scale infestation of fruit was also noted. No fruit counts could be done after harvest so beating samples were introduced.



Figure 3.4.3.2. Mites under calyx.



Figure 3.4.3.3. Sticky pipe-trap driven into the ground.

Beating

A white plastic sheet was held under a terminal branch with leaves. Using a length of ± 28 mm dia. PVC pipe, the branch was struck 5 times to dislodge mites. This was done on six branches on one side of three adjacent trees and then the mites on the sheet were counted with aid of a 5 diopter headloupe. This count was repeated three times on each side of the data row, yielding six counts per data station from 18 trees. Samples of the counted mites were placed in 70% ethanol to confirm identification.

Sticky Pipe-traps

Lengths of 40 mm dia. white plastic pipe, ± 400 mm long were prepared by sharpening one end to be driven into the soil. A sticky barrier was created on the top half of the pipe by wrapping a strip of cling wrap 75 mm high around the pipe and applying Tangle-Trap adhesive (20 mm wide) to the centre of the cling wrap to trap mites moving upwards. Four of these traps were placed at each data station (Fig. 3.4.3.3).

Sticky Branch-traps

Cling wrap ± 70 mm wide was wrapped around a lateral branch in the tree and coated with a ± 15 mm band of Tangle-Trap adhesive (Fig. 2.4.x.4). Four of these traps were placed at each data station to monitor mite movement in the tree on the main branch framework. Long-term branch-traps were introduced in January 2018 and evaluated in March 2018 when further branch-traps were installed and evaluated in May 2018.



Figure 3.4.3.4. A sticky branch-trap inside the canopy.



Figure 3.4.3.5. A pitfall trap (later discontinued).

Pitfall Traps

Two pitfall traps were placed per data station. These comprised 400 ml plastic honey jars containing ± 100 ml of a water/ethanol/glycol mix and embedded in the soil with the rim at ground level (Fig. 3.4.3.5). These were later discontinued.

Soil Samples

Three soil samples were taken per data station. A small spade was used to lift a 30 to 40 mm deep section of soil from under a tree. This sample was inserted into a 6 L bucket with as little disturbance of the soil as possible. The volume of the sample was about 1.5 L. A sticky barrier on duct tape was placed inside the bucket just under the rim to catch mites emerging from the soil and moving upwards.

All samples were taken at approximately two-month intervals for one complete season. Sampling dates were 2-5 June 2017, 14-16 August 2017, 30 October - 1 November 2017, 8-11 January 2018, 13-16 March 2018 and 15-17 May 2018.

Evaluation of field treatments

In 2018, treatments applied for evaluation in the navel orange blocks against the beetle mites included a Citrimist 1% oil spray on 13 August on three blocks of trees. A Hunter (chlorfenapyr 240 g/L SC) 45 ml/hl was applied on 24 October to two of the blocks sprayed with the oil in addition to another block. Citrimist 1% was again sprayed on 26 November to one of the blocks sprayed with the winter oil and another unsprayed block. The blocks of trees used per treatment were four to six rows wide. All treatments were applied by the grower using a commercial spray machine at around 4 000 L/ha with 8 000 L/ha for the summer oil. Two untreated blocks were also retained for comparison purposes. However, without our knowledge the grower also applied profenofos (500 ml/L EC) at 100 ml/hl and 4 900 L/ha to all citrus on the farm on 5 October 2018.

For evaluations, each treatment block was divided into two zones and within each zone, six terminal branches from three to four trees in three regions of the zone and on both sides of the central two rows in the block, were beaten over a white card. Counts of beetle mites were totalled for every 6 beats from the three to four trees. There were therefore six counts per zone and 12 counts per treatment. The first such beating evaluation was conducted on 24 October 2018 just before the Hunter sprays were applied and a second similar evaluation was conducted on 29 January 2019. On the latter date, 100 fruit were inspected per zone in each treatment block. These comprised 25 fruit from four positions per zone with two positions being on either side between the central two rows. The fruit were rated as being infested with beetle mite or not and calyces were lifted for inspection purposes.

Results and discussion

Surveys in 2017/8

The first samples taken in June 2017 showed that 35% of the fruit were infested despite a previous spray programme that contained seven acaricidal applications plus the inclusion of oil on several occasions. There were only negligible numbers of mites on the orchard floor with a mean of 1.46 mites per soil sample evaluated one month later, 0.19 mites per sticky pipe-trap and 0.8 beetle mites per pitfall trap (plus many other mites, beetles, flies and ants being caught, which led to this monitoring system being discontinued). The sticky branch-traps had an average of 0.38 mites per trap, indicating very little mite movement on the tree framework.

In August after harvest, results of the beating counts varied greatly between data stations. Total beetle mite numbers varied from 7 (Val 3) to 203 (Navels 3) with an average of 81 beetle mites per data station and 13.5 beetle mites per branch. Counts within data stations also varied with some large variations between row sides i.e., East versus West, but this was not consistent for all data stations. The sticky pipe-traps yielded no mites while the branch-traps yielded a mean of only 0.7 mites per trap, with the most mites caught on one trap being 11 (Navels 1). These counts indicate that at this time the beetle mites are present on the tree foliage and there is very little movement to and from the tree framework. The mean number of mites recovered from the soil samples was 0.25.

Counts for each data station in October are summarised in Table 3.4.3.1. Counts in the Valencia blocks were all low, except for the soil sample from V3c. In the navels, the beating counts were high, with N3 yielding the highest number of mites, but stick and branch counts were very low. These counts indicate that at this time, beetle mites were still present on the tree foliage and were already moving to the fruit. However, all the branch traps showed

no mites, indicating no movement to and from the tree canopy on the framework. Numbers of beetle mites emerging from the soil samples were variable with high numbers from two samples, V3c and N3a, yielding 12 and 9 mites respectively. The soil samples were then moved to Tulgren funnels and a further 58 mites were extracted from N3. This showed that there were mites in the soil in some places but there was no tendency for these mites to move upwards on the pipe-traps or onto the branch-traps. When fruit were inspected there was no presence of red scale and only 1.3% infested with mealybug.

Table 3.4.3.1. Results of sampling between 30 October and 1 November 2017

Orange type	Fruit infestation (%)	Total number beetle mites			
		Beating per station	Pipes	Branch	Soil after Tulgren
Valencia 1	4	9	0	0	0
Valencia 2	6	2	0	0	1
Valencia 3	2	3	0	0	15
Block F	22	37	0	0	7
Navels 1	28	102	1	0	8
Navels 2	32	34	1	0	2
Navels 3	24	143	1	0	68
Navels 4	0	28	1	0	5
MEANS	14.8	44.8	0.2	0.0	4.4

Counts for each data station in January 2018 are summarised in Table 3.4.3.2. Fruit and beating counts were generally high in the navels and had increased in comparison with the survey in October. N3 again yielded the highest number of mites, as it has for the previous two surveys. No mites were recorded for V2, possibly due to the application of Hunter to this area in October by the grower. Although most branch traps showed no mites, from 1 to 8 mites were recorded on 10 traps, indicating some movement taking place both to and from the tree canopy, although these numbers are low relative to the numbers obtained with beating. Mite numbers in the soil samples were generally higher than previously, perhaps due to the summer rains, and there were low numbers of mites on the branch traps, although no indication that they were moving only in the direction from the trunk outwards.

Table 3.4.3.2. Results of sampling between 8 and 11 January 2018

Orange type	Fruit infestation (%)	Total beetle mites			
		Beating per station	Pipes	Branch	Soil
Valencia 1	12	21	0	1	1
Valencia 2	0	0	0	0	3
Valencia 3	2	4	7	2	7
Block F	32	65	1	9	2
Navels 1	30	207	0	7	16
Navels 2	52	176	1	8	11
Navels 3	58	295	0	2	16
Navels 4	50	110	0	0	10
MEANS	29.1	109.8	0.4	0.9	2.8

Woolly whitefly numbers were observed in navel blocks 2 and 3 in both October and January surveys but beetle mite numbers in navels block 1 were similar to levels in blocks 2 and 3 so it did not appear that the mites were increasing in number due to the presence of honeydew or sooty mould. Mean mealybug infestation of fruit also declined in January to 0.8% while fruit infestation levels of beetle mites increased.

Counts for each data station in March 2018 are summarised in Table 3.4.3.3. Fruit infestation and beating counts were again high in the navels and low in the Valencias. Beating yielded more than twice the number of mites than the previous survey with very high numbers in the navels. In Block F, 92% of the navels were infested and around 70% of the fruit in the other navel blocks. Branch traps and stick traps showed even less mites than the previous survey, again indicating very little movement to and from the tree canopy. Each of the long-term branch traps, exposed for 64 days, showed more mites than the four traps exposed for ±42 h. However, when related to time, the results are similar. V2 showed the least mites, probably due to the application of Hunter by the grower to this area.

Table 3.4.3.3. Results of sampling between 13 and 15 March 2018

Orange type	Fruit infestation (%)	Total beetle mites				
		Beating per station	Pipes	Branch	Branch-L.Term	Soil
Valencia 1	22	48	0	0	-	10
Valencia 2	2	9	0	0	-	2
Valencia 3	16	18	0	0	0	18
Block F	92	306	1	1	9	11
Navels 1	72	844	0	2	5	1
Navels 2	70	365	0	3	23	2
Navels 3	70	658	1	4	11	21
Navels 4	76	358	2	1	12	20
MEANS	52.5	325.8	0.2	0.3	10.0	3.5

It is possible that the May 2018 counts were affected by the cooler weather and wet conditions around sampling time, although the grower had applied some treatments to the blocks. Results for each data station are summarised in Table 3.4.3.4. Fruit infestation in the Valencias increased except for V2 that stayed low. Fruit infestation in the two navel areas not harvested showed a slight decrease. Beating counts were generally lower than the previous survey except for V2 where beetle mite numbers have been increasing since the Hunter application. Branch-traps and pipe-traps again caught very few mites. The long-term branch-traps, exposed for 61 days, also caught few mites relative to the regular branch-traps exposed for ±42 h.

Applications by the grower of Envidor (10 ml/hl water) between the March and May surveys had little effect in reducing mite numbers but Mitigate (150 ml/hl) and Agrimec Gold (8.6 ml/hl) plus Citrimist (200 ml/hl) caused 30-80% reduction in infestation.

Table 3.4.3.4. Results of sampling between 15 and 17 May 2018

	Total beetle mites
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Orange type	Fruit infestation (%)	Beating per station	Pipes	Branch	Branch-L.Term	Soil
Valencia 1	54	33	0	0	-	2
Valencia 2	2	34	1	1	-	2
Valencia 3	32	30	0	0	-	0
Block F	52	70	0	1	11	2
Navels 1	58	256	2	2	11	0
Navels 2	Harvested	259	1	0	2	0
Navels 3	Harvested	130	1	0	7	5
Navels 4	64	336	0	0	16	9
MEANS	43.7	143.5	0.2	0.1	9.4	0.8

Figures summarising the trends in fruit infestation (Fig. 3.4.3.6) and beetle mites in beating samples (Fig. 3.4.3.7) over the complete sampling period are given below and show increasing numbers during the summer rainy season. However, there was no explanation as to where the mites were coming from as they were clearly not moving into the tree from the soil. Most of the mites in the canopy appeared to be adults so perhaps they were blowing up the valley on the wind from somewhere else and surviving on algae, lichen or fungi that may occur in that climate.

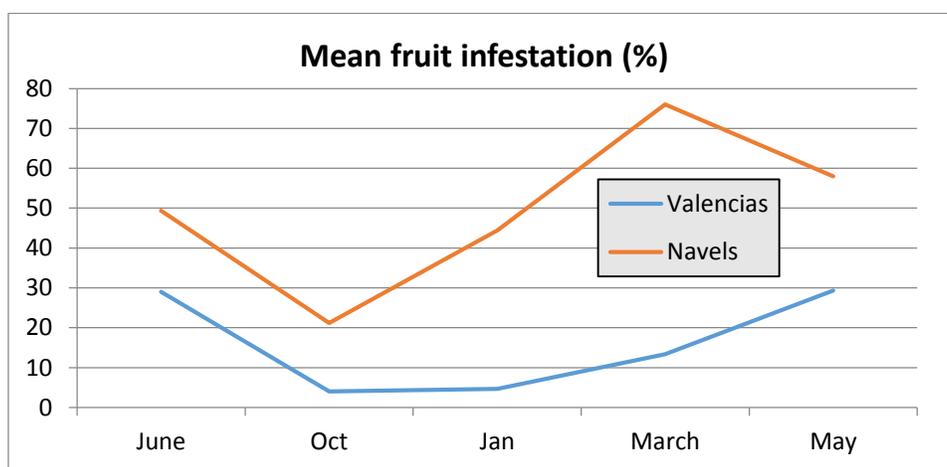


Figure 3.4.3.6. Percentage fruit infestation at different sampling dates through 2017-8

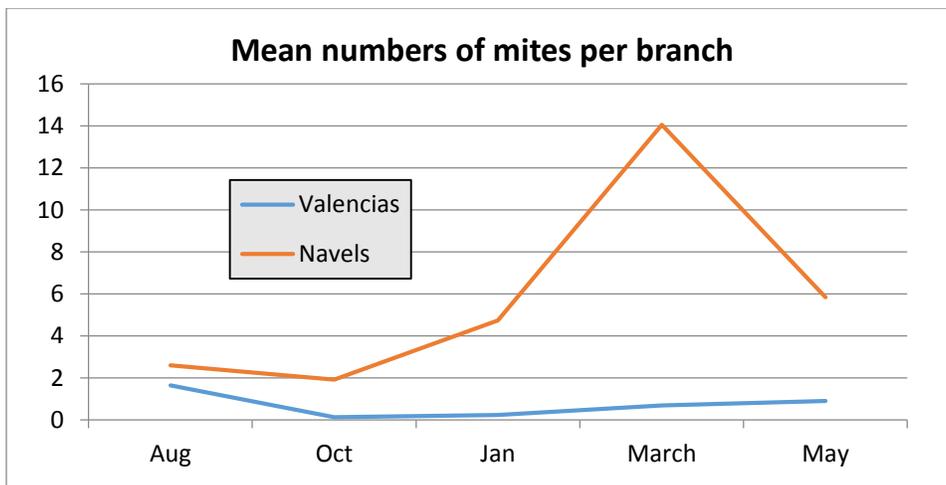


Figure 3.4.3.7. Mean numbers of mites per branch from beating samples at different dates through 2017-8

Evaluation of field treatments

Results from beating samples on 24 October 2018 were very surprising with only one beetle mite being collected after beating 72 branches per treatment, including the control. On enquiry we then discovered that all blocks had been sprayed with profenofos which was clearly very effective in eliminating the beetle mites. Nevertheless, we proceeded with a second evaluation on 29 January 2019 after all treatments had been applied. These results showed that 3% of the fruit in the control treatment was infested but there was zero infestation in all the other treatments. The results from beating showed some recovery of mite numbers in all treatments except the winter oil followed by Hunter where no mites were found (Fig. 3.4.3.8).

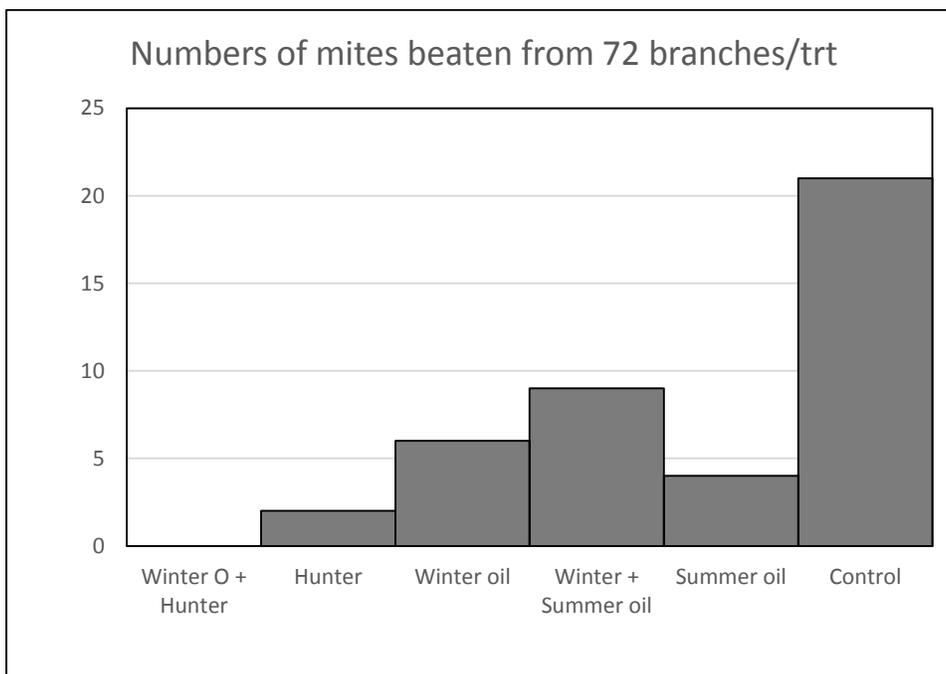


Figure 3.4.3.8. Total numbers of mites recovered per treatment after beating 72 branches on 29 January 2019

Although the grower's application of profenofos interfered with the evaluation of the planned treatments in our trial it demonstrated that this would be an excellent way of controlling this mite and the treatment may not be required every season. If full cover oil sprays are included in the spray programme and possibly the use of Hunter for

thrips, the extremely high fruit infestation levels that were found in our surveys would be prevented and further exports will not be jeopardised.

Conclusion

The unusual case of the beetle mite *Siculobata sicula* causing export rejections was confirmed when fruit infestation in the orchard was found to exceed 70% in mid-summer. The mites appear to survive in the outer canopy without any movement into the tree from the soil or noticeable movement on the main framework branches. It is possible that the mites are blowing into the citrus trees from another host plant because most on the canopy appeared to be adults. There was no obvious source of honeydew or sooty mould for them to feed on but perhaps there are sources of algae or fungi on the foliage that they can survive on. Research on control measures showed that profenofos was very effective and full cover oil sprays or medium cover sprays with Hunter, or both, would largely control the pest.

Technology transfer

A poster was presented at the CRI Citrus Research Symposium in August 2018 and a presentation at the Entomological Society Congress in Umhlanga in July 2019.

Future research

No further research is planned on this oribatid mite.

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3.4.4 FINAL REPORT: Augmentation of *Orius thripoborus* for citrus thrips control

Project 1191 (April 2018 – March 2019): Sean Moore, Wayne Kirkman, Mellissa Peyper (CRI), Candice Coombes, Tammy Marsberg, Sonnica Albertyn, Martin Hill (Rhodes University), Shimon Steinberg, Rami Friedman, Amit Sade, Amir Grosman and Eldad Peer (BioBee)

Summary

Both *Orius thripoborus* and *Amblyseius swirskii* are known to be predators on certain species of thrips, amongst other insects. If it can be demonstrated that augmentation of these species can contribute towards suppression or control of citrus thrips in the field, this may provide a valuable tool for use in commercial citrus orchards. Although augmentation trials with *Orius* were previously conducted by Tim Grout, and no establishment was recorded, conducting similar trials again were thought to be justified, as BioBee has developed mass rearing technology for both natural enemies and the simultaneous application of Artemia cysts, providing an alternative food source, may be beneficial. *Orius* and *A. swirskii* were released on citrus seedlings in tunnels and in a cage on several occasions, at differing densities and simultaneously with Artemia cysts. No establishment of either species was recorded on any occasion. The reasons for this are not clear, but could be unfavourable temperatures (too hot initially and too cool for the last release), residues of sprays on trees (only potentially for the first release)

or the lack of new flush on the trees for oviposition by the predators. Independent trials conducted by BioBee revealed a similar lack of establishment with both species. Consequently, the project has been terminated, despite the initial plan to conduct field trials.

Opsomming

Albei *Orius thripoborus* en *Amblyseius swirskii* is bekend as predatore op sekere blaaspootjie spesies en ander insekte. As dit bewys kan word dat loslating van hierdie spesies tot die onderdrukking van sitrusblaaspootjie in die veld kan lei, kan hierdie 'n waardevolle praktyk vir gebruik in kommersiële boorde word. Alhoewel loslatings proewe met *Orius* voorheen deur Tim Grout gedoen is en geen vestiging aangeteken is nie, het ons gemeen dat verdere proewe regverdig word omrede BioBee massateel tegnologie vir albei spesies ontwikkel het, en die gesamentlike toediening van *Artemia* sists, as 'n alterniewe kosbron, voordelig kan wees. *Orius* en *A. swirskii* is op sitrussaailing in tonnells en in 'n veldhok teen verskillende digthede en met *Artemia* sists en verskillende geleenthede losgelaat. Geen vestiging van enige van die twee spesies is op enige geleentheid aangeteken nie. Die redes hiervoor is nie duidelik nie maar kan ongunstige temperature wees (oorspronklik te warm en vir die laaste loslating te koud), residue op bome (moontlik net die eerste loslating) of 'n tekort aan jong groei op die bome vir eierlegging deur die predatore. Ons sal binnekort besluit of ons gaan voortgaan met die oorspronklik beplande veldloslatings. Onafhanklike proewe wat deur BioBee uitgevoer is het soortgelyk geen vestiging van beide spesie gewys nie. Gevolglik is die projek beëindig, ondanks die oorspronklike plan om veldproewe te doen.

Introduction

There is a dearth of soft options for control of citrus thrips, *Scirtothrips aurantii*, particularly for use in IPM orchards and for use after spring, when harsh broad-spectrum thripicides are likely to cause repercussions of other pests. *Orius thripoborus* is recognised as an effective naturally occurring predator of citrus thrips in South Africa (Grout & Stephen, 1999). BioBee is developing the technology for mass rearing of *Orius thripoborus*. This provides us with the opportunity to test whether augmentation of this species can be used for control of citrus thrips.

This was initially investigated by Grout & Stephen (1999). They concluded that a) releases of 10 or 30 adults, or 10 nymphs per citrus tree had no impact on thrips infestation of fruit, and neither did double releases of similar numbers per tree; b) numbers of *Orius* recovered by beating six days after the second release were negligible in all treatments and the same as the control, indicating that the predators had dispersed; c) survival of *Orius* from adult to egg in the rearing process was only 20-30%; d) the high cost of rearing would make the product prohibitively expensive.

Orius laevigatus has been commercialized by BioBee since the early 1990s. Since then it has been used successfully as a biocontrol agent against Western flower thrips, *Frankliniella occidentalis*, in Israel and Europe, in greenhouse vegetables such as sweet pepper and eggplant, and several other crops in which the flowers possess available pollen (Chambers et al., 1993).

BioBee South Africa is developing the technology for mass rearing of *Orius thripoborus*. It will therefore be possible to release higher numbers and more frequently than did Grout & Stephen (1999). The problem experienced with rapid dispersal of the predatory bugs can be countered by the simultaneous application of *Artemia* cysts. These are dormant eggs of brine shrimps, *Artemia* spp., which are mass produced globally and used for rearing *Orius* spp. and other biocontrol agents (Arjis and De Clercq, 2001). This food supply may not only reduce the dispersion of *Orius*, but could also enable the build-up of *Orius* numbers as a preventive standing army in the orchard.

If it can be demonstrated that *Orius* augmentation can contribute towards suppression or control of citrus thrips in the field, this may provide a valuable tool for use in commercial citrus orchards. Although *Orius* is likely to be affected by residues of certain broad-spectrum insecticides, by releasing these bugs, it may become possible to adopt a softer thripicide programme, both achieving satisfactory suppression of thrips and avoiding a detrimental

impact on the released *Orius*. The trial also will provide an opportunity to test the efficacy of *Amblyseius swirskii*, released with *Artemia* cysts, to control citrus thrips.

Stated objectives

- A. Mass rear sufficient numbers of *Orius thripoborus* for greenhouse and field trials.
- B. Conduct greenhouse trials on citrus thrips infested citrus seedlings to determine approximate release densities of *Orius* for field trials.
- C. Conduct field augmentation trials with *Orius* against citrus thrips.
- D. Determine whether the application of *Artemia* cysts can improve the establishment of *Orius* and provide biocontrol of thrips with *Orius*.

Materials and methods

Five treatments, four experimental and a control, were set up in shaded greenhouse tunnels at Rhodes University (Table 3.4.4.1).

Table 3.4.4.1. Established treatments and tunnel location

Treatment No.	Treatment description	Tunnel	I-button present
1	100 <i>Orius</i> per ten trees	C (on opposite ends)	Yes, only one placed by treatment 1
2	200 <i>Orius</i> per ten trees	C (on opposite ends)	
3	100 <i>Swirskii</i> per tree	A (1 st half of tunnel)	Yes
4	100 <i>Swirskii</i> per tree & 100 <i>Orius</i> per ten trees	A (2 nd half of tunnel)	Yes
5	Control (no treatment)	B	Yes

Each treatment comprised 10 actively flushing citrus seedlings. The citrus seedlings were collected from the nursery and had not been treated with chemicals four-weeks prior to the natural enemies being released (Table 3.4.4.2).

Table 3.4.4.2. Chemicals applied to citrus seedlings used in this study. Natural enemies were released onto these trees four weeks after the last treatment (18.04.2018).

Date of spray	Chemical
23.01.2018	Chlorpyrifos
29.01.2018	Spinetoram
06.02.2018	Copper and abamectin
07.03.2018	Fenpyroximate and spirotetramat
21.03.2018	Profenofos

Orius and *Swirskii* were applied following instructions provided by BioBee, from which the insects were received. Total insects per treatment were released in two tranches, on 18 April 2018 and 25 April 2018. On each date, half the total number of insects per treatment were released. A further application of *Orius* only occurred on 23 May 2018 – 50 *Orius* were applied to treatment 1; 100 to treatment 2. Prior to insect release and every week thereafter, 0.1 g of *Artemia* cysts were applied to each tree by first gently spraying the trees with a handheld sprayer, followed

by sprinkling the cysts evenly over the leaves of the trees, as shown by Adriaan Serfontein of BioBee who was present during the first natural enemy release. Each week, following the first release of insects, all 10 trees per treatment were searched thoroughly using magnifying glasses for the presence of possible *Orius* or *Swirskii* eggs, nymphs or adults. Temperature within the tunnels was also monitored during the study using i-buttons.

A further release of 100 *Orius* per tree on eight potted trees in a 3 m x 3 m cage was conducted on 23 May at CRI PE. Artemia cysts were simultaneously applied.

Results and discussion

Orius and *Swirskii* establishment was unsuccessful. On only one occasion was a single *Orius* observed in treatment 2, whilst only two and one *Swirskii* were observed in treatments 3 and 4, respectively (Table 3.4.4.3). Temperature readings recorded in the tunnels are presented graphically (Figures 3.4.4.1 to 4). The trial was terminated on 8 June 2018.

Table 3.4.4.3. Recorded observations for *Orius* and *Swirskii* at different times post-release.

Evaluation time post first release	Observations
<p>One week (24.04.2018)</p> <p><i>First half batch released 18.04.2018</i></p>	<p>No <i>Orius</i> or <i>Swirskii</i> recorded.</p> <p>Other: It was noted that the leaves of the trees had been sprayed with water and thus may have forced the insects to seek shelter elsewhere. This problem was resolved prior to further releases and did not occur again.</p>
<p>Two weeks (02.05.2018)</p> <p><i>First half batch released 18.04.2018</i> <i>Second half batch released 25.04.2018</i></p>	<p>One <i>Orius</i> was observed in treatment 2, on the plastic sheeting behind the trees.</p> <p>Hundreds of mites were observed. However, distinguishing between <i>Swirskii</i> and the co-packaged prey mites, was not made. Given that most of the mites counted were within close proximity to the sachet and moved relatively slowly, counted mites were suspected to be prey mites, not <i>Swirskii</i></p>
<p>Three weeks (10.05.2018)</p>	<p>No <i>Orius</i> was observed. Two and one <i>Swirskii</i> were observed in treatments 3 and 4, respectively.</p> <p>Other: It was suggested that flowering plants in the nearby vicinity be actively searched for the presence of <i>Orius</i>. This was done, but no <i>Orius</i> were observed. In addition, pictures of newly applied <i>Artemia</i> cysts were taken for evaluation a week thereafter to assess whether feeding had occurred. Cysts were also examined for the presence of mould. None was observed.</p>
<p>Four weeks (15.05.2018)</p>	<p>No <i>Orius</i> or <i>Swirskii</i> were observed.</p> <p>Other: Pictures of one-week old <i>Artemia</i> cysts were taken and compared. Feeding was not apparent.</p>
<p>Five weeks (22.05.2018)</p> <p><i>A third release of 50 and 100 Orius to treatment 1 and 2, respectively, occurred on 23 May 2018</i></p>	<p>No <i>Orius</i> or <i>Swirskii</i> were observed.</p>
<p>Six weeks (31.05.2018)</p>	<p>No <i>Orius</i> or <i>Swirskii</i> were observed.</p>
<p>Seven weeks (07.06.2018)</p>	<p>No <i>Orius</i> or <i>Swirskii</i> were observed on either the citrus seedlings or surrounding flowering vegetation. This evaluation was conducted by BioBee personnel, Adriaan Serfontein and Rami Friedman.</p> <p>The project has been terminated.</p>

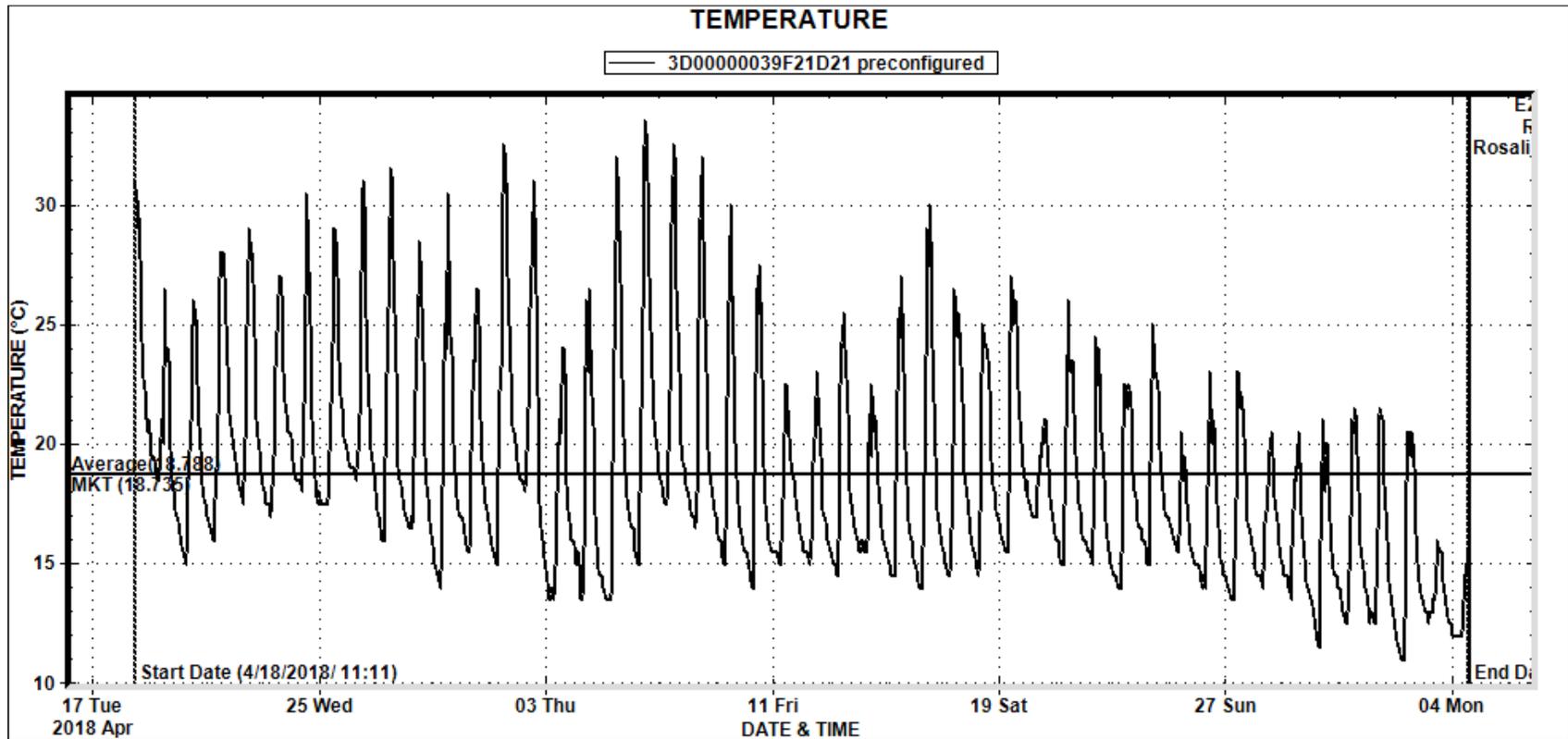


Figure 3.4.4.1. Temperature readings recorded between the period 18.04.2018 and 07.06.2018 for Tunnel C in which both *Orius* treatments (treatment 1 and 2) were held.

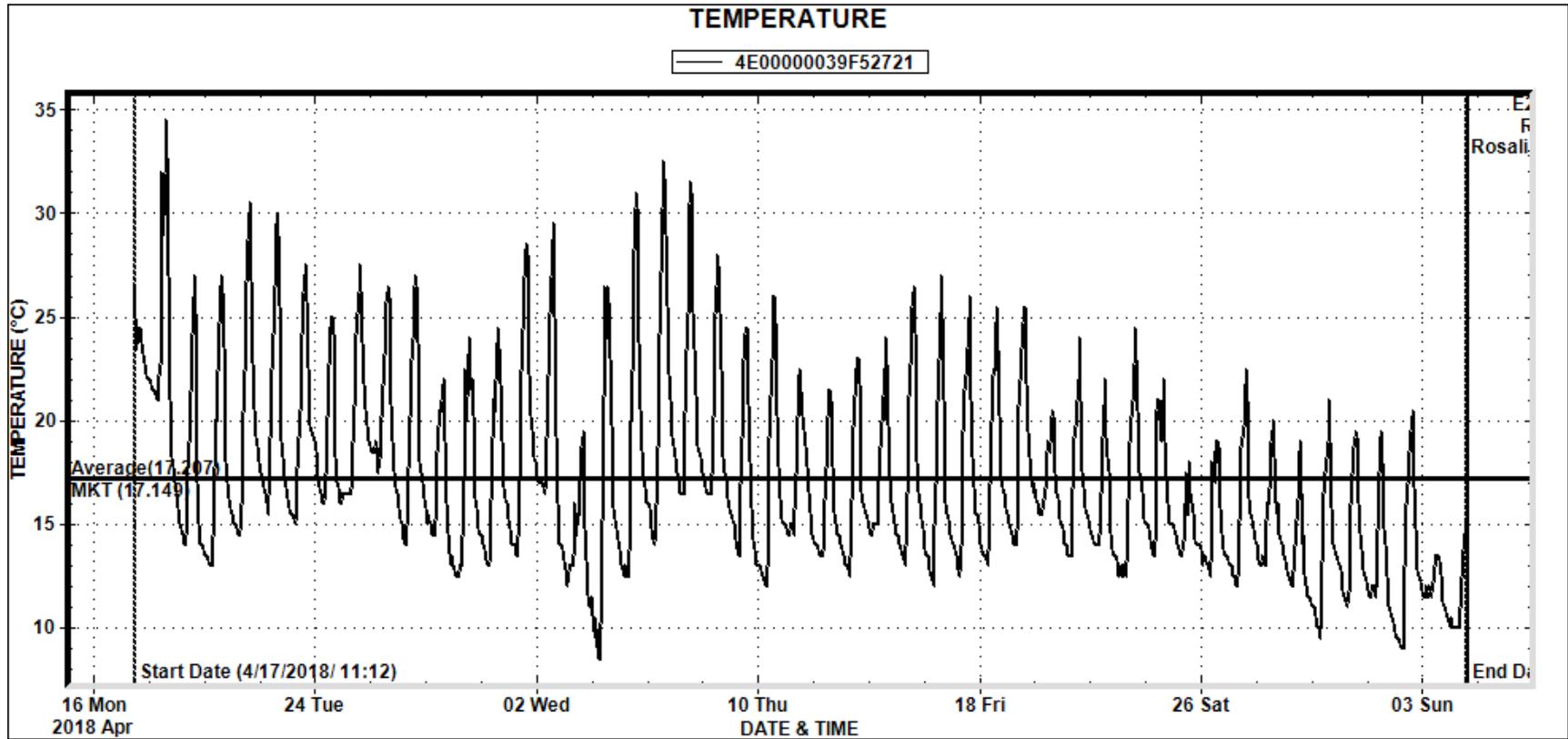


Figure 3.4.4.2. Temperature readings recorded between the period 18.04.2018 and 07.06.2018 for Tunnel A, for the *Swirskii* only treatment (treatment 3).

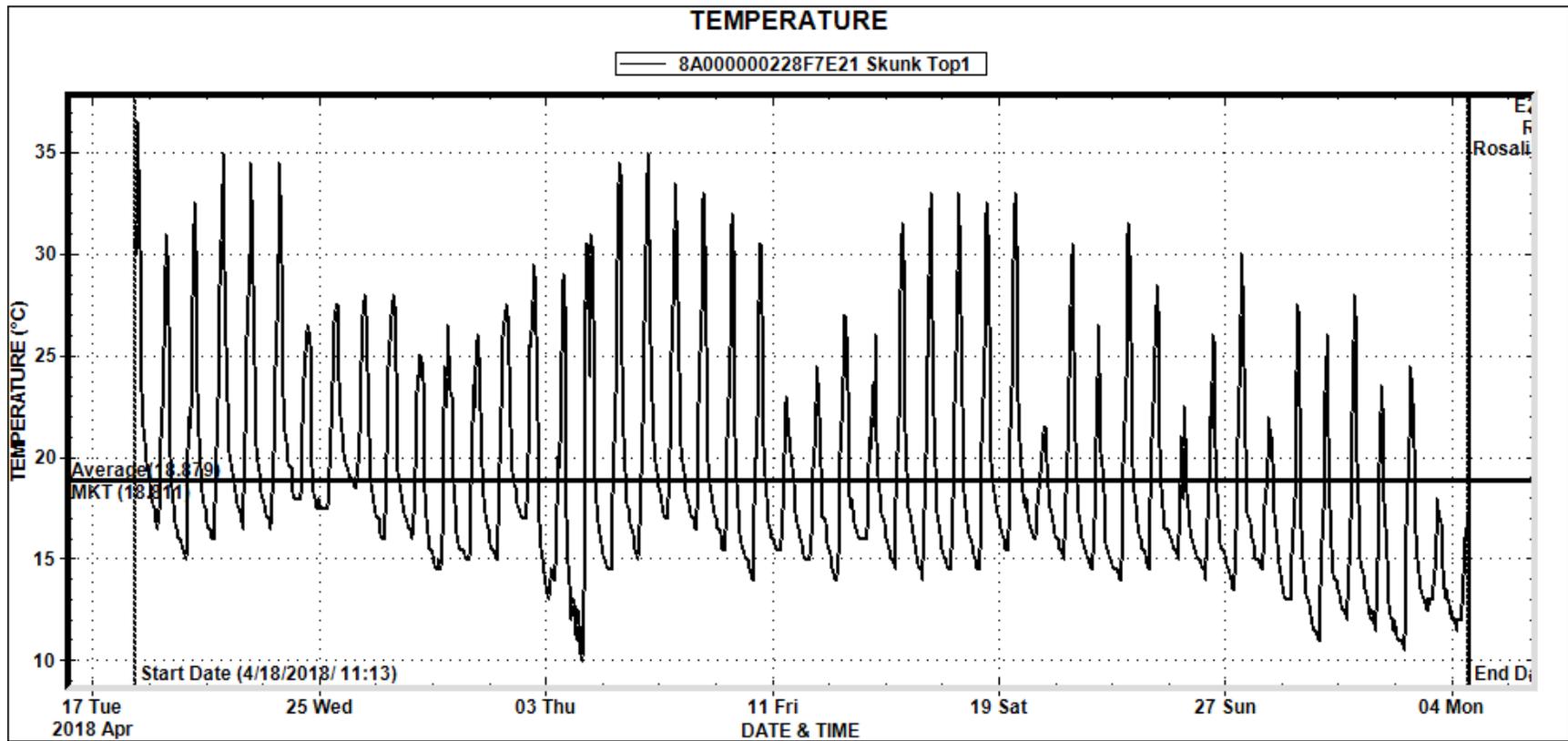


Figure 3.4.4.3. Temperature readings recorded between the period 18.04.2018 and 07.06.2018 for Tunnel A, for the *Swirskii* & *Orius* treatment (treatment 4).

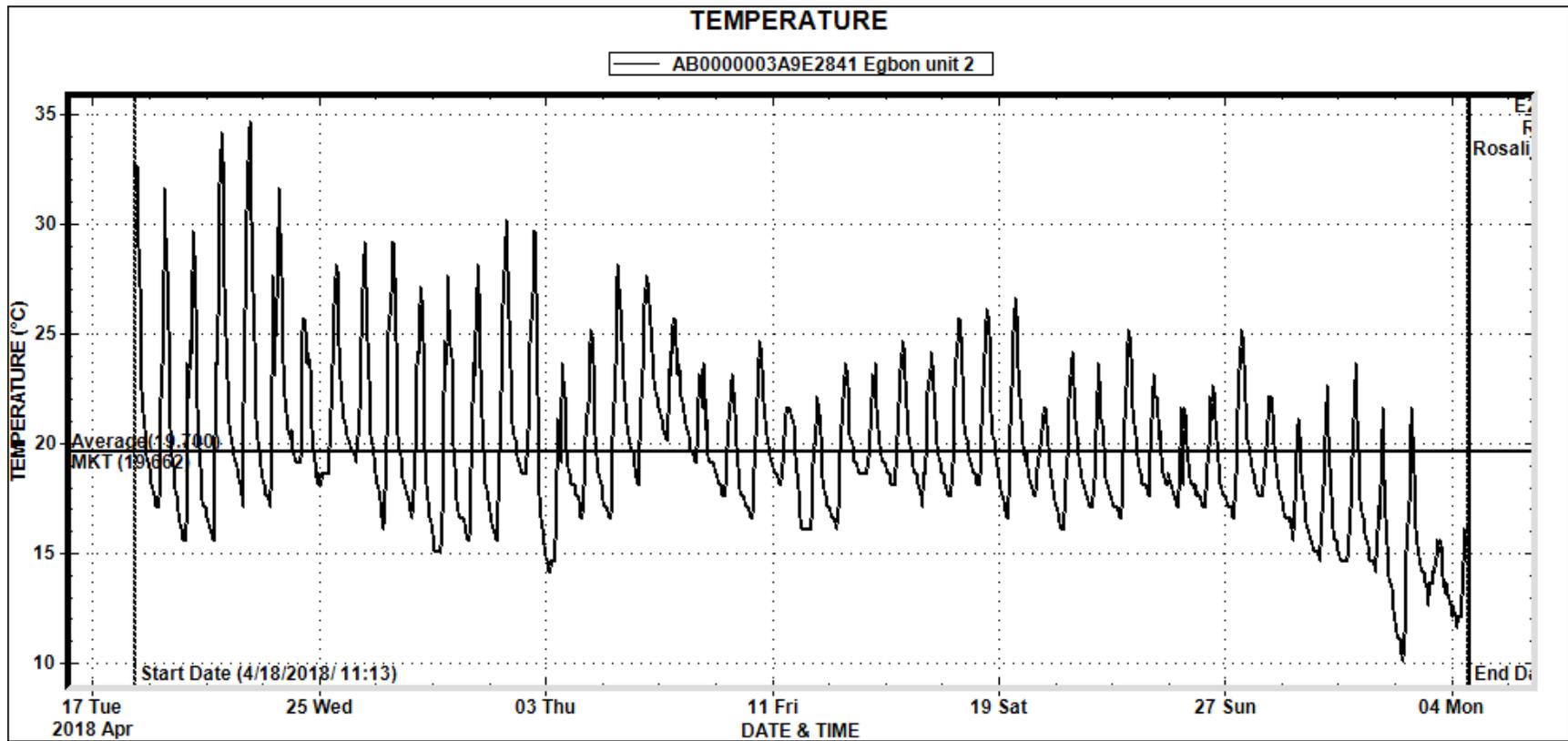


Figure 3.4.4.4. Temperature readings recorded between the period 18.04.2018 and 07.06.2018 for Tunnel B, in which only the control trees were held.

In the 3 m x 3 m cage trial, once again, no *Orius* could be found with subsequent surveys.

The reasons for the lack of establishment (tunnels and cage) could be unfavourable temperatures (too hot initially and too cool for the last release), residues of sprays on trees (only potentially for the first release) or the lack of new flush on the trees for oviposition by the predators.

Conclusion

No establishment of either *Orius* or *A. swirskii* was recorded in tunnel and cage trials. Similar trials subsequently conducted by BioBee, similarly revealed no establishment of either species on citrus trees in a protected environment. Consequently, the project was terminated prematurely i.e. without the planned execution of field trials.

Future research

No future research is planned.

Technology Transfer

None.

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3.4.5 FINAL REPORT: Control of Asian Citrus Psyllid, vector of Huanglongbing

Project 1158 (2016/7 – 2018/9) by Aruna Manrakhan (CRI), Glynnis Cook (CRI), Rochelle Clase (CRI), Tim Grout (CRI), Herbert Wiehe (Domaine de Labourdonnais, Mauritius), Preaduth 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* Kuwayama (Hemiptera: Liviidae), an effective vector of Huanglongbing, is currently known to be present in southern Tanzania. It is a question of time when ACP will reach South Africa. In preparation for the potential introduction of ACP in South Africa, trials on management of the pest were carried out in Mauritius where it is present. A treatment package consisting of sequential applications of three systemic insecticides: acetamiprid, imidacloprid and acephate registered for control of the citrus trioza *Trioza erytreae* (Del Guercio) (Hemiptera: Triozidae) in South Africa was tested in a citrus orchard. The efficacy of three sticky traps: Alpha Scents lime green ACP, Alpha Scents yellow and Chempac yellow for monitoring of ACP was also evaluated in citrus orchards. A plant based attractant (ACP Pherolure) was tested in combination with the Alpha Scents ACP trap. Incidence of ACP was generally 50% lower in plots under the treatment package compared to the control (untreated plots). However, the lower numbers of ACP in the treated plots compared to untreated plots did not consistently occur between replicates. At low populations of ACP, the Alpha Scents ACP and Alpha Scents yellow traps were more effective than the Chempac yellow

sticky traps in capturing ACP. The addition of ACP Pherolure on the Alpha Scents ACP trap did not improve catches of ACP. The studies in Mauritius demonstrated that the insecticides used for control of *T. erytrae* in South Africa would partially suppress ACP. For more effective control of ACP, additional measures including application of other insecticides would be necessary. Emergency registration of the insecticides tested in this study and other insecticides known to be effective against ACP in other parts of the world should be sought. Tests on improving detection of ACP should continue.

Opsomming

Die Asiatiese sitrus bladvloei (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), wat die effektiewe vektor van Huanglongbing is, is tans teenwoordig in die suide van Tanzanië. Dit is 'n kwessie van tyd voordat ACP Suid-Afrika sal bereik. Ter voorbereiding vir die moontlike verspreiding van ACP na Suid-Afrika is daar ondersoek ingestel na die bestuur van die plaag in Mauritius, waar die pes teenwoordig is. 'n Behandelingspakket wat bestaan uit sekwensiële toedienings van drie sistemiese insekdoders: asetamiprid, imidakloprid en asefaat, wat geregistreer is vir beheer van die sitrus bladvloei, *Trioza erytrae* (Del Guercio) (Hemiptera: Triozidae) in Suid-Afrika, is getoets in 'n sitrusboord. Die doeltreffendheid van drie klewerige lokvalle: 'Alpha Scents lime green ACP', 'Alpha Scents yellow' en 'Chempac yellow' is ook geëvalueer in sitrusboorde, vir die monitering van ACP. 'n Plant-gebaseerde lokval (ACP-Pherolure) is getoets in kombinasie met die 'Alpha Scents ACP'-lokval. Voorkoms van ACP was oor die algemeen 50% laer in eksperimentele areas onder die behandelingspakket in vergelyking met die kontrole (onbehandelde area). ACP getalle binne herhalings van behandelde areas, was egter nie altyd laer as die in onbehandelde areas nie. Tydens lae insidensie van ACP was die geel lokvalle van 'Alpha Scents ACP' en 'Alpha Scents yellow' meer effektief met die vangs van ACP as die 'Chempac-yellow' lokvalle. Die byvoeging van 'ACP Pherolure' tot die ACP-lokval van 'Alpha Scents' het nie die vangs van ACP verbeter nie. Die studies in Mauritius het getoon dat die insekdoders wat gebruik word vir die beheer van *T. erytrae* in Suid-Afrika, ACP gedeeltelik onderdruk. Vir meer effektiewe beheer van ACP sal addisionele maatreëls, insluitende die toepassing van ander insekdoders nodig wees. Noodregistrasie van die insekdoders wat in hierdie studie getoets was, en ander insekdoders bekend vir effektiewe beheer van ACP in ander dele van die wêreld, moet versoek word. Toets van produkte om verbeterde opsporing tegnieke vir ACP daar te stel, moet voortgaan.

Introduction

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is an important pest of citrus in many parts of the world due to its effective ability to vector Huanglongbing (HLB), a devastating disease of citrus, caused by the phloem limited bacteria *Candidatus Liberibacter asiaticus* (Las) (Hall et al., 2013, Halbert and Manjunath, 2004, Grafton-Cardwell et al., 2013). The combined presence of ACP and Las in citrus cultivation areas has been shown to be devastating to citrus production as is currently the case for the citrus industry of Florida, USA, with high production and revenue losses (Hodges and Spreen, 2012) due to high incidence of Las. ACP is of Asian origin and currently occurs in many citrus production regions in the world including Asia, North America and South America (Hall et al., 2013). ACP has been spreading across North America and Central America (Hall et al., 2013). The spread of ACP has been attributed mainly to the movement of infested plant materials (Hall et al., 2013). In 2016, the occurrence of ACP was first reported on the African continent, more precisely in Morogoro, Tanzania (Shimwela et al., 2016). Subsequent surveys conducted in Tanzania and neighbouring Kenya have confirmed a wider distribution of ACP in these countries, with the pest even occurring at altitudes of over 1600m above sea level (Rwomushana et al., 2017). Las was not found in any of the ACP specimens sampled in the most recent East African surveys (Rwomushana et al., 2017). However, Las is present in Ethiopia (Saponari et al., 2010) and it is a question of time when the vector and the pathogen will meet. ACP can also vector the African form of HLB or African Greening caused by *Candidatus Liberibacter africanus* (Laf) (Lallemand et al., 1986) which is present in East and southern Africa (Aubert, 1987). The reports of ACP about 2000 km from the northern borders of South Africa are concerning and have, as such, increased the level of threat of introduction of ACP in South Africa. Both Asian and African forms of HLB are best managed by vector control. It was thus deemed important to conduct some proactive research on the control of ACP.

The biology of ACP renders it a difficult pest to manage (Grafton-Cardwell et al., 2013), possibly more difficult to manage than the indigenous citrus trioqid *Trioza erytreae* (Del Guercio) (Hemiptera: Triozidae). ACP has a high intrinsic growth rate, is tolerant of temperature extremes and transmits HLB rapidly (Grafton-Cardwell et al., 2013). Adult females of ACP lay eggs exclusively on young flush points and nymphs develop on immature leaves (Chavan and Summanwar, 1992). ACP adults, on the other hand, feed on young stems and leaves of all development stages (Hall et al., 2013).

As with the control of *T. erytreae* in South Africa, soil applied systemic insecticides were found to be effective and are recommended for control of ACP in parts of the world where the pest occurs (Setamou et al., 2010, Rogers and Shawer, 2007, Ichinose et al., 2010, Grafton-Cardwell et al., 2013, Gatineau et al., 2010). In field trials conducted by Setamou et al. (2010), efficacy of control of soil-applied neonicotinoid insecticide imidacloprid was found to vary between 92.7% and 100%. In the same trials, interestingly, control levels of ACP were correlated with titres of imidacloprid in leaves with high levels of suppression occurring when titres in leaves were well above 200 ppb which occurred for up to 11 weeks after application (Setamou et al., 2010). The dosage of imidacloprid used in the trials by Setamou et al. (2010) was approximately 1.6 times lower than the recommended dosage of imidacloprid in citrus orchards in South Africa. Besides systemic insecticides, foliar sprays of broad spectrum insecticides such as chlorpyrifos (organophosphate), fenpropathrin (pyrethroid) and oxamyl (carbamate) on dormant trees before bud break were found to be effective in suppressing adult ACP numbers for 5-6 months (Qureshi and Stansly, 2010).

In South Africa, the main measures to manage *T. erytreae* and Laf which it vectors, include soil drenching with dimethoate or imidacloprid and trunk treatments with either methamidophos or acetamiprid (Grout, 2012). On bearing trees, application of the systemic dimethoate Rogor EC can only be used up to 50% petal fall (Grout, 2012). Whilst treatments should be applied preventatively on non-bearing trees, the treatment threshold on bearing trees is when one or more trioqid eggs and/or nymphs are found on new growth or when one of more adult trioquids are caught on yellow traps (Grout, 2012). Spray treatments of some organophosphates are also recommended for control of psyllids but only for trees with a trunk diameter in excess of 150 mm (Grout, 2012).

In view of the potential incursion or introduction of ACP in South Africa, the question is whether the control measures which are recommended for *T. erytreae* in South Africa will be as effective against ACP and against transmission of the greening disease. Moreover, it will be important to know the efficacy of control of some new measures against ACP.

Since ACP does not occur in South Africa, studies to determine efficacy of management measures on the pest have to be carried out in areas where the pest occurs. The study on management measures for ACP was conducted in Mauritius where it is present (Garnier and Bove, 1992). In Mauritius, both *Liberibacter* species: Las and Laf also occur (Garnier et al., 1995).

Stated objectives

- A. To evaluate the effectiveness of citrus trioqid control measures used in South Africa for control of ACP.
- B. To evaluate the effectiveness of novel measures for control of ACP.
- C. Compare efficacy of visual traps for detection of ACP

Materials and methods

1. Efficacy of control measures for ACP

The aim of this trial was to evaluate the efficacy of a treatment package, consisting of three sequential systemic insecticide applications, on populations of ACP. Two of the registered insecticides are currently recommended by Citrus Research International (CRI) for control of *T. erytreae*. One insecticide is a new product which was registered for control of *T. erytreae* in 2018.

Experimental site

The field trial was conducted between October 2016 and September 2017 in a 5-year-old orchard of *Citrus sinensis* (L.) Osbeck (cv. Valencia Late) (0.6 ha) (S 20° 03' 54.44" E57° 37' 23.02") situated in a commercial farm, Domaine de Labourdonnais Ltée, in the north of the Republic of Mauritius. The trees were grafted on rough lemon, *Citrus jhambiri* Lush., and planted at intervals of 5m x 5m. The average canopy surface area of the trees in the orchard was estimated at 6 m² (tree height [2 m] x canopy diameter [3m]). Each tree in the orchard had 3-4 trunks. Each trunk diameter of each tree was estimated at 65 mm. Prior to this trial, there were no specific control measures for ACP in the orchard.

Treatment package

The treatment package consisted of (1) a trunk application of acetamiprid (Rado™ 20 SL with acetamiprid at 200 g/L, Kirsch Co. Ltd, Mauritius) at 13 ml per tree in October 2016, (2) an application of imidacloprid (Premiseal 30.5 SC with imidacloprid at 305 g/L, Kirsch Co. Ltd, Mauritius) by soil drenching at a rate of 10 ml per tree in November 2016, and (3) a trunk treatment with acephate (Spectra stem 350 AL with acephate at 350 g/L, Spectrum Research Services, South Africa) at a rate of 3 ml per trunk in April 2017.

This treatment package was compared to an untreated control.

Experimental layout

The orchard was divided equally in eight plots such that each plot contained 20-30 trees. Four of the plots were under the above treatment package and there were four untreated control plots. There were therefore four replicates of the treatment package and four replicates of the untreated control. Treated and untreated replicate plots were randomized within the orchard (Fig. 3.4.5.1).

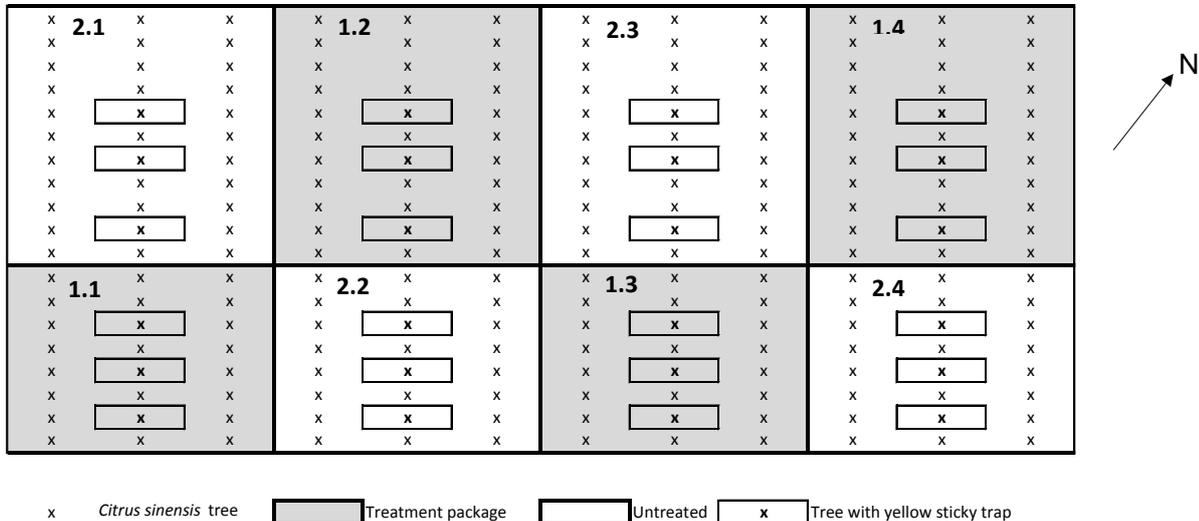


Figure 3.4.5.1. Sketch of layout of field trial testing efficacy of a chemical treatment package for control of *D. citri*. Plots 1.1, 1.2, 1.3 and 1.4 were under the treatment package of sequential application of acetamiprid, imidacloprid and acephate. Plots 2.1, 2.2, 2.3 and 2.4 were untreated.

Trapping

Efficacy of the treatment on populations of ACP was assessed by weekly counts of adult ACP on yellow sticky traps (Chempac (Pty) Ltd, Suider Paarl, South Africa). Three yellow sticky traps were placed in the middle row of each treated and untreated plot (Fig. 3.4.5.1). Traps were placed one week before the start of treatment in treated and untreated plots. Trapping was conducted for one year in all plots from October 2016 until

September 2017. Every week, traps were removed and replaced. Traps were shipped back to CRI Nelspruit for psyllid identification and counts.

Identification and analysis of ACP samples

Traps collected were checked under a stereomicroscope for ACP adults. ACP was identified using features described in Burckhardt (2007). ACP adults were characterized by their forewing patterns which consisted of brown spots spreading to the outer margin and a white gap at the apex of the wing separating two brown areas.

In order to determine fluctuations of infective ACP samples across the weeks, the presence of *Candidatus Liberibacter asiaticus* (Las) in ACP samples collected from traps was determined using Real-time PCR. ACP samples were analysed individually between October 2016 and June 2017. Thereafter all samples of ACP from the same replicate plot of a treatment were pooled prior to analysis. ACP were removed from sticky traps using HistoChoice clearing agent (Merck) and DNA extractions were done using a modified CTAB (hexadecyltrimethylammonium bromide) extraction protocol as previously described (Cook et al, 2014). Real-time PCR for the specific detection of 'Ca.L. asiaticus' was done using primers and probe described by Li et al. (2006).

2. Efficacy of visual traps for detection of ACP

In a second study, the efficacy of different trap types for monitoring of ACP was determined. The study was divided in two parts: (1) Evaluation of different colour traps for monitoring of ACP and (2) Evaluation of a baited and unbaited sticky trap for monitoring of ACP.

The evaluation of different colour traps for monitoring of ACP was conducted for four weeks over two separate time periods: (1) October 2017- January 2018 (15 weeks) and (2) January- February 2019 (4 weeks). The evaluation of baited and unbaited traps for monitoring of ACP was carried out for four weeks between February and March 2019.

Study site and layout

All trapping studies were conducted in three pummelo, *Citrus maxima* (Burm.) Merrill, orchards in a commercial farm, Domaine de Labourdonnais Ltée, (S20° 04' 18.90" E57° 37' 02.92") in the north of Mauritius. The orchard sizes varied between 0.3 and 0.6 ha.

Study 1: Colour traps

Three double-sided traps were evaluated: (1) Chempac yellow sticky trap (8.5 cm x 20 cm) (Chempac (Pty) Ltd, Suider Paarl, South Africa), (2) Alpha Scents yellow sticky trap (18 cm x 14 cm) (Insect Science (Pty.) Ltd, Tzaneen, South Africa) and (3) Alpha Scents lime green ACP trap (18 x 14 cm) (Insect Science (Pty.) Ltd, Tzaneen, South Africa).

The dominant wavelengths of the three traps were determined using a Lovibond LC100 colorimeter (European Technology, Ripalta Cremasca, Italy) at a laboratory of Agricultural Research Council, Nelspruit, South Africa. The traps were not exposed in the field prior to the colorimeter measurements. Three measurements of lightness, colour attributes, chroma and hue angle of each trap type were taken and compared to three readings of the same parameters in two white reference samples.

Study 2: Baited and unbaited ACP traps

Two types of ACP traps were evaluated: (1) lime green ACP trap (18 cm x 14 cm) baited with ACP Pherolure (undisclosed plant based attractants) and (2) unbaited lime green ACP trap (18 cm x 14 cm). The ACP Pherolure was dispensed from a bulb-like septum. For the field comparison, the ACP Pherolure was placed in a cylindrical plastic basket (2.5 cm diameter and 5 cm height) and hung on top of one side of a sticky card (Fig. 3.4.5.2).

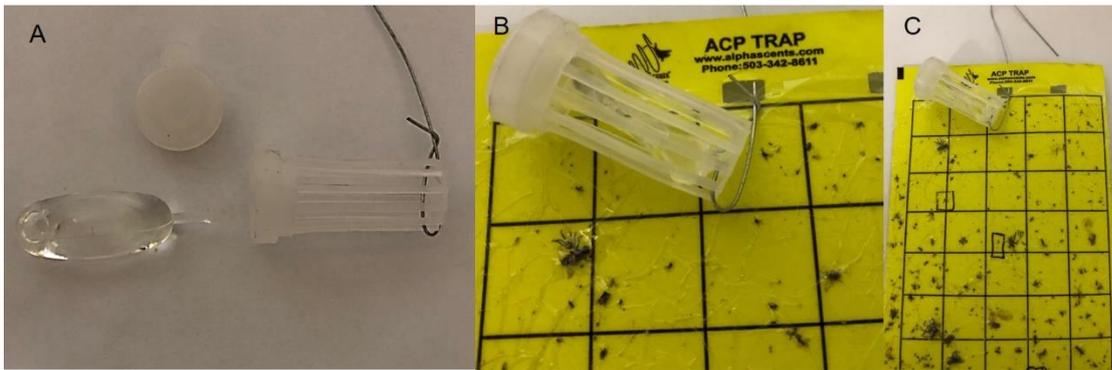


Figure 3.4.5.2. ACP trap with ACP Pherolure. (A) Placement of septum with lure inside basket with the pointed end of the septum facing the bottom end of the plastic basket; (B) Placement of ACP basket containing ACP Pherolure on top of the ACP trap; (C) Position of basket containing ACP Pherolure on top of ACP trap.

Experimental layout

Each trap was suspended at 1-1.5 m above ground on the outside of the canopy of a tree selected in each of the four border rows in each orchard. Each border row therefore contained one of each trap type evaluated. There were in total four traps of each type per orchard and 12 traps of each type in the three orchards. The positions of the different trap types along each border row of each orchard were randomised during placement and were not rotated within the border rows across the weeks. Traps were spaced at 10 - 15 m intervals along the border row.

Trap servicing and identification of psyllids.

The sticky traps were collected and replaced with fresh traps weekly. When baited and unbaited ACP traps were evaluated, only the sticky cards were replaced with fresh ones every week. The ACP Pherolure, on the other hand, was not replaced across the weeks.

Data analysis

For the trial on efficacy of control measures, the ACP catches in each replicate plot of a treatment were first summarised as numbers of ACP per trap per day. This was derived by dividing the total numbers of ACP captured by the product of total number of traps (3) in that replicate plot and the number of trap exposure days (number of days between placement and servicing). This was carried out in order to obtain standardised quantitative data on adult ACP populations. For the statistical analysis, all ACP count data (ACP per trap per day) which were above 0 in each replicate plot of each treatment were coded as 1 (presence) and all the count data that were 0 were coded as 0 (absence). A logistic regression was used to determine effects of treatment (treatment package and untreated), effects of time (sampling week) and effects of replicate on presence and absence of ACP. The interaction between replicate and treatment was also determined. For the logistic regression analysis, only data collected after the initiation of treatment were used.

The percentage of Las infectivity in ACP was determined from October 2016 to June 2017 when individual ACP specimens from each trap were tested.

For the trial on efficacy of traps for monitoring ACP, only weeks with positive catches (all trap types) were considered in the analysis. For the trial comparing the three types of colour traps, only 6 weeks out of 15 sampling weeks were used. For the trial comparing baited and unbaited ACP traps, all four weeks of sampling were used. Trapping data for both trials were converted to ACP catches per trap per day for each trap of each trap type. Since both trapping data sets did not follow a normal distribution (Shapiro-Wilk test, $P < 0.05$), non-parametric tests (Kruskal Wallis and Mann-Whitney) were used to determine differences between trap types in both trapping trials.

Results

1. Efficacy of control measures for ACP

Incidence of ACP was generally lower in plots under the treatment package consisting of sequential applications of acetamiprid (in October 2016), imidacloprid (3 weeks later) and acephate (5 months later) (Fig 3.4.5.3, Tables 3.4.5.1 & 2). The odds ratio of the untreated versus the treatment package was estimated at 1.91 which signifies that ACP was about two times more likely to be in untreated plots than in treated plots. It was difficult in this study to quantify the individual effects of each treatment within this treatment package. All treatments applied were systemic insecticides and as such would likely have a cumulative impact on populations of ACP. Replicate and time also influenced ACP incidence (Table 3.4.5.2). There was a higher incidence of ACP in the north-western plots than in the south-eastern plots (Table 3.4.5.1 & Fig. 3.4.5.1). There was a significant interaction between replicate and treatment which signifies that treatment was not always significant between the replicates. ACP was captured almost year round in the study site but the population was at its highest in August (as observed in the untreated plots) and at its lowest from December 2016 until mid-January 2017 (Fig. 3.4.5.3). During the latter period, there were five consecutive weeks when no ACP was captured in 24 traps checked on a weekly basis (Fig. 3.4.5.3). Infective ACP samples were found across the study period. Infectivity of ACP populations with Las was lower between October and December 2016 (mean percentage infectivity: $16.97\% \pm 10.30\%$) and peaked between April and June 2017 (mean percentage infectivity: $54.17\% \pm 14.38\%$).

Throughout this study no *T. erythrae* was recorded on the traps. However, another *Trioza* species, possibly *T. litseae* Bordage, was recorded weekly on traps in the citrus orchard. High catches of this *Trioza* spp. were recorded in the study orchard: a total of 2 189 specimens from October 2016 to May 2017. The identity of the *Trioza* species was not confirmed.

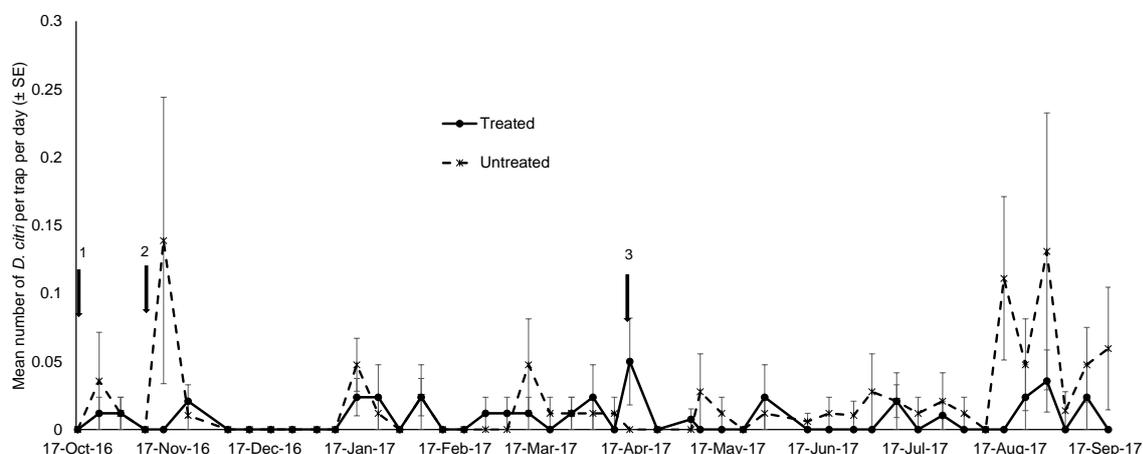


Figure 3.4.5.3. Catches of ACP on double sided Chempac yellow sticky traps between October 2016 and September 2017 in blocks under a treatment package and in untreated blocks in a Valencia orange orchard at Domaine de Labourdonnais Ltée, Republic of Mauritius. The treatment package consisted of (1) a trunk application of acetamiprid in October 2016, (2) an application of imidacloprid by soil drenching in November 2016, and (3) a trunk treatment with acephate in April 2017 as indicated by the black arrows.

Table 3.4.5.1. Presence of ACP in four replicate plots under (1) a treatment package of sequential application of acetamiprid, imidacloprid and acephate and (2) control/untreated in a Valencia orange orchard at Domaine de Labourdonnais Ltée, Republic of Mauritius. ACP was considered present (coded as 1) in a replicate plot when the ACP catches per trap per day was above 0. ACP was considered absent (coded as 0) in a replicate plot when the ACP catches per trap per day was 0.

Treatment	Replicate Plot number	Sum of counts over the study period (October 2016-September 2017)	
		Presence (1)	Absence (0)
Treatment package (1)	1.1	2	45
	1.2	15	32
	1.3	0	47
	1.4	7	40
Untreated (2)	2.1	22	25
	2.2	3	44
	2.3	15	31
	2.4	0	47

Table 3.4.5.2. Logistic Regression Analysis of incidence of ACP as a function of treatment, replicate, time and interaction between treatment and replicate.

Source	<i>df</i>	χ^2	<i>p</i>
Treatment	1	213.74	<0.0001
Replicate	3	213.05	<0.0001
Time	45	252.96	<0.0001
Treatment x Replicate	3	257.78	<0.0001
Model evaluation			
Likelihood ratio test	52	331.57	<0.0001
Score	52	138.48	<0.0001
Wald	52	66.34	0.09
Goodness of fit test			
Hosmer-Lemeshow	8	9.91	0.27

2. Efficacy of visual traps for detection of ACP

Very few ACP adults were captured during trials where different colour traps were evaluated. Only a total of 10 ACP adults were captured (on all traps of all trap types) between October 2017 and January 2018. Between January and February 2019, only 2 ACP adults were captured in total (all traps of all trap types). These low

prevailing numbers of ACP during the trials as such limit the conclusions of the comparative study of the performance of different trap types for detection monitoring of ACP.

Nonetheless at these low populations, significant differences in captures were found between trap types (Kruskal Wallis test: $df=2$, $p=0.03$) (Fig. 3.4.5.4). There was no capture of ACP in the Chempac yellow traps. It was clear that the two Alpha Scents traps (both yellow and lime green) were more sensitive for detection of ACP than the Chempac yellow trap. The dominant wavelengths of Chempac yellow, Alpha Scents yellow and Alpha Scents lime green were 580, 578 and 574 nm respectively.

In the trapping trial comparing baited and unbaited ACP traps, a total of 29 ACP adults were captured over the four weeks of trapping in the same orchards. There were no significant differences in catches between the two types of traps (Mann-Whitney test, $p=0.36$). The mean (\pm SE) numbers of ACP per trap per day in the unbaited ACP trap and baited ACP trap were 0.042 ± 0.02 and 0.045 ± 0.03 respectively.

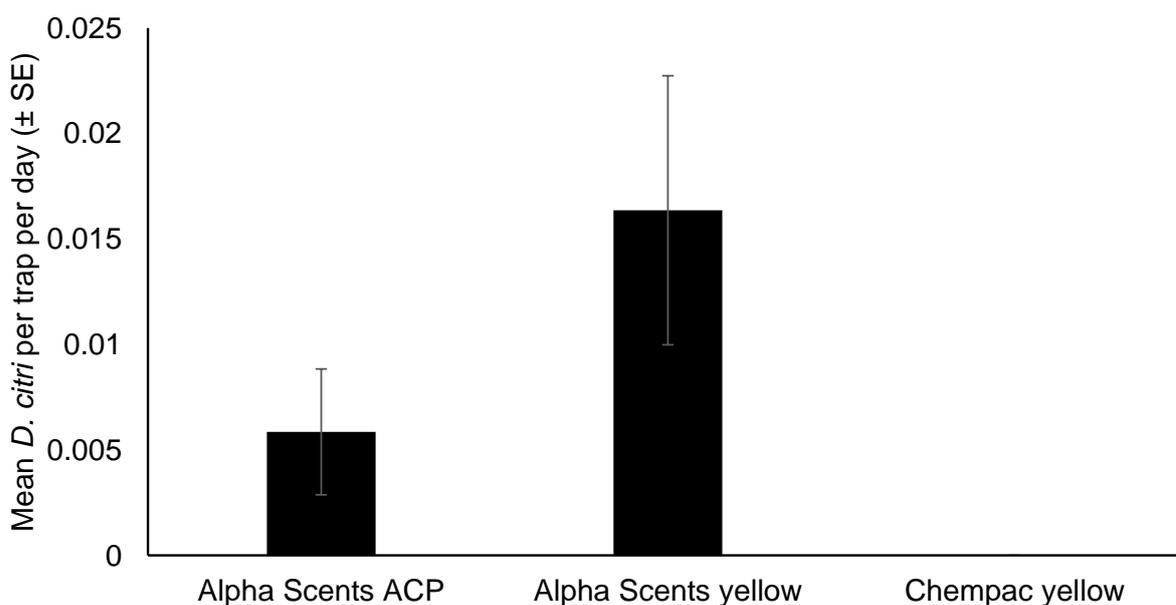


Figure 3.4.5.4. Catches of ACP on three types of double-sided sticky traps: Alpha Scents ACP lime green trap, Alpha Scents yellow trap and Chempac yellow trap, evaluated in pummelo orchards at Domaine de Labourdonnais Ltée Mauritius.

Discussion

The treatment package consisting of a sequential application of three systemic insecticides registered for control of *T. erythrae* in South Africa: acetamiprid (neonicotinoid), imidacloprid (neonicotinoid) and the novel acephate (organophosphate), generally kept ACP adult numbers low. These insecticides were applied in a sequence such that they would provide long term control of ACP from the start of application until at least harvest in that orchard (planned for May 2017) and would at the same time ensure adherence to the pre-harvest intervals specified for citrus in South Africa (acetamiprid: 150 days, imidacloprid: 212 days and acephate: 40 days). Imidacloprid has a long term suppression action on ACP and high titres of this insecticide remain in citrus leaf tissues for up to 12 weeks (Setamou et al., 2010, Langdon et al., 2018). The insecticides or chemistry groups used in the treatment package target the nervous system of the vector and at specified doses would kill the insect upon either feeding or contact (Boina and Bloomquist, 2015). Among the three insecticides used in the treatment package, imidacloprid has been the most studied one in terms of its effect on ACP (Gatineau et al., 2010, Ichinose et al., 2010, Langdon and Rogers, 2017, Langdon et al., 2018, Setamou et al., 2010). Imidacloprid has been shown to have both lethal and sublethal effects on ACP (Boina et al., 2009, Langdon and Rogers, 2017). Concentrations of imidacloprid required for ACP mortality by contact and ingestion were recently determined (Langdon and Rogers, 2017). Higher concentrations of imidacloprid are required to effect mortality of ACP by ingestion than by contact (Langdon and Rogers, 2017). The latter

authors also found that titres of soil applied neonicotinoid like imidacloprid in citrus foliage in commercial orchards in Florida rarely reached levels to effect kill of ACP. At sublethal concentrations though, imidacloprid deterred feeding in adult ACP (Boina et al., 2009). ACP adults were found to have lower frequency and duration of feeding on citrus plants that were treated with imidacloprid by soil drenching (Serikawa et al. 2012). In a long term field study in Vietnam, monthly trunk application of imidacloprid in a *Citrus reticulata* Blanco (cv King mandarin) orchard effectively reduced ACP adult populations by over 90% compared to an untreated control (Gatineau et al., 2010). Similarly, in an experimental citrus orchard in Texas, Setamou et al. (2010) recorded significantly lower numbers of ACP adults (more than 7 times lower) in blocks treated with imidacloprid by soil drenching than in untreated blocks. In this study, the differences in ACP adult numbers between untreated control and a treatment package which included imidacloprid were much smaller. While the experimental design used in this study (smaller plots combined with the patchy distribution of ACP) could have played a role in a lower magnitude of differences between treated and untreated plots, it is also possible that the once off application of each systemic product might not have been effective enough to highly suppress ACP. Nonetheless, it would be important for the South African citrus industry to request emergency registration of the chemicals tested in this study as well as registration of other chemicals which are allowed on citrus in South Africa and which are known to control ACP in other parts of the world (Qureshi et al., 2014, Boina and Bloomquist, 2015).

Originally in the project, a second part of the study was proposed. The effects of the treatment package on control of ACP and prevention of Las were to be determined. This would have required evaluation of the treatment package on disease free citrus trees in Mauritius which could not be obtained there. Import of disease free citrus seedlings from South Africa was not allowed in Mauritius. The Mauritius authorities however agreed for the preparation of disease free citrus plants under quarantine conditions. *Citrus sinensis* x *Poncirus trifoliata* (L.) Raf, Carrizo citrange, rootstock seeds were therefore imported from South Africa. Most of the rootstock seedlings however died in the quarantine glasshouse in Mauritius. Therefore, the second part of the study could not be conducted. In the long term study in Vietnam, referred to earlier, Gatineau et al. (2010) found that Las prevalence was lower but not totally absent in the sweet orange orchard treated with imidacloprid. Although control of ACP would not be able to totally prevent Las, it would play an important role in reducing spread of Las by ACP.

Early detection of ACP for effective control of the pest would be important in citrus production areas. Although there are new developments on attractants for ACP, for example volatiles (a blend of mycene, ethyl butyrate and p-cymene) derived from plants and acetic acid derived from ACP sex pheromone (Zanardi et al., 2018, Coutinho-Abreu et al., 2014), monitoring of ACP is still largely being done by one or a combination of the following methods: visual sampling, stem tap sampling and trapping using yellow sticky cards (Monzo et al., 2015). In this study, we determined the efficacy of different colour sticky cards that were available in South Africa for monitoring of ACP. In field evaluation in pummelo orchards in Mauritius, we found that the larger Alpha Scents traps currently available at Insect Science were better than the smaller Chempac yellow sticky traps for detection of the pest. In studies done in citrus orchards in Texas and Florida in USA, the Alpha Scents yellow and Alpha Scents lime green ACP traps also captured numerically higher number of ACP compared to the other colour traps tested at a particular time of the year (Hall et al., 2010). Since the Alpha Scents yellow and ACP traps performed better than the Chempac yellow sticky traps in this study, the former traps should be the ones recommended for surveillance of ACP in South Africa and other southern African countries. The ACP Pherolure from Insect Science which was tested with the Alpha Scents ACP trap did not increase the performance of the visual trap. It would be important that these newly discovered attractants be further evaluated on ACP in countries where ACP is currently present.

Conclusion & future research

The insecticides recommended and registered for control of *T. erytrae* in citrus in South Africa would be able to partially suppress ACP in the field. For more effective suppression of ACP, other control measures would have to be applied. It would be important in the near future that all the insecticides tested here be registered for control of ACP. Registration of other insecticides allowed in citrus in South Africa and known to be effective against ACP in other parts of the world should also be sought. Furthermore, the application rates of systemic

insecticides and horticultural practices in citrus orchards would have to be fine-tuned for more effective control of ACP.

The Alpha Scents ACP and yellow sticky traps should be used for early detection of ACP in view of their better performances vis-à-vis the Chempac yellow sticky traps. Potential attractants such as acetic acid and the blend of plant volatiles should be tested with the Alpha Scents ACP and Alpha Scents yellow sticky traps in the future.

Technology transfer

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3.4.6 FINAL REPORT: Suitability of entomopathogenic fungal isolates for microbial control of citrus pests: biological control and effects of formulation

Summary

Research over the past decade, has identified promising native isolates of entomopathogenic fungi (EPF) for their potential to control key citrus insect pests in South Africa. From 62 isolates identified, one *Beauveria bassiana* and two *Metarhizium anisopliae* isolates were selected for further studies and subsequent development into biocontrol agents, based on virulence. Although field trials against a key citrus pest, false codling moth (FCM), have been positive, efficacy against citrus mealybug and thrips in field trials has been disappointing. Understanding the full array of biological traits of these fungal isolates for selecting the most suitable candidates for controlling citrus pests is paramount, rather than just focussing on virulence. Temperature tolerance (radial growth at 6-40°C), humidity requirements (virulence against FCM at 12-98% RH) and UV sensitivity (germination after exposure to UV radiation for 15-120 min at 0.3 W/m²) were studied in the laboratory with seven selected fungal isolates and two commercial mycoinsecticides. The lower and upper thermal thresholds for isolates were 6 and 34°C, respectively, with optimal growth between 26 and 28°C. In the humidity bioassays, and at standard concentrations tested, pupal mortalities were not affected by humidity, but were higher for the *M. anisopliae* isolates and mycoinsecticides (63-92%). These findings suggested that temperature and humidity are not likely to impair the control potential of any of these promising isolates in citrus orchards. In contrast, conidia of all isolates and mycoinsecticides (unformulated) were extremely sensitive to UV radiation; 2 h exposure killed conidia of tested isolates, 1 h delayed germination for 48 h, whilst the formulated mycoinsecticides were highly tolerant when tested for 1 h. Thus, emphasising the need for a suitable UV protectant formulation of these fungi or different application strategy, for success against *Planococcus citri* and *Scirtothrips aurantii*. The endophytic potential of the seven isolates on citrus seedlings were also investigated, however, results were not promising.

This study was undertaken as a doctoral degree, which has subsequently been submitted for examination. Manuscripts are currently in preparation.

Opsomming

Navorsing oor die afgelope dekade het belowende inheemse isolate van entomopatogeniese swamme (EPS) geïdentifiseer vir hul potensiaal om belangrike sitrusinsekplae in Suid-Afrika te beheer. Uit 62 isolate geïdentifiseer, is een *Beauveria bassiana* en twee *Metarhizium anisopliae* isolate geselekteer vir verdere studies en daaropvolgende ontwikkeling as biologiesebeheermiddels, gebaseer op virulensie. Alhoewel veldproewe teen 'n sleutel sitrusplaag, valskodlingmot (VKM), positief was, was die doeltreffendheid teen sitruswitluis en blaaspootjie in veldproewe teleurstellend. Om die volle verskeidenheid biologiese eienskappe van hierdie swamisolate te verstaan om die geskikste kandidate vir die beheer van sitrusplae te kies, is uiters belangrik, eerder as om net op virulensie te fokus. Temperatuur toleransie (radiale groei teen 6-40°C), humiditeit vereistes (virulensie teen VKM teen 12-98% RH) en UV-sensitiwiteit (ontkieming na UV-bestraling vir 15-120 min teen 0.3 W/m²) van sewe geselekteerde swamisolate en twee kommersiële EPS produkte is in die laboratorium bestudeer. Die onderste en boonste hitte drempels vir isolate was onderskeidelik 6 en 35°C, met optimale groei tussen 25 en 29°C. In die humiditeits-biotoetse, en by standaardkonsentrasies wat getoets is, was die pupil sterftes nie deur humiditeit beïnvloed nie, maar was hoër vir die *M. anisopliae*-isolate en mycoinsekticides (63-92%). Hierdie bevindings het voorgestel dat temperatuur en humiditeit waarskynlik nie die beheerpotensiaal van enige van hierdie belowende isolate in sitrusboorde sal benadeel nie. In teenstelling hiermee was conidia van alle isolate en mycoinsekticides (onformuleer) uiters sensitief vir UV-straling; 2 uur blootstelling vermoor konidie van getoëerde isolate, 1 uur vertraagde ontkieming vir 48 uur, terwyl die geformuleerde mycoinsecticides hoogs verdraagsaam was toe dit vir 1 uur getoets word. So, beklemtoon die behoefte aan 'n geskikte UV-beskerende formulering van hierdie swamme of verskillende aansoekstrategieë vir sukses teen *Planococcus citri* en *Scirtothrips aurantii*. Die endofitiese potensiaal van die sewe isolate op sitrusplantjies is ook ondersoek, maar die resultate was nie belowend nie.

Introduction

The study proposed here had its source from work done by Goble (2009) who isolated 62 strains of EPF from within and around conventional and organic citrus orchards in the Eastern Cape, mostly in the genera, *Beauveria* and *Metarhizium*. Relative virulence of 21 out of the 62 strains was compared against the soil-dwelling life stages of *Thaumatotibia leucotreta*, *Ceratitis capitata* and *Ceratitis rosa* at a standard rate (1×10^7 conidia/mL) (Goble *et al.*, 2011), thus identifying the most promising isolates with which to conduct further research. Out of the 12 fungal isolates, three isolates, two *M. anisopliae* (G 11 3 L6 and FCM Ar 23 B3) and one *B. bassiana* (G Ar 17 B3) were identified as showing the greatest potential against FCM (Coombes *et al.*, 2013 & 2015). Consequently, all further research and developmental work has been conducted with these three isolates, selected purely on the basis of virulence. This includes not only field trials against FCM (Coombes *et al.*, 2013 & 2015), but also laboratory trials against citrus mealybug (*Planococcus citri*) and citrus thrips (*Scirtothrips aurantii*) (Chartier-Fitzgerald, 2014), and red scale (*Aonidiella aurantii*) (Upfold *et al.*, 2016) and field trials against mealybug and thrips (Grout *et al.*, 2015). Although results from field trials against FCM have been extremely positive (Coombes, 2016), efficacy against mealybug and thrips in field trials has been disappointing (Grout *et al.*, 2013). Consequently, this has highlighted the importance of understanding the full array of biological traits of fungal isolates for selecting the most suitable candidates for development for biological control of citrus pests, rather than just focussing on virulence. For example, it is well documented that certain isolates are more temperature (Vidal *et al.*, 1997; Rodriguez *et al.*, 2009), humidity (Luz & Fargues, 1997) or UV (Fernandez *et al.*, 2015) tolerant than others. A relatively recent discovery is also the endophytic benefit offered by certain isolates of EPF (Backman & Sikora, 2008). The term endophyte is used to define fungi (or bacteria) occurring inside plant tissues without causing any apparent symptoms in the plant (Wilson, 1995). Fungal endophytes have been detected in hundreds of plants, including many important agricultural commodities (Larran *et al.*, 2002; Vega *et al.*, 2008). Several roles have been ascribed to fungal endophytes, including providing protection against herbivorous insects (e.g. Breen, 1994), plant parasitic nematodes (e.g. Elmi *et al.*, 2000), and plant pathogens (e.g. Wicklow *et al.*, 2005). Some or all of these factors could play a role in the suitability and success of an EPF for biological control, particularly for those pests that occur, and are targeted on the foliage and twigs of citrus trees, such as citrus thrips and mealybugs.

Objectives

Objectives A-D are applicable for seven of the 12 isolates identified by Goble *et al.* (2011), three *M. anisopliae* var. *anisopliae* (G 11 3 L6, FCM Ar 23 B3, G OL R8) and four *B. bassiana* (FCM 10 13 L1, G 14 2 B5, G B Ar 23 B3, G AR 17 B3) and two commercial products (Broadband® a.i. *B. bassiana* PPRI 5339 and Real IPM a.i. *M. anisopliae* CIPE 69). Objective E was not undertaken.

- A. Determine temperature tolerance
- B. Determine UV sensitivity
- C. Determine moisture requirements
- D. Determine endophytism
- E. Determine effect of formulation on the biological traits previously assessed (temperature, moisture and UV sensitivity)

Materials and methods

Please refer to the final thesis for a more detailed description of the experimental procedure and statistical analyses.

A. Temperature tolerance trial

Conidia harvested from 2 weeks' sporulating cultures, previously passed through FCM fifth instars, were suspended in 20 mL sterilised distilled water containing 0.01% Tween 20. Conidial suspensions were plated on SDA medium and incubated at 27°C for three days in order to obtain mycelial growth. Discs (6 mm diameter) of the unsporulated mycelia were singly transferred to the centre of 90 mm Petri dishes containing the same

nutrient mixture and placed upside down. Five replicate Petri dishes per isolate were incubated for 15 days in dark chambers at 98% RH (using saturated salt solution of Potassium sulphate) for each of the 10 temperatures studied (6, 8, 16, 20, 25, 27, 29, 35, 40 ± 1°C). The experiment was repeated twice with each replicate originating from different cadaver-culture plates. Colonies grew linearly over the 15-day incubation period. Radial measurements from the 3rd to the 15th day fitted a linear model $y = vt + b$, where the slope of the model (v), indicated the growth rate (velocity in mm/day) at an incubation temperature t (Quedraogo *et al.* 1997). Regression analysis was therefore conducted for each isolate/temperature combination. A generalised β function of a nonlinear model, (Bassanezi *et al.* 1998) was fitted to the growth rates under the different temperatures to evaluate the influence of temperature on fungal growth rates (Y). Parameters generated from the nonlinear model, which included the minimum, maximum and optimum temperatures for growth as well as growth rates at the optimal temperatures were contrasted using Pairwise t-test with Bonferroni adjustment.

B. UV sensitivity trial

Three fungal isolates were irradiated at a time due to limited space in the irradiation chamber. Stock cultures of each isolate were subcultured on SDAC and incubated for 12-15 days at 27°C, 60% RH, on a 12 h photoperiod. Conidia were then harvested from colonies, suspended in sterile distilled water supplemented with 0.01% Tween 20 (sdt H₂O), adjusted to 1×10⁵ conidia/ml using a haemocytometer and immediately used for inoculation. Conidial suspensions of the mycoinsecticide products were also prepared using sdt H₂O and adjusted to 1×10⁵ conidia/ml. For each isolate/product, a 50 µL suspension was spread on SDA plate (polystyrene, 60 × 15 mm) supplemented with 0.002% Benomyl (25% active ingredient) at four replicates for three exposure periods including controls. Within 30 min after inoculation, plates were exposed to simulated full spectrum sunlight (295-780 nm) produced by a lamps in a Q-SUN[®] Xe-3-HC xenon test chamber (Q-Lab Corporation, Westlake, OH, USA) at 0.3 W/m² for 15, 30 and 60 min. The corresponding total doses at these periods were 0.7, 1.3 and 2.6 KJ/m², respectively at 28.44 ± 0.61°C and 44.49 ± 3.19% RH. Preliminary tests indicated 60 min as an appropriate maximum exposure period, as complete conidia inactivation occurred for six isolates tested after only 2 h exposure to this irradiance. This irradiance set point is less than the erythema-weighted noon irradiance of 44.60 mW/m² during winter (June) in Port Elizabeth (PE) (33°59'4.28"S, 25°36'37.75"E) (South Africa Weather Service 2018), but importantly, approximates the Quaitte-weighted noon irradiance of 350 mW/m² during winter in São José dos Campos, South-eastern Brazil (Dias *et al.* 2018), with a similar climate to the southern citrus producing region of South Africa. The Quaitte-weighted irradiance in the Q-SUN[®] Xe-3-HC at 0.6 W/m² (unweighted irradiance set point), was found to be 1335 mW/m², and equally approximated noon summer irradiance (1300 mW/m²) in São José dos Campos, South-eastern Brazil (Dias *et al.* 2018; Luo *et al.* 2017). Therefore, the irradiance used in this study may be lower than summer solar irradiance in southern citrus producing region in South Africa. Control plates were covered with aluminium foil to block UV radiations. After irradiation, the plates were incubated in the dark at 21°C. The number of germinated (conidia with germ tubes) and non-germinated conidia per plate, out of 300 conidia, were evaluated at 24-27 and 48-51 h post irradiation. The trial was repeated three times for each isolate with fresh conidial suspensions. Percentage germination, relative to unirradiated controls was calculated for each fungal isolate/product. Data were analysed using a generalised linear model (GLM) with gamma error distribution (link = "identity"). A three-way analysis of variance was applied to the results of the GLM and subsequently contrasted using Tukey's HSD post hoc ($P < 0.05$).

C. Humidity trial

Cultures from FCM-passaged fungal isolates were incubated for two weeks at 27°C, 60% RH on a 12 h photoperiod. Conidia harvested from these cultures were suspended in 20 mL sterile 0.01% Tween 20 solution and adjusted to two concentrations (1×10⁵ and 1×10⁷ conidia/mL). For each concentration per isolate, 40 FCM fifth instars distributed in four 90 mm, sterile plastic Petri plates (10 larvae each) were topically inoculated with approximately 1 mL of the inoculum per plate with a spray bottle. Control insects (10 fifth instar in four replicated Petri plates) were treated with 0.01% Tween 20 only. After drying insects in Petri dishes for 1 hour under a laminar flow hood, each larva was placed in glass vial, corked with cotton wool and transferred to sealed containers with respective saturated salts solutions corresponding to a range of relative humidities (12%, 43%, 75% and 98%). Containers were then incubated in separate test chambers at 27°C. The number of live and

dead larvae, pupae and adult FCM were recorded daily ceasing 10 days after first adult eclosion was recorded. For most isolates, this termination date was between 20-21 days post inoculation. Cadavers were removed daily, surface sterilised and incubated at ambient conditions to confirm if death was due to mycosis. The experiment was replicated three times for each concentration and humidity level, with each replicate originating from a different passaged culture of the same isolate. Adult mortality in all bioassays was very low (< 2%) and were rarely mycosed following incubation. Analysis was thus restricted to larval and pupal mortality only. For each concentration tested, mortality and survival data for all isolates and controls at each humidity level tested over the 21-day period were fitted to a logistic regression in a generalized linear model with binomial error distributions. The efficacy of each fungal isolate across the humidity levels tested, was also analysed using logistic regression. If statistical differences amongst treatments in the model were found, pairwise comparisons with Tukey's HSD contrast ($P < 0.05$), were performed. The LT_{50} values for all isolates at each humidity tested were also computed from these models.

D. Endophytism trials

For each isolate, conidia (1×10^7 conidia/mL) were applied either directly to the leaves or soil of 3-month old potted citrus seedlings (C35 citrange or Swingle citrumelo rootstocks). Each treatment, including the control, consisted of 28 seedlings (14 plants per inoculation method). Seedlings were evaluated 14-15 days after inoculation and again 30-31 days' post-inoculation. From each treatment, root, leaf and stem samples were taken, surface sterilised, inoculated on SDAC Petri plates and monitored for fungal outgrowth of the respective isolates. All statistical analyses were conducted in R version 3.4.0 (R Core Team 2016). Briefly, data were analysed using logistic regression in a generalised linear mixed model and contrasted with Tukey's HSD if statistical differences were reported.

Results and discussion

Please refer to the final thesis for more detailed results and discussion thereof.

Task table

Objective / Milestone	Achievement
<u>July-Dec 2016</u> A: Conduct temperature tolerance trials	A: Completed. Results presented in Table 3.4.6.1.
<u>Apr-Jun 2017</u> C: Conduct humidity trials D ₁ : Prepare for endophytism trials	C: Completed. Results presented in Table 3.4.6.3 and 4. D: Completed. Results presented in Table 3.4.6.5.
<u>Jul-Sep 2017</u> D ₂ : Initiate endophytism trials	D: Completed. Results presented in Table 3.4.6.5.
<u>Oct-Dec 2017</u> D ₃ : Continue with endophytism trials	D: Completed. Results presented in Table 3.4.6.5.
<u>Apr-Jun 2018</u> B ₁ : Initiate UV sensitivity trials D ₃ : Continue with endophytism trials	B: Completed. Results presented in Table 3.4.6.2 D: Completed. Results presented in Table 3.4.6.5
<u>Jul-Sep 2018</u> E ₁ : Initiate formulation trials	E: Not undertaken.

B ₂ : Continue with UV trials	B: Completed. Results presented in Table 3.4.6.2
<u>Oct-Dec 2018</u> E ₂ : Continue formulation trials	E: Not undertaken.
<u>Jan-Mar 2019</u> None provided	Thesis submitted for examination; Manuscripts in preparation

A: Temperature tolerance trials (Table 3.4.6.1)

In agreement to the reported mesophilic growth characteristics of EPF (Quedraogo *et al.* 1997; Ekesi *et al.* 1999; Bugeme *et al.* 2008; Tumuhaise *et al.* 2018), all *B. bassiana* isolates grew between 8 and 33°C and optimally at 27 to 28°C. The *M. anisopliae* isolates exhibited similar temperature growth profiles as the *B. bassiana* isolates, growing at 16 to 34°C and optimally at 26 to 27°C. At the optimal temperatures (T_{opt}), the three indigenous *M. anisopliae* isolates tested, (G 11 3 L6, FCM Ar 23 B3 and G OL R8), recorded significantly higher radial growth rates (TY_{opt}), (3.15-3.42 mm/day) than the *B. bassiana* isolates (0.69-2.44 mm/day) and the two commercial isolates (1.72-2.55 mm/day). *Beauveria bassiana* isolate, G Ar 17 B3, exhibited the least radial growth at all growth temperatures (data not shown), including the optimal temperature. The maximum temperature for growth, T_{max}, ranged between 32 to 33°C and 33 to 34°C for *B. bassiana* and *M. anisopliae* isolates, respectively (Table 3.4.6.1). Analysis of historical weather data for the past decade (2008-2017) in four orchards within the Sunday's River Valley citrus producing region in the Eastern Cape, (where the EPF field trials were undertaken Coombes *et al.* 2016) revealed their average annual air temperature to be 17.7°C (<https://www.wunderground.com>). The average temperature in the spring and summer months is 19.5°C and typically ranges between 20-39°C; soil temperature during these months, at 10 cm depth where subterranean stages of FCM occur and would be controlled by EPF, is 19.4°C and ranges between 13.3-24.2°C (Coombes 2015; Coombes *et al.* 2016). Temperatures within orchards in warmer provinces are slightly higher. For instance, the annual air temperature in Olifants River Estate, Limpopo, where the EPF field trials against citrus thrips and mealybugs were conducted (Grout *et al.* 2015) is 21.9°C, with spring and summer temperatures usually between 22.9-40.9°C (<https://www.wunderground.com>). The temperature profiles of these isolates suggest that air temperatures in the spring and summer months in both the cooler and warmer South African provinces will not limit fungal growth when these isolates are developed into mycoinsecticides. Since temperature is an important persistence and efficacy impacting factor for fungal entomopathogens (Jaronski 2010), this outcome can be considered positive for their commercialisation in South Africa. However, air temperatures above 34°C (i.e. the upper thermal growth limit of these isolates) are also recorded in orchards in both cooler and warmer citrus producing regions. Hence mineral and vegetable oils such as canola oil, soy and sesame oil, Naturo[®] (Mola & Afkari 2012; Paixão *et al.* 2017; Oliveira *et al.* 2018) could be considered as adjuvants in future formulation studies for an improved thermotolerance.

Table 3.4.6.1. Estimated parameters (\pm standard error) from the modified β function¹ (Bassanezi *et al.* 1998) fitted to vegetative growth data of *Beauveria bassiana* and *Metarhizium anisopliae* isolates.

		Estimated parameters ⁶			
Species	Isolates	Topt (°C) ²	TYopt (mm/day) ³	Tmax (°C) ⁴	Tb3 ⁵
<i>B. bassiana</i>	G Ar 17 B3	28.31 (0.83) a	0.69 (0.02) f	32.07 (0.05) e	0.38 (0.16) b
<i>B. bassiana</i>	G B Ar 23 B3	28.24 (0.17) ab	2.44 (0.09) c	32.05 (0.02) e	0.35 (0.04) b
<i>B. bassiana</i>	G 14 2 B5	27.44 (0.59) abc	1.81 (0.03) d	32.91 (0.40) bcd	1.32 (0.45) a
<i>B. bassiana</i>	FCM 10 13 L1	26.91 (0.58) abc	1.46 (0.05) e	32.43 (0.17) cde	0.96 (0.19) ab
<i>B. bassiana</i>	Bb PPRI 5339	26.19 (0.20) c	1.72 (0.02) d	32.07 (0.03) de	0.61 (0.05) ab
<i>M. anisopliae</i>	G 11 3 L6	26.33 (0.11) bc	3.42 (0.10) a	33.93 (0.14) a	0.98 (0.05) ab
<i>M. anisopliae</i>	FCM Ar 23 B3	26.90 (0.11) abc	3.15 (0.08) a	32.98 (0.16) bc	0.74 (0.07) ab
<i>M. anisopliae</i>	G OL R8	26.27 (0.10) c	3.23 (0.06) a	33.45 (0.16) ab	0.93 (0.07) ab
<i>M. anisopliae</i>	Ma ICIPE 69	27.27 (0.21) abc	2.55 (0.03) b	32.50 (0.12) cde	0.48 (0.06) b

¹The generalized β function is given by $Y(T) = TY_{opt} / ((T - T_{min}) / (T_{opt} - T_{min}))^{\wedge} (Tb3 * ((T_{opt} - T_{min}) / (T_{max} - T_{opt})) * ((T_{max} - T) / (T_{max} - T_{opt}))^{\wedge} Tb3$, where $Y(T)$ is the fungal growth in mm/day (dependent variable) and T is the incubation temperature (independent variable). T_{min} is the lowest temperature for fungal growth, and was fixed at 6°C.

²Topt is the optimal temperature for fungal growth.

³TYopt is the fungal growth at the optimal temperature.

⁴Tmax is the highest temperature for fungal growth.

⁵Tb3 is the shape parameter which influences the temperature range around Topt in which the curve stays near TYopt.

⁶Means within columns with the same letter are not significantly different (Pairwise t-test, $P > 0.05$ with Bonferroni correction factor).

B: UV tolerance trials (Table 3.4.6.2)

2 h exposure to simulated solar radiation at UV irradiance of 0.3 W/m² (5.2 KJ/m²) killed conidia of six tested fungal isolates: G Ar 17 B3, G B Ar 23 B3, *B. bassiana* PPRI 5339, G 11 3 L6, FCM Ar 23 B3 and *M. anisopliae* ICIPE 69. Exposures to 15 min (0.7 KJ/m²) and 30 min (1.3 KJ/m²) simulated solar radiation were not detrimental to six of the indigenous isolates, as their relative conidial germination exceeded 86% after 24-27 h incubation. Accordingly, differences in susceptibility were indiscernible for these isolates at these exposure periods. However, germination was markedly delayed for 48-51 h in five of these indigenous isolates, following exposure for 60 min (2.6 KJ/m²), except in two relatively tolerant isolates, G Ar 17 B3 and G 14 2 B5, which exhibited over 93% relative germination, 24-27 h post incubation. Although full conidial germination was restored in six isolates after 48-51 h incubation (actual germination > 97% (data not shown)), FCM Ar 23 B3 remained inactivated, with less than 3% relative germination (Table 3.4.6.2). The commercial isolates showed similar susceptibility to the UV irradiance and exposure periods tested, however, the relative germination of their formulated products exceeded 90% when tested for 15 to 60 min. This study highlighted the extreme sensitivity of all seven isolates under investigation to UV radiation. One of the currently virulent *M. anisopliae* isolates, FCM Ar 23 B3 was the most susceptible to UV radiation, whilst the less virulent *B. bassiana* isolates, G Ar 17 B3 and G 14 2 B5 exhibited the greatest UV resilience. The irradiance set point used in this study, as previously mentioned, is likely to approximate winter noon irradiance in southern citrus producing regions in South Africa. Thus, persistence longer than the 1 h tolerant period will definitely be required for success against the targeted arboreal pests (citrus mealybugs and thrips). Some UV protectants that have protected fungal propagules in various EPF formulations studies such as vegetable oils, mineral oils, sunscreens should be considered in future formulation studies.

Table 3.4.6.2. Relative percentage germination (\pm standard error) of *M. anisopliae* and *B. bassiana* isolates/products after exposure to simulated solar radiation¹ and incubation for 24-27 h at 21°C.

Species	Isolate/product	Relative germination ² (%) at each exposure period		
		15 min	30 min	60 min
<i>M. anisopliae</i>	G 11 3 L6	99.09 (0.32) Aa	96.43 (0.77) Aa	4.68 (0.75) Bb
<i>M. anisopliae</i>	FCM Ar 23 B3	98.59 (0.39) Aa	97.02 (0.49) Aa	1.41 (0.83) Bc
<i>M. anisopliae</i>	G OL R8	98.36 (0.39) Aa	97.59 (0.54) Aa	7.51(1.01) Bb
<i>M. anisopliae</i>	Ma ICIPE 69	98.90 (0.25) Aa	99.21 (0.39) Aa	1.79 (0.41) Bc
<i>M. anisopliae</i>	Ma ICIPE 69*	99.25 (0.16) Aa	97.83 (0.63) Aa	92.79 (0.69) Aa
<i>B. bassiana</i>	G Ar 17 B3	99.64 (0.14) Aa	99.49 (0.19) Aa	93.06 (0.99) Aa
<i>B. bassiana</i>	G B Ar 23 B3	90.86 (0.87) Ab	60.19 (2.93) Ab	5.91 (0.71) Bb
<i>B. bassiana</i>	G 14 2 B5	97.33 (0.36) Aa	98.39 (0.44) Aa	97.77 (0.39) Aa
<i>B. bassiana</i>	FCM 10 13 L1	93.45 (0.64) Ab	86.21 (1.28) Aa	5.82 (0.91) Bb
<i>B. bassiana</i>	Bb PPRI 5339	56.39 (1.43) Ac	10.32 (1.84) Bc	2.02 (0.25) Cc
<i>B. bassiana</i>	Bb PPRI 5339*	98.56 (0.34) Aa	95.80 (0.77) Aa	90.27 (1.37) Aa

¹ Exposure to Xenon arc lamps from 295 to 780 nm at 0.3 W/m², 28.44 \pm 0.61°C and 44.49 \pm 3.19% RH.

²Means within each row with the same upper case letter are not significantly different (Tukey's HSD test, $P > 0.05$). Means within each column with the same lower case letter are not significantly different (Tukey's HSD test, $P > 0.05$).

* Commercial product

C: Humidity trials (Tables 3.4.6.3 and 3.4.6.4)

High mortalities were recorded for the *M. anisopliae* isolates and the two commercial isolates, regardless of humidity. At the highest tested concentration, three *M. anisopliae* isolates, G 11 3 L6, FCM Ar 23 B3 and G OL R8 induced pupal mortalities of 77.5%, 83.3% and 80.0% at 12% RH; 85.8%, 90.0% and 82.5% at 43% RH; 91.7%, 91.7% and 80.8% at 75% RH; and 85.8%, 90.8% and 82.5% at 98% RH, respectively. These mortalities did not differ significantly from isolates of the two commercial mycoinsecticides, Real IPM (75.8%, 83.3%, 82.5% and 85.8% at 12, 43, 75 and 98% RH respectively) and Broadband[®] (78.3%, 80.0%, 76.7% and 82.5% at 12, 43, 75 and 98% RH respectively) at all humidities tested. However, the LT_{50} (lethal time to cause 50% pupal mortality) for G 11 3 L6 and FCM Ar 23 B3 was significantly lower at high humidities (4.6-4.8 days at 43%, 4.1-4.2 days at 75% and 4.1-4.4 days at 98% RH) than at 12% RH (5.6-6.7 days). *Beauveria bassiana* isolates, G Ar 17 B3, GB Ar 23 B3, G14 2 B5 and FCM 10 13 L1, showed reduced virulence against FCM, with pupal mortalities of the highest tested concentration being 40.0%, 57.5%, 60.0% and 50.8% at 12% RH, 47.5%, 58.3%, 57.5% and 55.8% at 43% RH, 46.7%, 63.3%, 59.2% and 55.0% at 75% RH, and 45.8%, 60.0%, 60.8% and 58.3% at 98% RH, respectively. Similar to the results obtained in the highest concentration, pupal mortality for most isolates did not vary across the tested humidity levels with the lowest concentration (Details in Chapter 3 section 3.3.3 of thesis). The results from this study suggest that, the range of ambient relative humidities tested (12 to 98%), which conforms with the year round humidity levels in South African citrus orchards, will not negatively affect the virulence of any of the seven isolates when developed into mycoinsecticides. Additionally, this study, revealed that the most virulent isolate (G Ar 17 B3) against FCM soil-dwelling fifth instars in the field (Coombes *et al.* 2016), may no longer be suitable for further development due to its attenuation towards this pest. Thus, G Ar 17 B3 could be exhibiting similar attenuation as the other *B. bassiana* isolates used in this study, although this only occurred after 10 years. However, all three *M. anisopliae* isolates, G 11 3 L6, FCM Ar 23 B3 and G OL R8 were still virulent to pre-pupating FCM instars and seem favoured for development into mycoinsecticides. In previous studies, one of the currently virulent *M. anisopliae* isolates, FCM Ar 23 B3, which showed good control potential against FCM fifth instars under both laboratory and field conditions, showed similar control potential against citrus thrips and mealybug in the laboratory (Goble *et al.* 2011; Chartier Fitzgerald 2014; Chartier Fitzgerald *et al.* 2016; Coombes *et al.* 2016). It was therefore deduced from this study that if FCM Ar 23 B3 can induce similar virulence against thrips and mealybug, regardless of ambient humidity, then other abiotic constraints likely UV radiation led to their inefficacy against thrips and mealybug in the field. This assumption may be supported by the good control of FCM subterranean stages by this same isolate in the upper 10 cm depth of soil where UV radiation is virtually non-existent (Coombes *et al.* 2016). The influence of humidity on sporulation of isolates, which was not determined in this study, could be investigated in future as this will be vital for long-term survival of isolates.

Table 3.4.6.3. Mean pupal mortality (\pm standard error) of FCM fifth instars exposed to *M. anisopliae* and *B. bassiana* isolates at 1×10^7 conidia/mL and incubated at four RH levels (12, 43, 75 and 98%) at 27°C, 12 h photoperiod, over a 21-day period.

Species	Isolate/Treatment	Mean mortality (%) at the four humidity levels tested ¹			
		12%	43%	75%	98%
<i>M. anisopliae</i>	G 11 3 L6	77.50 (2.50) Bab	85.83 (2.21) ABa	91.67 (0.83) Aa	85.83 (0.83) ABa
<i>M. anisopliae</i>	FCM Ar 23 B3	83.33 (3.01) Aa	90.00 (2.89) Aa	91.67 (2.21) Aa	90.83 (1.67) Aa
<i>M. anisopliae</i>	G OL R8	80.00 (2.50) Aa	82.50 (1.44) Aa	80.83 (2.21) Aab	82.50 (2.50) Aa
<i>M. anisopliae</i>	Ma ICIPE 69	75.83 (2.21) Aabc	83.33 (1.67) Aa	82.50 (1.44) Aa	85.83 (2.21) Aa
<i>B. bassiana</i>	G Ar 17 B3	40.00 (4.33) Ad	47.50 (3.82) Ab	46.67 (5.07) Ad	45.83 (2.21) Ab
<i>B. bassiana</i>	G B Ar 23 B3	57.50 (3.82) Acd	58.33 (2.21) Ab	63.33 (2.21) Abd	60.00 (2.89) Ab
<i>B. bassiana</i>	G 14 2 B5	60.00 (1.44) Abd	57.50 (1.44) Ab	59.17 (1.67) Acd	60.83 (2.21) Ab
<i>B. bassiana</i>	FCM 10 13 L1	50.83 (2.21) Ad	55.83 (2.21) Ab	55.00 (2.89) Ad	58.33 (2.21) Ab
<i>B. bassiana</i>	Bb PPRI 5339	78.33 (3.33) Aab	80.00 (2.50) Aa	76.67 (5.47) Aabc	82.50 (2.89) Aa
	Control	5.00 (1.44) Ae	5.8 (0.83) Ac	5.00 (1.44) Ae	5.00 (1.44) Ac

¹ Means within each row with the same uppercase letter are not significantly different (Tukey's HSD test, $P > 0.05$). Means within each column with the same lower case letter are not significantly different (Tukey's HSD test, $P > 0.05$).

Table 3.4.6.4. Mean pupal mortality (\pm standard error) after exposure of FCM fifth instars to *M. anisopliae* and *B. bassiana* isolates at 1×10^5 conidia/mL and incubated at four RH levels (12, 43, 75 and 98%) at 27°C, 12 h photoperiod, over a 21-day period.

Species	Isolate/Treatment	Mean mortality (%) at the four humidity levels tested ¹			
		12%	43%	75%	98%
<i>M. anisopliae</i>	G 11 3 L6	62.50 (6.61) Bab	69.17 (5.83) ABa	80.00 (2.89) Aa	80.83 (3.01) Aa
<i>M. anisopliae</i>	FCM Ar 23 B3	67.50 (6.61) Aa	73.33 (3.01) Aa	71.67 (2.21) Aa	72.50 (1.44) Aa
<i>M. anisopliae</i>	G OL R8	66.67 (3.01) Aa	64.17 (4.41) Aab	71.67 (3.33) Aa	64.17 (3.01) Aab
<i>M. anisopliae</i>	Ma ICIPE 69	66.67 (6.01) Aa	75.83 (2.21) Aa	71.67 (3.33) Aa	75.00 (1.44) Aa
<i>B. bassiana</i>	G Ar 17 B3	25.00 (2.89) Bd	31.67 (3.63) ABc	38.33 (3.63) ABb	40.83 (3.01) Ac
<i>B. bassiana</i>	G B Ar 23 B3	38.33 (4.41) Acd	42.50 (2.89) Ac	43.33 (2.21) Ab	43.33 (4.64) Ac
<i>B. bassiana</i>	G 14 2 B5	45.00 (4.33) Abc	44.17 (3.63) Abc	45.00 (5.78) Ab	47.50 (1.44) Abc
<i>B. bassiana</i>	FCM 10 13 L1	40.00 (5.77) Acd	40.83 (4.17) Ac	39.17 (3.01) Ab	37.50 (1.44) Ac
<i>B. bassiana</i>	Bb PPRI 5339	65.83 (7.95) Aa	68.33 (3.63) Aa	70.83 (3.63) Aa	70.83 (3.01) Aa
	Control	5.83 (2.21) Ae	5.00 (1.44) Ad	6.67 (3.01) Ac	8.33 (2.21) Ad

¹ Means within each row with the same uppercase letter are not significantly different (Tukey's HSD test, $P > 0.05$). Means within each column with the same lower case letter are not significantly different (Tukey's HSD test, $P > 0.05$).

D: Endophytism trials (Table 3.4.6.5)

This pioneer study demonstrated that, the *M. anisopliae* isolates, G 11 3 L6 and FCM Ar 23 B3 can colonise leaves of C35 citrus seedlings at a low level (<12%) for a month, following leaf inoculation. Endophytic colonisation could not be established with any *B. bassiana* isolate and the two commercial isolates, following both inoculation methods investigated. Further studies are therefore required to determine whether endophytic colonisation can be achieved in mature trees and importantly, if colonisation of leaves could result in additional control of the target foliar insect pests as reported in other studies (Batta 2013; Resquín-Romero *et al.* 2016; Rondot & Reineke 2018).

Table 3.4.6.5. Percentage (\pm standard error) of plant parts colonised 14-15 days after inoculation with four *M. anisopliae* isolates at $24.85 \pm 0.06^\circ\text{C}$, 35.85 ± 0.34 % RH at a 12 h photoperiod.

Inoculation method	Mean colonisation (%) of the various plant parts ¹			
	Isolate	Leaves	Stem	Roots
Leaf spray	G 11 3 L6	10.00 (5.77) a	0.00 (0.00) a	0.00 (0.00) a
Leaf spray	FCM Ar 23 B3	11.43 (6.34) a	0.00 (0.00) a	0.00 (0.00) a
Leaf spray	G OL R8	1.43 (1.43) a	0.00 (0.00) a	0.00 (0.00) a
Leaf spray	Ma ICIPE 69	0.00 (0.00) a	0.00 (0.00) a	0.00 (0.00) a
Soil drench	G 11 3 L6	0.00 (0.00) a	0.00 (0.00) a	0.00 (0.00) a
Soil drench	FCM Ar 23 B3	5.87 (5.95) a	1.43 (1.43) a	0.00 (0.00) a
Soil drench	G OL R8	0.00 (0.00) a	0.00 (0.00) a	0.00 (0.00) a
Soil drench	Ma ICIPE 69	0.00 (0.00) a	0.00 (0.00) a	0.00 (0.00) a

¹ Means within each column with the same lower case letter are not significantly different (Tukey's HSD test, $P > 0.05$).

Conclusion

G 11 3 L6 and FCM Ar 23 B3 were the fastest growing isolates at all temperatures tested. These isolates are still highly virulent towards FCM; their efficacy is not limited by available humidity; and are able to transiently colonise citrus seedlings. However, they are highly sensitive to UV radiation. Formulation is therefore crucial if they are to be selected for use against arboreal citrus insects. G Ar 17 B3 and G 14 2 B5 were the most tolerant to UV radiations, however, the former grew poorly and slowly in the temperature tolerance trials. Again, based on the humidity studies, both isolates are less virulent on FCM. If suitable formulants are able to improve efficacy against key insect pests, then these isolates will be most suited for use against pests such as thrips and mealybugs. Nevertheless, formulation can equally improve the UV tolerance of the currently virulent isolates for product development.

Future research

UV radiation has been highlighted from this project to be the most detrimental abiotic factor likely to impede fungal efficacy when the virulent *M. anisopliae* isolates are developed into commercial products. Some of the promising UV protectants, particularly sunscreens and vegetable or mineral oil adjuvants will be investigated in future

formulation studies. A novel EPF encapsulation formulation, “single cell encapsulation via Pickering emulsion” (Yaakov *et al.* 2018), which could improve persistence on foliage will also be investigated.

Expected technology transfer

Talks or presentations

Acheampong, M.A., Hill, M.P., Moore, S.D. and Coombes, C.A. *Suitability of entomopathogenic fungal isolates for microbial control of citrus pest: Biological traits*. ESSA (Entomological Society of southern Africa) Congress, Umhlanga, Durban, 8-11 July 2019 (oral presentation, presenter: C. Coombes).

Acheampong, M., Hill, M.P., Moore, S.D. & Coombes, C.A. 2018. *Suitability of entomopathogenic fungal isolates for microbial control of citrus pests: biological traits*. 10th Citrus Research Symposium, Drakensberg, South Africa, 19-22 August (oral presentation, presenter: M. Acheampong)

Acheampong, M.A., Hill, M.P., Moore, S.D. and Coombes, C.A. *Suitability of entomopathogenic fungal isolates for microbial control of citrus pest: Biological traits*. Joint ESSA (Entomological Society of southern Africa)/ ZSSA (Zoological Society of southern Africa) Congress, CSIR ICC, Pretoria, 3-7 July 2017 (oral presentation, presenter: M. Acheampong).

Title for refereed paper: Three publications in preparation

Endophytism of *Beauveria bassiana* and *Metarhizium anisopliae* isolates from South African agricultural soils (Short communication)

Temperature and moisture requirements of *Beauveria bassiana* and *Metarhizium anisopliae* isolates from South African agricultural soils

UV tolerance of select entomopathogenic fungal isolates from South African agricultural soils

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3.4.7 PROGRESS REPORT: The efficacy of commercial entomopathogenic fungi products for control of citrus pests

Project 1174 (April 2017 – April 2019): Sean Moore, Wayne Kirkman, Mellissa Peyper (CRI) and Tamryn Marsberg (RU)

Summary

Currently at least three products based on entomopathogenic fungi (EPF) are actively marketed and sold for control of citrus pests in South Africa. Several other EPF products are known to be produced and sold in niche markets by much smaller companies. Although these products are not always registered for use against the pests being targeted, their usage in the industry is increasing. Claims of good control of a range of citrus pests are becoming more commonplace. However, these claims often conflict with the recorded experiences of scientists. This project aims to clarify the truth on the efficacy and usability of these products. During the previous season, no significant efficacy could be recorded against red scale, mealybug or thrips with a preventative programme using both *Beauveria bassiana* and *Metarhizium anisopliae* based products. Subsequently, a corrective trial was applied with three products at a site heavily infested with red scale and mealybug. Two of the products reduced the number of mealybug by 22.7% and 31.8% compared to the control. No efficacy against red scale was recorded. This project was to be terminated; however, a new *Isaria fumorosea* product with reported efficacy against the Asian citrus psyllid (ACP), *Diaphorina citri*, was obtained for testing. The intention was to test it against a pest such as psylla, aphids or woolly whitefly, which could be indicative of its efficacy against ACP. However, unfortunately no reasonable level of infestation of any of these pests could be found in the Eastern Cape. Consequently, the product was tested in an orchard with an extremely high mealybug infestation. Unfortunately, no significant efficacy was recorded. The product will be tested again next spring, when aphids appear on new flush.

Opsomming

Tans word minstens drie produkte gebaseer op entomopatogeniese swamme (EPS) aktief bemark en verkoop vir beheer van sitrusplae in Suid-Afrika. Dit is bekend dat verskeie ander EPS produkte deur kleiner maatskappye vervaardig word en in nis-markte verkoop word. Al is hierdie produkte nie altyd teen die teikenplae geregistreer nie, is hulle gebruik in die bedryf besig om toe te neem. Bewerings van goeie beheer van 'n reeks sitrusplae word al hoe meer algemeen, maar hierdie bewerings bots gereeld met die ondervindinge van wetenskaplikes in die bedryf. Gedurende die vorige seisoen kon geen beduidende doeltreffendheid aangeteken word teen rooidopluis, witluis of blaaspootjie met 'n voorkomende program deur beide *Beauveria bassiana* en *Metarhizium anisopliae* gebaseerde produkte te gebruik. Gevolglik is 'n korrektiewe proef toegedien met drie produkte by 'n perseel waar dopluis en witluis besmetting hoog was. Twee van die produkte het die aantal witluis met 22.7% en 31.8% verlaag in vergelyking met die kontrole. Geen doeltreffendheid is vir dopluis waargeneem nie. Die projek moes daarna beëindig word, maar 'n nuwe *Isaria fumororosea* produk met berigte doeltreffendheid teen Asiëse sitrusbladvlou (ABV), *Diaphorina citri*, is verkry vir proewe. Die bedoeling was om dit te toets teen plae soos sitrusbladvlou, plantluis of wollerige witvlieg, wat kan dui op die doeltreffendheid daarvan teen ABV. Ongelukkig kon geen toepaslike vlak van besmetting vir enige van hierdie plae verkry word in die Oos-Kaap nie. Gevolglik is die produk in 'n boord met 'n uiters hoë witluis besmetting getoets. Ongelukkig is geen betekenisvolle doeltreffendheid waargeneem nie. Die produk sal weer in die lente getoets word, wanneer plantluis op die nuwe groeipunte verskyn.

3.4.8 PROGRESS REPORT: Synergism and formulation of entomopathogenic fungi for foliar control of various citrus pests

Project 1188 by Sonnica Albertyn, Sam Prinsloo (RU), Sean Moore (CRI), Candice Coombes and Martin Hill (RU)

Summary

The South African citrus industry is in urgent need of crop protection products which are not disruptive to the natural enemies of insect pests. Entomopathogenic fungi (EPF) can possibly be used to fill this gap. Therefore, this study aims to determine and improve the ability of EPF to control mealybugs, thrips, red scale and the arboreal stages of false codling moth (FCM). Possible synergism with chemical control agents, botanical control agents and EPNs will also be investigated. Previous bioassays have been conducted to select the best EPF and EPN isolates for FCM and citrus mealybug control. Nematodes, *Steinernema yirgalemense* (92% mortality), *Heterorhabditis noenieputensis* (88% mortality) and the entomopathogenic fungal isolate, *Metarhizium anisopliae* (Ma2) (73% mortality) caused the highest mortality of citrus mealybug. One commercially available product containing *Beauveria bassiana* will also be tested for synergism with *S. yirgalemense* and *H. noenieputensis* for citrus mealybug control. *Steinernema yirgalemense* caused the highest mortality of FCM (99% mortality of larvae and 32% mortality of pupae). *Beauveria bassiana* G Ar 17 B3 caused the highest mortality of FCM (80% mortality) seven days after inoculation with the fungus. The three *Metarhizium anisopliae*, isolates, FCM AR met, MA2 and MA met caused similar mortality of FCM (67 to 73% mortality). Therefore, the MA2 isolate will also be tested for synergism with *S. yirgalemense* as it has been selected for potential commercial production and use. The efficacy of *S. yirgalemense*, *H. noenieputensis*, *M. anisopliae* FCM Ar 23 B3 and an additional nematode species *Steinernema jeffreyense* is currently (April/May 2019) being re-established prior to initiation of synergism trials with these EPN isolates and the EPF *M. anisopliae* FCM Ar 23 B3. Based on the training received from Antoinette Malan, the methodology varies slightly from previously. Briefly, plates inoculated with EPNs are retained for 48 h, after which all larvae are washed with distilled water to remove any external nematodes and separated into two groups, dead or alive, and placed into a sterile petri dish lined with moistened filter paper. Larvae are left for a further 48 h after which any dead larvae are dissected to confirm the presence or absence of nematodes, whilst alive larvae are placed into individual glass vials plugged with cotton wool and held until eclosion. Fungal bioassays

are conducted similarly, with the exception that larvae are dipped individually in conidial suspension and mortality/sporulation evaluated seven days' post-inoculation.

Opsomming

Die Suid-Afrikaanse sitrusbedryf 'n dringend behoefte aan gewasbeskermingsprodukte wat nie die natuurlike vyande van insekplae ontwig nie. Entomopatogeniese swamme (EPS) kan moontlik gebruik word om hierdie gaping te vul. Daarom beoog hierdie studie om die vermoë van EPS om witluise, blaaspootjies, dopluis en die boomlewende stadium van valskodlingmot (VKM) te beheer te bepaal en te verbeter. Moontlike sinergisme met chemiese beheermiddels, botaniese beheermiddels en entomopatogeniese nematodes (EPN) sal ook ondersoek word. Biotoeste is gedoen om die beste EPS en EPN isolate vir VKM en *Planococcus citri*, Sitrus-witluis beheer te kies. Nematodes, *Steinernema yirgalemense* (92% mortaliteit) en *Heterorhabditis noenieputensis* (88% mortaliteit) en die Ma2-swamisolaat, *Metarhizium anisopliae* (73% mortaliteit), het die hoogste mortaliteit van sitruswitluis veroorsaak. Een kommersieel beskikbare produk wat *Beauveria bassiana* bevat, sal ook getoets word vir sinergisme met *S. yirgalemense* en *H. noenieputensis* vir sitruswitluis beheer. *S. yirgalemense* het ook die hoogste mortaliteit van VKM veroorsaak (99% sterftes van larwes en 32% mortaliteit van papies). Die *Beauveria bassiana* isolaat G Ar 17 B3 het die hoogste mortaliteit van VKM (80% mortaliteit), sewe dae na behandeling met die swam veroorsaak. Die drie *Metarhizium anisopliae*, isolate, FCM AR, MA2 en MA, het soortgelyke mortaliteit van VKM veroorsaak (67-73% mortaliteit). Aangesien die MA2-isolaat gekies is vir potensieël kommersiële produksie sal die isolaat gebruik word om sinergisme met *S. yirgalemense* te bepaal. Die effektiwiteit van *S. yirmalmense*, *H. nieieputensis*, *M. anisopliae* FCM Ar 23 B3 en 'n addisionele nematode isolaat, *Steinernema jeffreyense*, word tans heropgestel (April / Mei 2019) voor die implementering van sinergistiese toetse met hierdie EPN-isolate en die EPF *M. anisopliae* FCM Ar 23 B3. Op grond van die opleiding van Antoinette Malan verskil die metodologie effens vantevore. Kortliks word plate wat met EPN's ingeënt word, vir 48 uur behou, waarna alle larwes met gedistilleerde water gewas word om enige eksterne aalwurms te verwyder en in twee groepe, hetsy dood of lewendig, geskei en in 'n steriele petrischaal met bevochtigde filterpapier geplaas. Larwes word nog 48 uur gelaat, waarna enige dooie larwes dissekteer om die teenwoordigheid of afwesigheid van nematodes te bevestig, terwyl lewende larwes in individuele glasbottels geplaas word wat met katoenwol gevul is en in eclosie gehou word. Swambiotipes word op dieselfde wyse uitgevoer, behalwe dat larwes individueel in konidiale suspensie gedoop word en mortaliteit / sporulasie geëvalueer word, sewe dae na inenting.

3.4.9 PROGRESS REPORT: IPM under nets in Mpumalanga Province

Project 1205 (April 2018 – December 2020) by Karlien Grobler (Komati Group), Martin Hill (RU) and Sean Moore (CRI)

Summary

Enclosed netting structures (20% shade) were erected over an orchard of Star Ruby grapefruit and an orchard of Mauritius litchi. Under the nets and in comparable orchards outside of the nets, three replicates of 10 data trees each were marked for regular monitoring. Scouting of citrus thrips (*Scirtothrips aurantii*), bollworm (*Helicoverpa armigera*), orange dog (*Papilio demodocus*) and citrus mealybug (*Planococcus citri*) was conducted in the citrus orchard. Thrips and mealybug populations were higher in the open orchard than under net while lepidopteran pests were found at similar levels. Pink wax scale (*Ceroplastes rubens*), Seychelles scale (*Icerya seychellarum*) and mango scale (*Aulacaspis tubercularis* Newstead) were scouted for in the litchi orchard. Simultaneously, monitoring of false codling moth (FCM) (*Thaumatotibia leucotreta*) and fruit fly species was conducted using traps in both crops. Litchi moth (*Cryptophlebia peltastica*) was additionally trapped in litchi orchards. Seychelles scale, pink wax scale, litchi moth trap counts and larval infestation were found to be present in higher populations under litchi shade net in comparison to the control. Mango scale did not seem to be any different under and outside of nets. Fruit fly trap catches and larval infestation were found to be higher in open orchards of both litchi and citrus compared to shade net orchards, however, overall FCM trap counts were higher in the netted citrus orchard, but larval infestation was found at similar levels. FCM trap counts and larval infestation were similar in the litchi netted

orchard and open orchard. Twenty citrus fruit per tree were graded on the tree according to CRI guidelines for export standards. 96.2% of the fruit from the netted orchard were categorized as class 1, with 3% in class 2 (due to wind scarring and mealybug damage), and 0.8% as damaged due to FCM and carob moth. 81.7% of the fruit from the open orchard were categorised as class 1 with 15.3% of the fruit graded as class 2 (due to wind scarring and mealybug damage) and 3% damaged due to FCM, carob moth, thrips and severe wind damage. Residue analyses conducted with citrus fruit showed higher concentrations (mg/kg) of residues in fruit from under shade net, compared to the open orchard, and the occurrence of two residues from fruit under nets that were not present in fruit outside nets.

Opsomming

Net strukture (20% skadu net) was opgerig oor 'n Star Ruby pomelo en Mauritius litchi boord. Drie rye van tien bome elk binne asook buite die nette op beide gewasse was geïdentifiseer vir moniterings doeleindes. Pes monitering op sitrus sluit in blaaspootjie (*Scirtothrips aurantii*), bolwurm (*Helicoverpa armigera*), lemoen skoenlapper (*Papilio demodocus*) en sitrus witluis (*Planococcus citri*). Blaaspootjie en witluis populasies was hoër buite die net in vergelyking met onder die net. Lepidoptera spesies se getalle was soortgelyk onder en buite die net struktuur. Die monitering van vals kodling mot (VKM) (*Thaumototibia leucotreta*) en vrugte vlieg spesies was in beide gewasse gemonitor deur middel van feromoon lokvalle. Litchi mot (*Cryptophlebia peltastica*) lokvalle was addisioneel in die litchi boord gehang. Pienk was dopluis (*Ceroplastes rubens*), Seychelles dopluis (*Icerya seychellarum*) en mango dopluis (*Aulacaspis tubercularis Newstead*) was gemonitor in litchi boorde. Seychelles dopluis, pienk was dopluis, litchi mot lokval tellings asook vrug infestasië vlakke was hoër in databome onder net as in die kontrole boord. Mango dopluis was in beide litchi boorde opgemerk in eweredige populasies. Vrugte vlieg lokval en vrug infestasië tellings was meer buite die net as onder net in beide gewasse. Totale VKM vangste was meer binne die sitrus net as buite in kontrole boord, maar die vrug infestasië vlak was dieselfde. In die litchi boord was die VKM vangste en vrug infestasië vlakke dieselfde onder net en buite die net. Twintig pomelos per boom op bogenoemde data bome was gegradeer volgens CRI se riglyne vir uitvoer standaard. 96.2% van die vrugte onder net was geklassifiseer as klas 1 vrugte, waar 3% in klas 2 geplaas was as gevolg van wind en witluis merke. 0.8% was beskadig as gevolg van VKM en karob mot. 81.7% van die vrugte in die kontrole boord was gekategoriseer as klas 1, en 15.3% in klas 2 as gevolg van wind en witluis skade. 3% van die vrugte was beskadig deur VKM, karob mot, blaaspoot en erge wind skade. Residu toets resultate toon hoër konsentrasies van chemiese aktiewe bestandele in vrugte onder net, asook twee addisionele aktiewe bestandele teenwoordig onder net teenoor vrugte in die oop kontrole boord.

3.4.10 PROGRESS REPORT: Determine the primary cause for mealybug repercussions under netting Project 1195 (2018/9 – 2019/20) by T G Grout and P R Stephen (CRI)

Summary

In citrus orchards under 20% shade net in both Australia and South Africa, mealybug has become a primary pest. This research is being conducted to determine the main reasons for this. Shade net structures at the Citrus Research Centre in Nelspruit were built, potted Valencia trees placed under the shade net and in the open adjacent to the structures and citrus mealybug cultures initiated. Plans to compare the growth rate of mealybug in parasitoid-proof containers outside the structures and under 20% white shade net have not been possible because the cultures heat up in the sun. Tremendous difficulty has also been experienced in preventing contamination of mealybug cultures with the parasitoid *Coccidoxenoides perminutus* but this has finally been resolved. Pest infestation of potted Valencia trees in the open adjacent to potted trees of the same age and source under 20% net at CRC, Nelspruit showed that under the net citrus red mite infestation was 75% higher, silver mite was 14% higher, red scale 33% higher and mealybug 6% higher. A photo-radiometer has been used to measure PAR and UV-B radiation in adjacent commercial orchards in the open and under 20% netting at various locations in Mpumalanga and Limpopo during summer. Results have been variable on different occasions but in general the nets reduce PAR by approximately 20% and UV-B by 26%. Leaf samples at the same commercial sites have been

taken on two occasions in the open and under net to test for levels of phenolics. These results are not yet available. Tests of chemical residues on fruit under nets and in the open during February and March showed little difference between samples under net and in the open except for a slight trend for some strobilurin residues to be slightly higher under net. None of these differences would have been problematic at harvest time.

Opsomming

Witluis het 'n primêre plaag in sitrusboorde onder 20% skadunet in beide Australië en Suid-Afrika geword. Hierdie navorsing is uitgevoer ten einde die belangrikste redes hiervoor vas te stel. Skadunet strukture by die Sitrus Navorsingssentrum (SNS) in Nelspruit is gebou, bome in potte is onder die skadunet geplaas, en in die oopte, naasliggend aan die strukture en sitrus, is witluis kulture begin. Planne om die groeitempo van witluis in parasitoïed-bestande houers buite die strukture en onder 20% wit skadunet te vergelyk, was nie moontlik nie omdat die kulture in die son verhit het. Uiterste moeite is ook gedoen in die voorkoming van kontaminasie van witluis kulture met die parasitoïed, *Coccidoxenoides perminutus*, maar dit is finaal opgelos. Plaag-infestasie van bome in potte in die oopte, naasliggend aan bome in potte van dieselfde ouderdom en bron onder 20% net by SNS, Nelspruit, het getoon dat sitrus roomyt-infestasie onder die net 75% hoër was, silwermyt 14% hoër was, rooidopluis 33% hoër was en witluis 6% hoër was. 'n Foto-radiometer is gebruik om PAR en UV-B bestraling in naasliggende kommersiële boorde in die oopte en onder 20% net by verskeie liggings in Mpumalanga en Limpopo gedurende die somer te meet. Resultate was by verskillende geleenthede variërend, maar oor die algemeen het die nette PAR met ongeveer 20% verminder en UV-B met 26%. Blaarmonsters by dieselfde kommersiële liggings is by twee geleenthede geneem, in die oopte en onder net, ten einde vir vlakke van fenole te toets. Hierdie resultate is nog nie beskikbaar nie. Toetse van chemiese residue op vrugte onder nette en in die oopte gedurende Februarie en Maart het min verskil tussen monsters onder net en in die oopte getoon, behalwe vir 'n mate van 'n neiging vir sommige strobilurien residue om effens hoër onder net te wees. Geen van hierdie verskille sal 'n probleem met oestyd wees nie.

3.4.11 PROGRESS REPORT: Improving biocontrol of woolly whitefly in the Western and Eastern Cape regions

Project 1194 (2017-20) by M J Gilbert and Claire Love (CRI)

Summary

Woolly whitefly, *Aleurothrixus floccosus*, is a relatively new pest to citrus in South Africa. It was discovered in the Western Cape in 2006. Since then it has spread to other parts of South Africa and can cause honeydew, sooty mould and the downgrading of citrus fruit. The parasitoid, *Cales noacki* Howard (Hymenoptera: Aphelinidae) was imported and bred by CRI in Nelspruit. Releases were then made in Mpumalanga and North-West Province to control *A. floccosus*. Unfortunately, the colony of *C. noacki* could not be maintained at Nelspruit and parasitoids were not available for release in the Western Cape. As a result, project 1194 was set up to sample woolly whitefly populations in the Western Cape and to identify any potentially useful parasitoids. Sampling of woolly whitefly was carried out on several farms and orchards near Robertson, Stellenbosch and Citrusdal during 2017 - 2019. The leaf samples were retained in the lab and emerging parasitoids were collected for identification. *C. noacki* emerged from the samples from all three locations (P.R. Stephen, personal communication). The Stellenbosch sample, despite only coming from two woolly whitefly-infested trees, provided the greatest number of adult parasitoids to date. A sampling of woolly whitefly-infested leaves from a netted orchard (Middeltuyn farm) in the area between Citrusdal and Clanwilliam also revealed that *C. noacki* was present there (identification confirmed by P.R. Stephen, personal communication). The parasitoid has therefore, by some unknown means, established in the Western Cape. Since then, no further samples of woolly whitefly could be found due to what seems to be a population decline. Whether this is due to the fact that the parasitoid has become established in the Western Cape, or to a lessening of the drought, or a combination of the two is not known. Nevertheless, any reported incidence of woolly whitefly outbreaks will continue to be investigated and sampling will be done in order to hopefully confirm the

presence of the parasitoid in other parts of the Western Cape. Insufficient parasitoid numbers has prevented the transfer of adults to the Eastern Cape to date.

Opsomming

Wollerige witvlieg, *Aleurothrixus floccosus*, is 'n relatiewe nuwe plaag op sitrus in Suid-Afrika. Dit was in die Wes-Kaap in 2006 ontdek. Vanaf dié tyd het dit na ander dele van Suid-Afrika versprei. *A. floccosus* kan heuningdoo, roetskimmel en die afgradering van sitrus vrugte veroorsaak. Die parasitoïed, *Cales noacki* Howard (Hymenoptera: Aphelinidae) was deur CRI in Nelspruit ingevoer. Loslatings was in Mpumalanga en Noord-Wes Provinsie gedoen om wollerige witvlieg te beheer. Ongelukkig het die *C. noacki* kolonie in Nelspruit uitgesterf en geen volwassenes was vir loslating in die Wes-Kaap beskikbaar nie. As gevolg daarvan is projek 1194 opgestel om wollerige witvlieg populasies in die Wes-Kaap te monitor en om enige potensieel nuttige parasitoïede te identifiseer. Die monsterring van wollerige witvlieg was op verskeie plekke en boorde naby Robertson, Stellenbosch en Citrusdal gedurende 2017 tot 2019 uitgevoer. Blaarmonsters was in die laboratorium gehou en enige parasitoïede wat uitgekam het was vir identifikasie weg gestuur. *C. noacki* is in al drie areas gevind (P. Stephen, persoonlike kommunikasie). Die Stellenbosch monster, alhoewel net van twee besmette bome, het die meeste volwasse parasitoïede opgelewer. 'N monster van besmette blare van 'n boord onder nette (Middel tuin plaas) tussen Citrusdal en Clanwilliam het ook *C. noacki* se teenwoordigheid in die area bevestig. Dus het die parasitoïed self deur onbekende metode in die Wes-Kaap gevestig. Vanaf daai tyd, kon geen ander monsters van wollerige witvlieg geding word omdat die populasie baie afgeneem het. Of dit het gebeur as gevolg van die vestiging van die parasitoïed, of 'n vermindering van die droogte, of 'n kombinasie van die twee faktore, is onbekend. Nogtans, enige uitbrekings van wollerige witvlieg sal ondersoek word en monsters sal geneem word om hopelik die teenwoordigheid van *C. noacki* in ander dele van die Wes-Kaap te bevestig. Onvoldoende parasitoïed getalle tot so ver die verskaffing van materiaal aan die Oos-Kaap voorkom het.

3.4.12 PROGRESS REPORT: New systemic insecticides for citrus

Project 1148 (2016/7 – 2019/20) by T G Grout and P R Stephen (CRI), and S M Faris (*icipe*)

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 citricidus* was used as an indicator pest for screening systemic insecticides on potted lemon trees. Potted lemon trees infested with aphids were used 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 seven days. Two dosages of sulfoxaflor as a drench gave the same result after seven days, although the mortality rate was slower. Results from an unregistered product, used in some countries as a soil drench for vegetables, were disappointing and a high dosage would be required. Another unregistered systemic used on citrus in the USA gave promising results as a soil drench. A field trial against cotton aphid on young citrus was attempted but populations were sporadic and results very variable. Collaborators at *icipe* in Kenya erected an insect-proof greenhouse at their Muhaka field station in SE Kenya where *Diaphorina citri* has established. However, infesting potted citrus plants within this greenhouse with *D. citri* for further screening trials has not been successful because numbers of *D. citri* found in the region have remained extremely low. If these pot trials can be conducted with citrus or perhaps *Berberis koenigii*, research will be expanded to young planted citrus trees.

Opsomming

Ten einde vir die aankoms van *Diaphorina citri* in Suid-Afrika voor te berei, moet ons meer sistemiese insekdoders vind wat gereeld in kwekerie en vir nie-draende bome gebruik kan word. Die bruin sitrus plantluis, *Toxoptera citricidus*, is as 'n indikatorplaag vir die evaluering van sistemiese insekdoders op suurlemoenbome in potte gebruik. Suurlemoenbome in potte, geïnfesteer met plantluis, is gebruik om 'n paar chemikalieë te evalueer. Die

geregistreeerde imidakloprid drenkbehandeling en onlangs geregistreeerde asefaat (Spectra Stem) stambehandeling, is as standaard gebruik en het gelei tot die afval van alle plantluse vanaf die blare binne sewe dae. Twee dosisse van sulfoxaflor as 'n drenkbehandeling, het dieselfde resultaat na sewe dae gegee, hoewel die sterftetempo stadiger was. Resultate van 'n nie-geregistreeerde produk, wat in sommige lande as 'n grondrenkbehandeling vir groente gebruik word, was teleurstellend en 'n hoë dosis sal benodig word. 'n Ander nie-geregistreeerde sistemiese produk wat op sitrus in die V.S.A. gebruik word, het belowende resultate as 'n grondrenkbehandeling gegee. 'n Veldproef teen katoen plantluis op jong sitrus is probeer, maar populasies was sporadies en resultate baie variërend. Samewerkers by *icipe* in Kenia het 'n insekbestande evalueringshuis by hul Muhaka veldstasie in SO Kenia opgerig waar *Diaphorina citri* gevestig is. Infestasië van sitrusplante in potte binne hierdie evalueringshuis met *D. citri* vir verdere evalueringsooie, was egter nie suksesvol nie, omdat getalle van *D. citri* wat in die area gevind is, uiters laag gebly het. Indien hierdie potproewe met sitrus of dalk *Bergera koenigii* uitgevoer kan word, kan navorsing na jong geplante sitrusbome uitgebrei word.

3.4.13 **PROGRESS REPORT: Controlling mites on budwood**

Project 1203 (2018/9-2019/20) by T G Grout and P R Stephen (CRI)

Summary

Citrus budwood often requires fumigation with methyl bromide on arrival in South Africa and this often kills many of the buds. In the search for a more benign means of control, preliminary work with fumigants Vapormate (ethyl formate) and carbon dioxide against citrus bud mite showed that Vapormate was more efficacious but that it was also more phytotoxic. Other time x dosage combinations could not be evaluated due to a difficulty in finding suitable sources of citrus bud mite. If a good source of these mites can be found, further research on carbon dioxide fumigation and dips in acaricide solutions will be conducted. Effective treatments will then need to be used on buds that are grafted to determine the percentage 'take' relative to untreated buds.

Opsomming

Sitrus okuleerhout vereis dikwels beroking met metielbromied met aankoms in Suid-Afrika en dit maak dikwels baie van die ogies dood. In die soeke na 'n meer vriendelike manier van beheer, toon voorlopige werk met berokingsmiddels Vapormate (etielformaat) en koolstofdioksied teen sitrus knopmyt, dat Vapormate meer effektief was, maar dat dit ook meer fitotoksies was. Ander tyd kon x dosis kombinasies nie geëvalueer word nie omdat dit moeilik was om geskikte bronne van sitrus knopmyt in die hande te kry. Indien 'n goeie bron van hierdie myte gevind kan word, kan verdere navorsing op koolstofdioksied beroking en doop in mytdoder-oplossings uitgevoer word. Effektiewe behandelings sal dan op ogies wat geïnkuleer is, gebruik word ten einde die persentasie 'vat' relatief tot onbehandelde ogies te bepaal.

4 PORTFOLIO: DISEASE MANAGEMENT

4.1 PORTFOLIO SUMMARY

Portfolio Manager: Jan van Niekerk (CRI)

Effective management of preharvest (soilborne, fruit and foliar and Citrus black spot), postharvest and graft transmissible diseases (GTD) of citrus is important for the sustainability of the industry. The Disease Management portfolio therefore consists of preharvest, postharvest and GTD programmes. The aim of all research programmes is 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.

Graft transmissible disease research is important to ensure that pathogen-free propagation material is supplied to the industry. This is done through the development of sensitive diagnostic tests for the different pathogens and

control strategies. Cross-protection is applied to mitigate the negative effects of citrus tristeza virus (CTV). Different CTV strains are therefore studied to determine which ones can be used for cross-protection. CTV is often a complex of strains and variants. Through the work done in project 1100 (PhD study of Glynnis Cook), strain diagnostics have progressed greatly, enabling single strain identification and characterization. Minor differences in DNA sequences between different variants of the same strain, were found to influence CTV symptom expression. This will assist in better understanding the genetic determinants for stem pitting. Within projects 968 and 1173, promising CTV sources are evaluated in field trials with sweet orange and soft citrus.

The performance of field cut plant material versus plant material provided by the Citrus Improvement Scheme (CIS) is being evaluated and early results clearly shows that trees made with CIS material are growing better and are healthier than trees made from field cut material (Project 1173). Another option to manage diseases, is the use of resistant or tolerant rootstocks. To identify these, project 1155 aims to test commercially important rootstock selections for viroid sensitivity in field trials. For this trial, plant preparation is underway for planting in spring 2019.

'Huanglongbing' (HLB) or 'Asian Greening' has not been reported in southern Africa yet. However, the confirmed presence of both '*Candidatus* Liberibacter asiaticus (Las) and *Diaphorina citri* on the African continent makes it important to be prepared for possible incursions. As part of this preparedness, accurate identification of the various Liberibacter species in citrus in Africa is important and requires specific diagnostics that is addressed in project 1200. Sniffer dogs was used with success for HLB detection in the USA and following on this, a local dog was imprinted to detect African Greening. Unfortunately, the working ability of the dog was not sufficiently accurate. Additionally, an uncorrected discipline problem with the dog further influenced reliability and led to the termination of project 1184. Project 1160 focusses on the development of an infectious citrus tristeza virus (CTV) clone to combat HLB. This constitutes a novel approach to use CTV as a vehicle to systemically deliver antimicrobial peptides or RNA 'signals' to arrest the insect vector or the Liberibacter pathogen of HLB.

Postharvest treatment of fruit is facing increasing challenges from export markets and clients in these markets. Investigating alternative treatment options or compounds are therefore very important. In project 123, the focus is specifically on testing products for sanitation of fruit. This will remain important as sanitation of fruit is becoming very important with increasing pressure on postharvest fungicides and reduction of allowed residues. Unfortunately, in the past season, none of the alternative products were shown to be effective. Application issues with certain PAA products were furthermore addressed in this project. Additionally, alternative actives to be used as replacement or in combination with current fungicides were investigated and this has led to the registration of azoxystrobin, an alternative for imazalil.

The phytochemical relationship between the CBS pathogen *Phyllosticta citricarpa* and different citrus types with variable sensitivity to CBS were investigated in project 1135. Results found that citrus types with different levels of sensitivity to CBS were clearly separated based on the volatile profiles of their rinds. As fruit develop from fruit set to harvest these profiles were furthermore found to change.

Development of pathogen resistance to current postharvest fungicides is important to understand. In project 1141 propiconazole (PPZ) was evaluated as an alternative for sour rot control. A baseline study on *Penicillium digitatum* and *G. citri-aurantii* isolates previously exposed to PPZ or imazalil revealed a low percentage of resistant *Galactomyces citri-aurantii* isolates. Among the *P. digitatum* isolates the percentage PPZ resistant isolates was much higher. Optimisation of PPZ aqueous applications were also done and found that in order to obtain 80% control of sour rot and green mould, a PPZ drench should be applied between 11 and 19 hours after harvest, depending on the citrus type being treated.

The removal of methyl bromide as a wood treatment has resulted in problems with pallet wood and fungal degradation of pallet bases during export. In project 1165, it was found that *Trichoderma* and *Fusarium* spp. were the dominant fungi found on degrading pallet bases. SOPP has been used as a preservative of the pallet wood

and this has led to unwanted residues on fruit. These residues can also be due to the insides of containers being sanitized with SOPP soaps. This aspect is further investigated with the help of PPECB.

Citrus brown rot caused by *Phytophthora nicotianae*, often cause significant postharvest losses. Currently no postharvest fungicide is registered in South Africa for the postharvest management of this disease. Project 1198 investigates the efficacy of fludioxonil and azoxystrobin to control this pathogen *in vitro* and postharvest on fruit. Previously exposed and unexposed *P. nicotianae* isolates were found to *in vitro* vary greatly in their sensitivity to these fungicides. In controlling brown rot on fruit, both fungicides were shown to have very good curative action when applied up to 12 hours after inoculation. Azoxystrobin was also found to have a very good protective ability. Further work is being done on mandarins and oranges.

In the preharvest research programme the research focus is on the management of soilborne pests and diseases as well as fruit and foliar diseases, including Citrus black spot (CBS). Aspects that are investigated is softer, more environmentally friendly control options, disease epidemiology, fungicide application technology and better chemical spray programmes.

From projects 762 and 1030 results are showing that pre-plant soil fumigation does have a positive effect on citrus tree growth in replant situations due to suppression of soilborne pathogens and nematodes. An algaecide was also tested in 1030 to treat *Phytophthora* spp. infection of nursery trees and were shown to improve the growth of the trees. In project 1068 a serious decline disease in the Eastern Cape production regions are under investigation. It was found that several pathogens, including *Neocosmospora (Fusarium)* spp. are involved along with other known stress pathogens of woody hosts. Drench treatments of seedlings with benomyl in a pot trial resulted in root systems of seedlings growing in soil from diseased orchards being bigger compared to other treatments and untreated seedlings. This treatment also promoted the growth of young trees in a diseased orchard to such an extent that it was statistically similar to trees growing in a fumigated portion of the orchard. Another foliar treatment with bioflavonoids were also found to promote the growth the young trees, similar to benomyl.

Project 1101 focused on the management of soilborne diseases in nurseries. Phosphonate foliar applications promoted the growth of the nursery trees while also reducing *Phytophthora nicotianae* root infections. Great variation in sensitivity of *Phytophthora nicotianae*, *Phytophthora citrophthora* and *Pythium* spp. towards mefenoxam was found, indicating that this fungicide should be used with caution in nurseries. Similarly, it was seen that among the isolates of these pathogens, variation exist with regards to chlorine sensitivity. A clear interaction between active chlorine concentration and exposure time was seen, with some isolates only being totally eliminated from water at a concentration of 6 ppm chlorine and a 60-minute exposure time. These findings have been incorporated in the nursery production manual for citrus nurseries. The search for potential biological control agents (BCA's) are conducted in project 1215 along with a study of the interaction between the different replant pathogens. New primers for the detection of abovementioned *Phytophthora* spp. were developed and are under validation. Some promising BCA's were furthermore identified. These include *Bacillus*, *Pseudomonas* and *Trichoderma* spp. that all significantly inhibited the mycelial growth of oomycete pathogens but were not as effective against *Neocosmospora* spp. Further testing of these organisms is also underway.

Due to its importance, major focus in this programme is placed on CBS, especially its epidemiology and management. Alternaria brown spot (ABS) is also receiving attention with regards to epidemiology, disease prediction and chemical control. In project 970, through spray trials, a promising new fungicide was identified for CBS control while in 750 improvements in the chemical control programme for this disease was achieved. This was done through an early season application of boscalid. Project 1132 and 1089 strove to evaluate low volume agro-chemical applications and LiDAR (Light Detection and Ranging) technology to measure tree canopy density. It was clearly seen that lower volume applications are not effective in controlling insect pests such as mealybug and red scale compared to high volume applications. This was mostly found to be due to better spray uniformity achieved by the higher volumes. The LiDAR technology was shown to detect differences in canopy densities but

poor correlation between the LiDAR and manual density measurements was seen. However, the importance of pruning in improving spray deposition inside the tree when applying low volumes was clearly illustrated.

Projects RCE-6 and RCE-8 investigated the epidemiology of CBS with the aim of improving the prediction models used in CRI PhytRisk. Good results were achieved in RCE-6 while some new models were developed in RCE-8. These improvements were incorporated in project RCE-7 that focused on the further validation of CRI PhytRisk. In this project a prediction model for ABS was also added to this online prediction service, leading to a major improvement in the decision making tool available to growers. The use of shade netting is furthermore becoming more important in citrus production and project 1187 studied their effect on ABS and CBS epidemiology. Depending on production area it was seen that the nets have a different effect on the two diseases, in some instances leading to a higher potential for disease development. Further studies will be done to clearly elucidate the effect of nets on these two diseases. Results from project 1186 also made a contribution to CBS control, confirming that fruit do become more resistant to infection the more mature they become, making fungicidal protection after March unnecessary.

PORTEFEULJE-OPSOMMING

Effektiewe bestuur van voor-oes (grondgedraagd, vrug en blaar, en Sitrus Swartvlek), na-oes en ent-oordraagbare siektes ("GTD") van sitrus is belangrik vir die volhoubaarheid van die industrie. Die Siektebestuur Portefeuje bestaan dus uit voor-oes, na-oes en "GTD" programme. Die doel van alle navorsingsprogramme is om huidige industrie navorsingsbehoefte binne hierdie verskillende areas aan te spreek, maar ook om pro-aktief te wees ten einde navorsing te doen oor die bestuur van siektes wat verwag word om in die toekoms 'n uitdaging vir die industrie te wees.

Ent-oordraagbare siekte navorsing is belangrik ten einde te verseker dat patogeenvrye voortplantingsmateriaal aan die industrie verskaf word. Dit word gedoen deur die ontwikkeling van sensitiewe diagnostiese toetse vir die verskillende patogene, en beheerstrategieë. Kruisbeskerming word toegepas om die negatiewe effekte van citrus tristeza virus (CTV) te verminder. Verskillende CTV stamme word dus bestudeer om vas te stel watter vir kruisbeskerming gebruik kan word. CTV is dikwels 'n kompleks van stamme en variante. Deur die werk wat in projek 1100 gedoen word (PhD studie van Glynnis Cook), het stam diagnose baie gevorder, wat enkel stam identifikasie en karakterisering moontlik maak. Daar is gevind dat klein verskille in DNS volgordes tussen verskillende variante van dieselfde stam, CTV simptoom-uitdrukking beïnvloed. Dit sal daartoe bydra om die genetiese determinante vir "stem pitting" beter te verstaan. Binne projekte 968 en 1173 word belowende CTV bronne in veldproewe met soet lemoene en sagte sitrus geëvalueer.

Die prestasie van veld-gesnyde plantmateriaal teenoor plantmateriaal wat deur die Sitrus Verbeteringskema (SVS) voorsien word, word geëvalueer, en vroeë resultate dui duidelik daarop dat bome wat met SVS materiaal gemaak word, beter groei en gesonder is as bome wat van veld-gesnyde materiaal gemaak word (Projek 1173). 'n Ander opsie om siektes te bestuur, is deur die gebruik van weerstandbiedende of bestande onderstamme. Ten einde dit te identifiseer, het projek 1155 ten doel om kommersieel belangrike onderstam seleksies vir viroïed sensitiwiteit in veldproewe te evalueer. Plantvoorbereiding is vir hierdie proef onderweg vir plant in die lente van 2019.

'Huanglongbing' (HLB) of 'Asiatiese Vergroening' is nog nie in suidelike Afrika aangeteken nie. Die bevestigde teenwoordigheid van beide '*Candidatus* Liberibacter asiaticus (Las) en *Diaphorina citri* op die Afrika vasteland, maak dit belangrik om voorbereid te wees vir moontlike invalle. As deel van hierdie voorbereiding, is akkurate identifikasie van die verskeie Liberibacter spesies in sitrus in Afrika belangrik en vereis spesifieke diagnose wat in projek 1200 aangespreek word. Snuffelhonde is met sukses vir HLB opsporing in die V.S.A. gebruik, en volgende hierop, is 'n plaaslike hond opgelei om Afrika Vergroening op te spoor. Die werksvermoë van die hond was ongelukkig nie akkuraat genoeg nie. Verder het 'n dissipline probleem met die hond die betroubaarheid verder beïnvloed, wat tot die beëindiging van projek 1184 gelei het. Projek 1160 fokus op die ontwikkeling van 'n

infektiewe citrus tristeza virus (CTV) kloon om HLB te bestry. Dit bestaan uit 'n nuwe benadering om CTV te gebruik as 'n vervoermiddel om antimikrobiële peptiedes of RNS 'seine' sistemies af te lewer om die insekvektor te stop of die Liberibacter patoëen van HLB.

Na-oes behandeling van vrugte staar toenemende uitdagings vanaf uitvoermarkte en kliënte in hierdie markte in die gesig. Die ondersoek van alternatiewe behandelingsopsies of verbindings is dus baie belangrik. Projek 123 fokus spesifiek op die toets van produkte vir sanitasie van vrugte. Dit bly belangrik soos wat sanitasie van vrugte baie belangrik word met toenemende druk op na-oes fungisiedes en vermindering van toelaatbare residue. Die afgelope seisoen was geen van die alternatiewe produkte egter effektief nie. Toedieningskwessies met sekere PAA produkte is verder in hierdie projek aangespreek. Addisioneel is alternatiewe aktiewes wat as vervanging of in kombinasie met huidige fungisiedes gebruik kan word, ondersoek, en dit het tot die registrasie van azoxystrobin gelei, 'n alternatief vir imazalil.

Die fitochemiese verhouding tussen die SSV patoëen, *Phyllosticta citricarpa*, en verskillende sitrus tipes met variërende sensitiwiteit teenoor SSV, is in projek 1135 ondersoek. Resultate het getoon dat sitrus tipes met verskillende vlakke van sensitiwiteit teenoor SSV, duidelik geskei word op grond van die vlugtige profiele van hul skille. Soos wat vrugte vanaf vrugset tot oes ontwikkel, is gevind dat die profiele verder verander.

Dit is belangrik om die ontwikkeling van patoëenweerstand teen huidige na-oes fungisiedes te verstaan. In projek 1141 is propikonasool (PPZ) as 'n alternatief vir suurvrot beheer geëvalueer. 'n Basisvlak studie op *Penicillium digitatum* en *G. citri-aurantii* isolate wat voorheen aan PPZ of imazalil blootgestel is, het op 'n lae persentasie van weerstandbiedende *Galactomyces citri-aurantii* isolate gedui. Tussen die *P. digitatum* isolate was die persentasie PPZ weerstandbiedende isolate baie hoër. Optimalisering van PPZ waterige toedienings is gedoen en daar is gevind dat ten einde 80% beheer van suurvrot en groenskimmel te verkry, 'n PPZ drenkbehandeling tussen 11 en 19 ure ná oes toegedien moet word, afhangende van die sitrus tipe wat behandel word.

Die verwydering van metielbromied as 'n houtbehandeling het tot probleme met pallethout en swamafbraak van palletbasisse gedurende uitvoer gelei. Daar is in projek 1165 gevind dat *Trichoderma* en *Fusarium* spp. die dominante swamme was wat op afgebreekte palletbasisse gevind is. SOPP is as preserveermiddel van die pallethout gebruik en dit het tot ongewenste residue op vrugte gelei. Hierdie residue kan ook die gevolg wees van die binnekante van houers wat met SOPP sepe gesaniteer word. Hierdie aspek word verder met die hulp van PPECP ondersoek.

Sitrus bruinvrot, veroorsaak deur *Phytophthora nicotianae*, veroorsaak dikwels betekenisvolle na-oes verliese. Huidig is geen na-oes fungisied in Suid-Afrika vir die na-oes bestuur van hierdie siekte geregistreer nie. Projek 1198 ondersoek die effektiwiteit van fludioxonil en azoxystrobin om die patoëen *in vitro* en na-oes op vrugte te beheer. Voorheen blootgestelde en nie-blootgestelde *P. nicotianae* isolate varieer grootliks *in vitro* in hul sensitiwiteit teenoor hierdie fungisiedes. In die beheer van bruinvrot op vrugte, het beide fungisiedes getoon dat hulle 'n baie goeie uitwissende aksie het wanneer toegedien word tot 12 ure ná inokulasie. Azoxystrobin het ook 'n baie goeie beskermende vermoë getoon. Verdere werk word op mandaryne en lemoene gedoen.

In die voor-oes navorsingsprogram, val die navorsingsfokus op die bestuur van grondgedraagde plaë en siektes, asook vrug- en blaarsiektes, insluitende Sitrus Swartvlek (SSV). Aspekte wat ondersoek word is sagter, meer omgewingsvriendelike beheer-opsies, siekte-epidemiologie, fungisied toedieningstechnologie en beter chemiese spuitprogramme.

Resultate vanuit projekte 762 en 1030 toon dat vóór-plant grondberoking 'n positiewe effek op sitrus boomgroei in herplant situasies het, weens die onderdrukking van grondgedraagde patogene en aalwurms. 'n Algdoder is ook in 1030 getoets om *Phytophthora* spp. infeksie van kwekerybome te behandel, en het getoon dat die groei van die bome verbeter het. In projek 1068 word 'n ernstige agteruitgang siekte in die Oos-Kaap produksie-areas ondersoek. Daar is gevind dat verskeie patogene betrokke is, insluitende *Neocosmospora (Fusarium) spp.*,

tesame met ander bekende strespatogene van houtagtige gasHERE. Drenkbehandelings van saailinge met benomyl in 'n potproef het daartoe gelei dat die wortelsisteme van saailinge wat in grond van siek boorde groei, groter was as ander behandelings en onbehandelde saailinge. Hierdie behandeling het ook die groei van jong bome in 'n siek boord tot so 'n mate bevorder dat dit statisties soortgelyk was aan bome wat in 'n beroekte deel van die boord gegroei het. 'n Ander blaarbehandeling met bioflavonoïedes, het die groei van jong bome bevorder, soortgelyk aan benomyl.

Projek 1101 het op die bestuur van grondgedraagde siektes in kwekerye gefokus. Fosfonaat blaartoedienings het die groei van kwekerybome bevorder, en ook *Phytophthora nicotianae* wortel-infeksies verminder. Groot variasie in sensitiwiteit van *Phytophthora nicotianae*, *Phytophthora citrophthora* en *Pythium* spp. teenoor mefenoxam is gevind, wat daarop dui dat hierdie fungusied versigtig in kwekerye gebruik moet word. Soortgelyk is gesien dat variasie tussen isolate van hierdie patogene bestaan met betrekking tot chloor sensitiwiteit. 'n Duidelike interaksie tussen aktiewe chloor konsentrasie en blootstellingstyd is gesien, met sommige isolate wat slegs heeltemal in water uitgewis is teen 'n konsentrasie van 6 dpm chloor en 'n 60 minuut blootstellingstyd. Hierdie bevindinge is in die kwekery produksiehandleiding vir sitruskwekerye opgeneem. Die soeke na potensiële biologiese beheer-agente ("BCA's") word in projek 1215 uitgevoer, tesame met 'n studie van die interaksie tussen die verskillende herplant patogene. Nuwe inleiers vir die opspoor van bogenoemde *Phytophthora* spp. is ontwikkel en word gevalideer. Belowende biologiese beheer-agente is ook geïdentifiseer. Dit sluit *Bacillus*, *Pseudomonas* en *Trichoderma* spp. is wat almal die miseliumgroei van oömiseet patogene betekenisvol geïnhibeer het, maar was nie so effektief teen *Neocosmospora* spp. nie. Verdere toetsing van hierdie organismes is ook onderweg.

Weens sy belang, word groot fokus in hierdie program op SSV geplaas, veral sy epidemiologie en bestuur. Alternaria Bruinvlek (ABV) kry ook aandag met betrekking tot epidemiologie, siekte-voorspelling en chemiese beheer. Deur spuitproewe, is in projek 970 'n belowende nuwe fungusied vir SSV beheer geïdentifiseer, terwyl verbeteringe in die chemiese beheerprogram van hierdie siekte in 750 behaal is. Dit is deur 'n vroeë seisoen toediening van boscalid verkry. Projek 1132 en 1089 streef na die evaluasie van lae volume agro-chemiese toedienings en LiDAR ("Light Detection and Ranging") tegnologie ten einde boomlowerdigtheid te meet. Daar is duidelik getoon dat laer volume toedienings nie effektief in die beheer van insekplae soos witluis en rooidopluis, in vergelyking met hoë volume toedienings, is nie. Dit was grootliks te vinde weens die beter spuit-uniformiteit wat met hoër volumes verkry word. Daar is gevind dat die LiDAR tegnologie verskille in lowerdigthede waarneem, maar swak korrelasie tussen die LiDAR en handdigheidsmetings is waargeneem. Die belang van snoei in die verbetering van spuitneerlegging binne die boom wanneer lae volumes toegedien word, is duidelik aangedui. Projekte RCE-6 en RCE-8 het die epidemiologie van SSV ondersoek met die doel om die voorspellingsmodelle wat in CRI PhytRisk gebruik word, te verbeter. Goeie resultate is in RCE-6 bereik, terwyl 'n paar nuwe modelle in RCE-8 ontwikkel is. Hierdie verbeterings is in projek RCE-7 geïnkorporeer wat op die verdere validasie van CRI PhytRisk fokus. In hierdie projek is 'n voorspellingsmodel vir ABV ook tot hierdie aanlyn voorspellingsdiens gevoeg, wat tot 'n groot verbetering in die besluitnemingshulpmiddel, beskikbaar tot produsente, gelei het. Die gebruik van skadunet word verder meer belangrik in sitrusproduksie, en projek 1187 het hul effek op ABV en SSV epidemiologie bestudeer. Afhangende van produksie-area, is getoon dat die nette 'n verskillende effek op die twee siektes het, en in sommige gevalle tot 'n hoër potensiaal vir siekte-ontwikkeling lei. Verdere studies sal gedoen word om duidelik die effek van nette op hierdie twee siektes uit te klaar. Resultate vanuit projek 1186 het ook 'n bydra tot SSV beheer gemaak, deur te bevestig dat vrugte meer weerstandbiedend teen infeksie word soos wat hul ryp word, en sodoende is fungusiedbeskerming ná Maart onnodig.

4.2 PROGRAMME: GRAFT TRANSMISSIBLE DISEASES

Programme coordinator: G. Cook (CRI)

4.2.1 Programme summary

Vegetative propagation carries the potential to disseminate pathogens including bacteria, viruses and viroids. The supply of pathogen-free propagation material is reliant on sensitive diagnostic capabilities, but additional control

strategies are required for insect transmitted pathogens. Cross-protection is one such management strategy applied to mitigate the damaging effects of citrus tristeza virus (CTV), especially in grapefruit. A research facet, within the Graft Transmissible Diseases (GTD) programme, is to investigate the effect of CTV strains and to determine which are ultimately required for cross-protection. CTV is a complex of strains and variants and CTV strain diagnostics has progressed significantly, enabling identification and characterisation of single-strain sources. Mild and severe variants of one strain were identified and results suggest that minor sequence differences are responsible for significant differences in symptom expression. These findings will assist in the identification of the genetic determinants for stem pitting. This work formed part of a PhD study that was finalised in project 1100. Field trials to assess the field performance of promising CTV sources in sweet orange and soft citrus are reported on in projects 1173 and 968.

A comparative trial to test the horticultural performance of field-cut propagation material compared to material supplied by the Citrus Improvement Scheme (CIS) is underway and preliminary results demonstrate better growth and tree health using CIS propagation material (Project 1074).

The use of cultivars and rootstocks that exhibit tolerance or resistance to pathogens is part of disease management. Commercial or potentially important rootstock selections will be tested for viroid sensitivity in a field trial. Plant preparation is underway for trial planting in the spring of 2019 (Project 1155).

'Huanglongbing' (HLB) or 'Asian Greening' has not been reported in southern Africa yet. However, the confirmed presence of both '*Candidatus* Liberibacter asiaticus (Las) and *Diaphorina citri* on the African continent necessitates preparation for an incursion event. Identification of various Liberibacter species in citrus in Africa requires specific diagnostics for correct identification and this is addressed in project 1200.

Following the success of sniffer dogs for HLB detection in the USA, a dog was imprinted to detect African Greening, but the working ability of the dog did not demonstrate sufficient accuracy. A discipline problem, which was not corrected, influenced the reliability of the dog and project 1184 was therefore terminated.

The development of an infectious citrus tristeza virus (CTV) clone to combat HLB is a novel approach to use CTV as a vehicle to systemically deliver antimicrobial peptides or RNA 'signals' to arrest the insect vector or the Liberibacter pathogen of HLB. Progress is reported in project 1160.

Programopsomming

Vegetatiewe voortplanting besit die potensiaal om patogene te versprei, insluitend bakterieë, virusse en viroïede. Die verskaffing van patogeenvrye enthout is afhanklik van sensitiewe diagnostiese vermoëns, maar bykomende beheerstrategieë word benodig vir insekoordraagbare patogene. Kruisbeskerming is een van die bestuurstrategieë wat toegepas word om die skadelike effekte van sitrus tristeza-virus (CTV) te verminder, veral in pomelo's. Navorsing, binne die GTD-program (Graft Transmissible Diseases), is daarop gerig om die effek van CTV-rasse te ondersoek en om te bepaal watter uiteindelik benodig word vir kruisbeskerming. CTV is 'n kompleks van rasse en variante. Diagnostiek om CTV-rasse te identifiseer het aansienlik verbeter wat die identifikasie en karakterisering van enkelrasbronne moontlik maak. Beide ligte en strawwe variante van een CTV ras is geïdentifiseer en resultate dui daarop dat geringe verskille in basisvolgordes verantwoordelik is vir beduidende verskille in simptome uitdrukking. Hierdie inligting sal help met die identifisering van genetiese volgorde-bepaling vir stamgleuf. Hierdie werk het deel gevorm van 'n PhD-studie wat in projek 1100 gefinaliseer is. Veldproewe om die veldprestasie van belowende CTV-bronne in soetlemoene en sagte sitrus te evalueer word in projekte 1173 en 968 gerapporteer.

'n Vergelykende veldproef om die tuinbouprestasie van veldgesnyde voortplantingsmateriaal te vergelyk met materiaal verskaf deur die Sitrusverbeteringskema (SVS) word uitgevoer en voorlopige resultate toon beter groei en boomgesondheid van bome gemaak met SVS enthout (Projek 1074).

Die gebruik van kultivars en onderstamme wat verdraagsaamheid of weerstand teen patogene toon, is deel van siektebestuur. Kommersiële of potensieel belangrike onderstamseleksies sal getoets word vir viroïed sensitiviteit in 'n veldproef. Plantvoorbereiding geskied vir proefplanting in die lente van 2019 (Projek 1155).

'Huanglongbing' (HLB) of 'Asiese Vergroening' is nog nie in suidelike Afrika opgespoor nie. Die bevestigde teenwoordigheid van beide 'Candidatus' *Liberibacter asiaticus* (Las) en *Diaphorina citri* op die Afrika-kontinent noodsaak voorbereiding vir moontlike inbeweging van die patoëen en/of vektor. Identifisering van verskeie *Liberibacter* spesies in sitrus in Afrika vereis spesifieke diagnostiek vir korrekte identifikasie wat in projek 1200 aangespreek word.

Na aanleiding van die sukses van bloedhonde vir HLB-opsporing in die VSA, is 'n hond opgelei om Afrika Vergroening op te spoor, maar die werkvermoë van die hond het nie voldoende akkuraatheid getoon nie. 'n Dissipline probleem, wat nie reggemaak is nie, het die betroubaarheid van die hond beïnvloed en projek 1184 is gevolglik beëindig.

Die gebruik van 'n CTV kloon om antimikrobiese peptiedes of RNA 'seine' in die plant te lewer, om die insekvektor of die *Liberibacter*-patoëen van HLB te beheer, is 'n unieke benadering. Vordering hiermee word in projek 1160 bespreek.

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

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

Summary

To understand the strain components of citrus tristeza virus (CTV) required 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. The aim of this study was to identify citrus tristeza virus single-strain isolates of different strains, to characterise them biologically and determine full-genome sequences. These characterised CTV isolates were used in a complementation study to investigate possible synergistic interactions affecting stem-pitting on grapefruit. Complete viral genomes of eight single-strain isolates were determined during the study. Two commercial grapefruit cultivars, 'Star Ruby' and 'Marsh', were used in a glasshouse trial to evaluate the ability of specific strains to induce stem-pitting in single or mixed infections. Evaluation over four years showed that symptom expression of mild strains did not result in altered symptom expression when in combination with each other, demonstrating that there was no additive effect on stem-pitting expression with multiple mild strains. Relative quantitation of the strains in 'Marsh' and 'Star Ruby' plants indicated that the individual strain concentrations were not significantly altered when in combination with the other strains. A valuable discovery made within this project was the characterisation of two variants of the T68 strain, derived from the same GFMS12 source, but displaying differences in stem-pitting severity in grapefruit. This finding demonstrates the co-existence of severe and mild variants of the same strain in a single source and provides an explanation for the presumed strain segregation event observed for the GFMS12 cross-protection source that resulted in the discontinuation of the source for use in cross-protection of grapefruit. The characterization of these variants will assist in the identification of the sequence determinants for stem-pitting in grapefruit.

Additionally, Next Generation Sequencing (NGS) was used for diagnostics in citrus viral pathology and small RNA (sRNA) sequencing was used to investigate the citrus host response to CTV and viroid infection. Differential regulation between infected and healthy samples of plant miRNAs was shown.

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 sitrusgasheer. Die doel van hierdie studie was om die sitrus tristeza virus enkel-ras isolate van verskillende rasse te identifiseer, om hulle biologies te karakteriseer en volledige-genoom basisvolgordes te bepaal. Hierdie gekarakteriseerde CTV-isolate is verder gebruik in 'n komplementeringsstudie om moontlike sinergistiese interaksies te ondersoek wat stamgleuf beïnvloed. Vollengte virale genome van agt enkelras-isolate is tydens die studie bepaal. Twee kommersiële pomelo kultivars, 'Star Ruby' en 'Marsh', is in 'n glashuisproef gebruik om die vermoë van spesifieke rasse te evalueer om stamgleuf in enkel- of gemengde infeksies te veroorsaak. Evaluering oor vier jaar het getoon dat simptome uitdrukking van ligte rasse nie gelei het tot 'n veranderde simptome uitdrukking wanneer hul in kombinasie met mekaar voorgekom het nie. Dit is belangrik om aan te toon dat daar geen toevoegende effek op stamgleuf uitdrukking met veelvoudige isolate was nie. Relatiewe hoeveelhedsbepaling van die rasse in 'Marsh' en 'Star Ruby' plante het aangedui dat die individuele raskonsentrasies nie beduidend verander in kombinasie met die ander rasse nie. 'n Waardevolle ontdekking wat in hierdie projek gemaak is, was die karakterisering van twee variante van die T68-ras, afkomstig van dieselfde GFMS12-bron, maar met verskille in stamgleuf uitdrukking in pomelo's. Hierdie bevinding demonstreer die gelyktydige bestaan van strawwe en ligte variante van dieselfde ras in een bron en verskaf 'n verduideliking vir die vermoedelike ras segregasie gebeurtenis waargeneem in die GFMS12 kruisbeskerming bron, wat gelei het tot die staking van die bron vir gebruik as kruisbeskerming van pomelo's. Die karakterisering van hierdie variante sal verder help met die identifisering van die volgorde-bepaling van stamgleuf in pomelos. Daarbenewens is metagenomiese volgende-generasie volgordebepaling (NGS) toegepas vir diagnostiese doeleindes in sitrus en klein RNA (sRNA) is gebruik om die sitrus gasheer se reaksie op CTV- en viroïed-infeksie te ondersoek. Differentiële uitdrukking van plant-miRNAs is gewys tussen besmette en gesonde monsters.

Introduction

Citrus tristeza virus (CTV) is endemic in southern Africa and was responsible for significant losses within the local citrus industry (McClellan 1956). CTV was considered the most destructive disease of citrus worldwide prior to the spread of Huanglongbing (HLB) in the Americas (Moreno et al. 2008; Roistacher and Moreno 1991). The sour orange rootstock was widely used due to its resistance to *Phytophthora* root-rot and its superior horticultural performance, but it is the only rootstock susceptible to tristeza disease. Propagation of sweet orange, grapefruit or mandarin scions on sour orange, in the presence of certain CTV strains, causes quick decline or tristeza disease. The South African citrus industry experienced major constraints with this rootstock since the initiation of the industry (Marloth 1938) and moved to CTV tolerant rootstocks such as rough lemon and the trifoliolate hybrids. Despite the local industry's use of less sensitive rootstocks, the virus was still a limiting factor in the production of sensitive citrus types. CTV causes severe stem-pitting in grapefruit which leads to a gradual tree decline and is often associated with lower production and decrease in fruit size.

To minimize losses due to CTV the South African Citrus Improvement Scheme (CIS) implemented CTV cross-protection. This is a management strategy which uses mild CTV sources to mitigate the effect of severe CTV strains. The use of cross-protection has been mostly successful, but cases of cross-protection breakdown have occurred.

CTV is a complex of strains and strain variants (Harper 2013). This complexity and the influence of the citrus host on symptom expression complicates the elucidation of the mechanism of cross-protection. A strain specific exclusion mechanism was demonstrated and proposed as a possible mechanism for cross-protection (Folimonova et al. 2010), but it is uncertain whether this is the mechanism solely responsible for cross-protection.

This study explored the possibility of synergistic associations between certain CTV variants and identified severe and mild isolates of a CTV strain which will aid in the identification of the sequence determinants for stem-pitting of CTV in grapefruit.

Objectives

The aim of the study was to identify and characterise single-strain CTV isolates and variants of a single-strain to investigate their effect, singly or in combination on stem pitting expression in commercial grapefruit cultivars.

The following objectives were set out to achieve this aim:

- Develop diagnostic assays to detect all known CTV strains.
- Identify and/or isolate CTV single-strain isolates.
- Characterise single-strain isolates by full-genome sequence determination and comprehensive biological characterisation on a standardised host range.
- Develop strain-specific RT-qPCR assays for relative quantification of five CTV isolates.
- Evaluate symptom expression of strains, inoculated singly and in various combinations, in two commercial grapefruit cultivars over time.
- Investigate possible strain interactions by determining individual strain concentrations in the constructed populations by using RT-qPCR.
- Interrogate biological and genetic differences of two variants of one strain, derived from the same source plant.
- Evaluate the effect of variants of a strain on stem pitting in grapefruit, in a glasshouse trial and an existing field trial.
- Investigate the citrus host response to CTV with NGS.

Materials and methods

The project was conducted over a four-year period and materials and methods are fully reported in a PhD thesis and various publications. Brief descriptions or referencing to published methods are presented.

Characterisation of CTV sources

Identification of single strain isolates

Methods published in (Cook et al. 2016a; Cook et al. 2016b)

Biological characterisation of single infection sources

Seven CTV strains were inoculated to a full Garnsey biological indicator host range and rated for symptom severity (Cook 2019).

Full-genome sequence determination

Full-genome sequences of CTV isolates were determined by direct Sanger sequencing of overlapping genome regions (Cook 2019; Cook et al. 2016b).

Single-strain isolate evaluation in four grapefruit cultivars

Four single-strain CTV isolates, Maxi (VT strain), GFMS12-8 (T68 strain), LMS6-6 (HA16-5 strain) and B390-5 (RB strain, group 2) were evaluated in two pigmented; 'Star Ruby' and 'Nel Ruby', and two white; 'Marsh' and 'Duncan', grapefruit cultivars for their ability to induce stem pitting. Plants were cut-back at various intervals to evaluate stem pitting and one shoot of new growth was allowed to grow out. Plants were evaluated after four growth intervals (Cook 2019; Cook et al. 2016b).

Characterisation of single and mixed strain CTV infections in grapefruit

Pathogenicity assessment of single and mixed infections

Two commercial grapefruit cultivars, 'Star Ruby' and 'Marsh' were bud-grafted to Rough lemon rootstocks according to normal nursery practices. Five single-strain isolates were used for a population study which included 'Maxi' (VT strain), GFMS12-8 (T68 strain), LMS6-6 (HA16-5 strain), B389-1 (RB strain, group 1) and B389-4 (RB

strain, group 2). Thirty-one different combinations were inoculated with four replicates. Trees left un-inoculated served as controls. The scions were cut back four internodes above the last inoculation point three weeks' post-inoculation and one shoot of new growth was allowed to grow from the top bud. Transmission success was determined using strain-specific RT-PCRs. Plants were cut back at yearly intervals for stem-pitting evaluation after which one shoot of new growth was again allowed to grow. Bark was removed from the cut stems and evaluated for stem-pitting severity. At the final cut-back in 2018, samples from four seasons were simultaneously evaluated to ensure uniformity of the evaluation (Cook 2019).

Relative quantitation of CTV components

Strain-specific RT-PCR assays and a relative quantitative protocol was developed using three reference genes. Relative quantification of strain components of selected trial samples was done to investigate possible strain interactions, discernible by altered strain concentrations (Cook 2019).

T68 variants of GFMS12 differ in stem pitting severity in grapefruit

Biological evaluation of two T68 sub-isolates of GFMS12

The sub-isolation of GFMS12-8 from the original GFMS12 source was previously reported (van Vuuren et al. 2000) and GFMS12-1.3 was sub-isolated in this study by single aphid transmissions (Cook 2019). These sub-isolates, derived from different propagation sources of GFMS12, were comparatively tested on the 'Garnsey' host range. An additional set of Duncan seedlings were inoculated as a validation test and evaluated for stem pitting after four months. Isolate GFMS12-1.3 was also inoculated to 'Star Ruby' grapefruit on Rough lemon rootstocks. Limited plants were available and GFMS12-8 was not tested in parallel, but was tested extensively on this host previously.

Full-genome sequence determination of GFMS12-1.3 and GFMS12-8

Full-genome sequences for isolates GFMS12-8 and GFMS12-1.3 were obtained by amplifying overlapping genome segments and direct Sanger sequencing. Both sequences were verified by Next Generation Sequencing (NGS) at the Genetics Department of Stellenbosch University. Independent RNA extractions were performed for this purpose.

Diagnostic differentiation of T68 variants

Primers were designed to amplify a region encompassing five single nucleotide polymorphisms (SNPs) found within a short section of ORF p33 of the GFMS12 T68 variants. Amplicons of this region were sequenced to identify the dominant variants found in the GFMS12 source plants and sub-isolates (Cook 2019).

Bioinformatic pipeline for virus diagnostics

Methods published in (Jooste et al. 2017; Visser et al. 2016a)

Generation of small RNA profiles to investigate host response to CTV and bioinformatic comparison of NGS datasets generated from different nucleic acids extracts for diagnostic use

Small RNA was isolated from grapefruit plants inoculated with the T3 CTV strain and used for sRNA and total RNA NGS for both diagnostics and host response investigation. Healthy and CTV-infected grapefruit plants were used and viral status was confirmed with RT-PCRs. These plants, consisting of two cultivars, 'Star Ruby' and 'Marsh', each with 3 healthy and 3 infected replicates, were established in a greenhouse and allowed to develop a shoot length of around 35 cm before harvesting for RNA extraction. Total RNA was extracted from phloem material using a CTAB method. The quality of the total RNA was validated by means of BioAnalyzer analysis. For each sample a small RNA (sRNA) library (18-30 nts in length) and a paired-end ribo-depleted transcriptome library was sequenced to generate 10 million reads per library. After adapter trimming, high-quality sRNA reads were retained for analysis. Detailed methods published in (Visser et al. 2016b; Visser et al. 2017).

Results and discussion

Characterisation of CTV sources

Identification of single strain isolates.

CTV strain-specific detection assays were improved to facilitate detection of known strains and were used to identify single-strain isolates (Cook et al. 2016a). The single-strain status of eight isolates were confirmed and full-genome sequences determined. A Neighbor Network construction including these genomes and those available on GenBank, is presented in Figure 4.2.2.1 and displays the positioning of each genome in relation to other available CTV genomes, based on sequence interrelatedness.

Biological characterisation of single infection sources using the Garnsey biological indicator host range.

Seven single-strain isolates were evaluated on the 'Garnsey' hosts for their ability to induce symptoms over a period of seven months. Table 4.2.2.1 summarizes the reactions of each isolate on the various citrus hosts. Isolates, apart from T3-KB were evaluated as mild isolates.

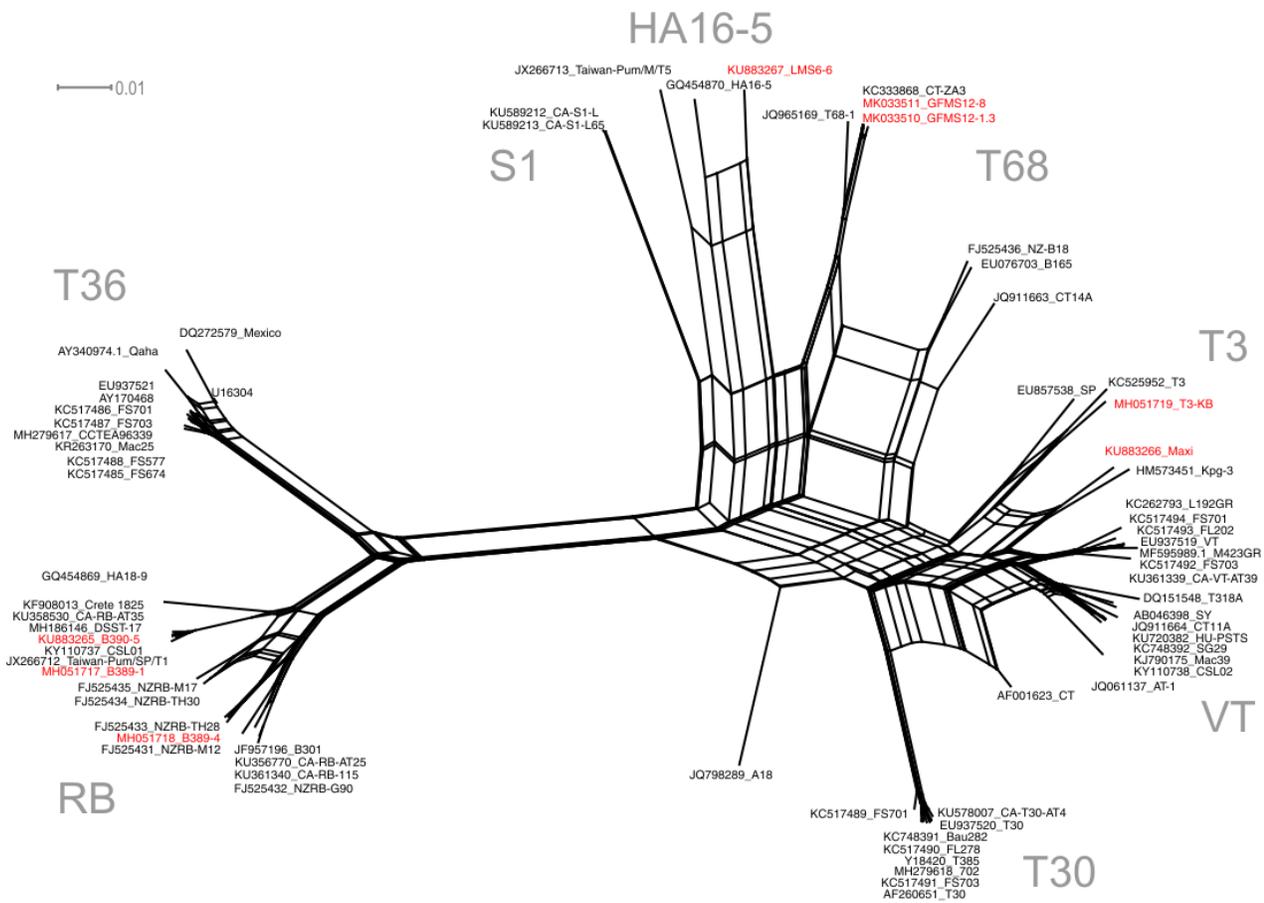


Figure 4.2.2.1. A Neighbor Network construction of complete genomes of citrus tristeza virus including genomes of this study indicated in red text; B389-1, B389-4, B390-5, GFMS12-8, Maxi, LMS6-6, T3-KB and GFMS12-1.3. Strain clusters are indicated in grey text.

Table 4.2.2.1. Virulence indexing of single-strain CTV isolates based the 'Garnsey' host range disease index per host and cumulative score (Σ DI).

Average DI per citrus host²

CTV Isolate	Strain	ML (x1)	SW/SO (x2)	SO (x3)	DGF (x4)	MV (x5)	ΣDI
Un-inoculated control	0	0	0	0	0	0
B389-1	RB	1.5	0	0.5	1.0	0	3.0
B389-4	RB	1	0	0.6	0	0	1.6
B390-5	RB	1.5	0	0	1.3	0	2.8
GFMS12-8	T68	2.2	0	0	2.9	0	5.1
Maxi	VT	3	0	0	1.9	0	4.9
LMS6-6	HA16-5	1.4	0	2	3.4	0	6.9
T3-KB	T3	2.9	5	4.1	8.3	0	20.4

^z Individual symptom were rated as 0 = no symptoms, 1 = mild, 2 = moderate and 3 = severe. The component scores for individual symptoms for each host were averaged and the composite score multiplied by the weight factor for each host as indicated. Symptoms scored per host were as follows: ML = 'Mexican' lime, vein clearing and stem-pitting (SP); SW/SO = sweet orange/sour orange, stunting/decline; SO = sour orange, seedling yellows (SY) and stunting; DGF = 'Duncan' grapefruit, SY, SP stunting; and MV = 'Madam Vinous', SP and stunting.

Single-strain isolate evaluation in four grapefruit cultivars

The virulence of four single-strain isolates was characterised on a grapefruit host range in a glasshouse trial. These isolates did not induce severe stem-pitting in any of the four grapefruit cultivars over a four year monitoring period. Detailed results are presented in (Cook 2019).

These comprehensively characterised CTV isolates are valuable tools as reference isolates and can be used in comparative analyses with other single-strain isolates to determine relative severity. These isolates are also useful for complementation studies to study population dynamics. Ultimately, the isolates can be used to identify components useful for cross-protection.

Characterisation of single and mixed strain CTV infections in grapefruit

Pathogenicity assessment of single and mixed infections

Symptom expression of single-strains, GFMS12-8 (T68), Maxi (VT) and LMS6-6 (HA16-5) was similar to findings of the previous trial. Mild to moderate stem pitting was observed with GFMS12-8 and Maxi, whereas LMS6-6 did not induce stem pitting. However, the two RB variants, B389-1 (RB2) and B389-4 (RB1) differed in expression from isolate B390-5 (RB2), used in the previous trial, and displayed mild to no stem pitting whereas isolate B390-5 was previously associated with mild to moderate stem pitting. The average stem pitting for different treatment combinations over four years are graphically presented in Figure 4.2.2.2 for 'Star Ruby' (A) and 'Marsh' (B). The purpose of this trial was to investigate possible strain interactions impacting stem pitting. However, no treatment combinations displayed statistically different stem pitting in either 'Star Ruby' or 'Marsh' compared to the single infection treatments. Detailed results are available in Cook 2019.

Relative quantitation of CTV components

Potential inter- and intra-strain interactions were investigated by relative quantitative determination of strain concentrations. Five isolates belonging to four CTV strains; T68, VT, HA16-5 and RB, including two isolates of the RB strain, were used.

Real-time, quantitative, strain-specific assays were developed and calibrated using a universal CTV assay which enabled the quantitative determination of strain components of the constructed populations. Overall, the strains were found within specific concentration ranges, which differed between the two hosts. Individual strain concentrations were not affected by the presence of heterologous strains in any of the strain combinations, but variants of the RB strain appeared to be in tension and were not detected simultaneously, suggesting spatial separation in the plant.

This study was not able to demonstrate strain interactions that impacted symptom expression in any additive manner. Complex mixtures of mild strains did not adversely affect the cultivars. Neither were inter-strain interactions, resulting in altered strain concentrations observed, but probable intra-strain segregation was noted. Details are reported in (Cook 2019).

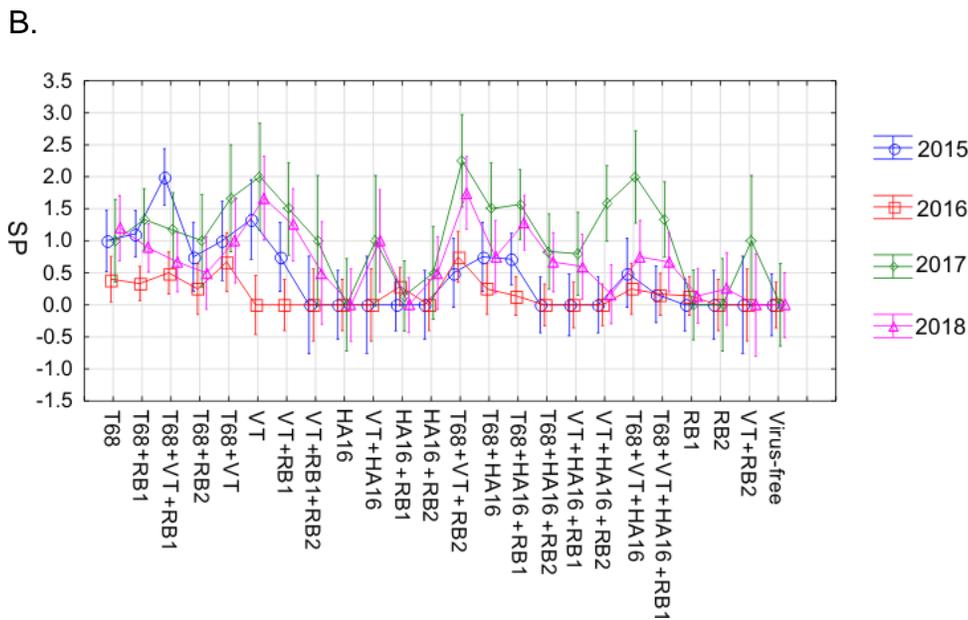
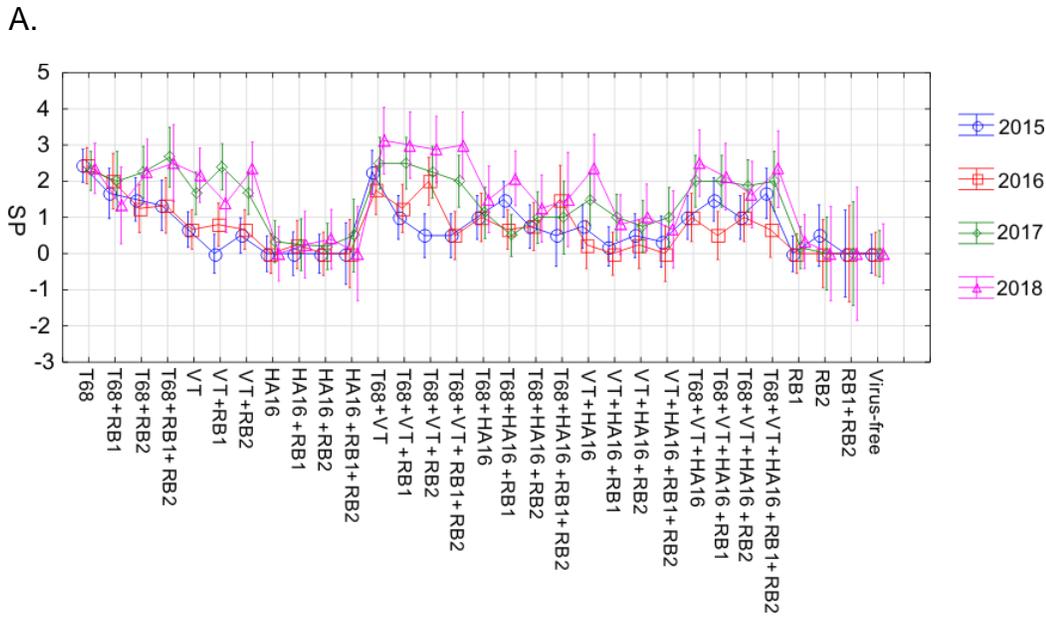


Figure 4.2.2.2. Average stem-pitting rates for ‘Star Ruby’ (A) and ‘Marsh’ (B) grapefruit inoculated with various combinations of four strains (two variants of the RB strain) in four consecutive yearly evaluations. Vertical bars denote 95% least squares confidence intervals for treatment means.

Rating scale: 0 = no stem-pitting, 1 = few (less than 10) shallow pits over the length of the cut stem, 2 = numerous (more than 10) shallow pits, 3 = few deep pits, 4 = frequent deep pits in close proximity to each other and covering a whole section of the stem, 5 = honeycomb-like pitting or porous wood pitting.

T68 variants of GFMS12 differ in stem pitting severity in grapefruit

Biological evaluation of two T68 sub-isolates of GFMS12

Both T68 sub-isolates GFMS12-1.3 and GFMS12-8 were rated as mild strains according to the 'Garnsey' disease index as they are both non-decline isolates that do not induce seedling yellows on either sour orange or 'Duncan' grapefruit, nor do they induce stem pitting on sweet orange. However, GFMS12-1.3 induced moderate to severe stem pitting on 'Duncan' grapefruit (Figure 4.2.2.3).

GFMS12-1.3 also induced severe stem pitting on 'Star Ruby', but was not tested in parallel with GFMS12-8 due to the limited availability of plants. However, the same stem pitting severity was not observed for GFMS12-8 in numerous evaluations in 'Star Ruby' grapefruit. A direct comparison of sub-isolates GFMS12-1.3 and GFMS12-8 was therefore only done in 'Duncan' grapefruit. These isolates are therefore differentiated by their relative stem pitting severity in this host.



Figure 4.2.2.3. Stem-pitting of sub-isolates GFMS12-1.3 and GFMS12-8. (A) GFMS12-1.3 and (B) GFMS12-8 in 'Duncan' grapefruit, four months after inoculation (C) GFMS12-1.3 in 'Star Ruby' grapefruit. *Arrows indicate less prominent stem pitting.*

Sequence analysis of T68 sub-isolates GFMS12-8 and GFMS12 1.3

Complete genome nucleotide sequences for sub-isolates GFMS12-1.3 and GFMS12-8 were determined and validated independently by NGS. Sequences were deposited in GenBank under the accession numbers MK033510 and MK033511, respectively. The GFMS12-8 (MK033511) sequence shares 99.7% sequence identity

with GFMS12-1.3 (MK033510). The type member of the strain, T68-1 (JQ965169), is closely related to both sequences and shares 97.3% nucleotide identity with them.

The differences between the genomes of GFMS12-8 and GFMS12-1.3 were interrogated and 39 SNPs were found over the length of the genome. Eighteen SNPs were present in open reading frame 1a (ORF1a) of which 10 were non-synonymous. Two synonymous SNPs were present in the RNA-dependent RNA polymerase (RdRp), five SNPs in ORF p65 of which four were non-synonymous, three non-synonymous SNPs in ORF p61, three synonymous SNPs in ORF p27, one synonymous and one non-synonymous SNP was found in the p20 and p23 ORF, respectively. A further six SNPs were observed in ORF p33 of which five were non-synonymous and these were in relatively close proximity to each other. This section was targeted as a diagnostic region to discriminate the T68 variants.

Pathogenicity determinants for stem pitting have not been defined, but the combined expression of ORFs p33, p18 and p13 were shown to impact stem pitting (Tatineni and Dawson 2012). It is noteworthy therefore, that five non-synonymous SNPs were found in ORF p33. It will be of value to further investigate this region as a stem pitting determinant of CTV.

Bioinformatic pipeline for virus diagnostics

An automated detection pipeline for citrus viruses based on the bioinformatic tool, EDNA (E-probe Diagnostic Nucleic Acid Analysis) was developed. A workflow was designed and software used to perform certain steps in a pipeline for virus diagnostics based on next-generation sequencing (NGS) data. Broadly the pipeline developed e-probes based on unique virus genomic regions and used these probes to screen NGS data for viral presence. In addition to establishing an e-probe based bioinformatics pipeline for NGS-based virus detection, a graphical user interface (GUI), named Truffle, which supports the pipeline was developed. The e-probes based pipeline along with its GUI, proved to be more computationally and time efficient. The software is available online at (<http://truffle.sourceforge.net>).

Generation of small RNA profiles to investigate host response to CTV and citrus dwarfing viroid

Small RNA analysis showed plant micro-RNAs (miRNAs) were differentially expressed when comparing healthy and infected plants. Differential expression analysis of the grapefruit transcriptome was performed in order to identify genes involved in the CTV-host interaction. The data from all samples were mapped against the genomes of seven different citrus-infecting viroid species and indicated the additional presence of citrus dwarfing viroid (CDVd) in the CTV infected samples. The symptoms observed as well as the variation in miRNA expression could therefore not be ascribed solely to CTV infection.

Bioinformatic comparison of NGS datasets generated from different nucleic acids extracts was used to determine the genome representation in the data. Two different data types sRNA and total RNA were compared. From the bioinformatic analysis results it was clear that ribo-depleted total RNA at 1 million reads had full genome representation for CTV.

Conclusion

Sequence determination of CTV genomes and diagnostic capabilities to distinguish strains, facilitate the linkage of biological expression to specific genetic components. Characterisation of CTV sources, used in cross-protection, was previously reliant on the sub-isolation and biological characterisation of components of these populations, but lacked the current strain-identification diagnostic capabilities. The maintenance of isolates and sub-isolates of GFMS12 provided the opportunity to understand the possible reasons for the failure of GFMS12 as a cross-protection source in grapefruit. This study demonstrated that the original GFMS12 source contains the T68 strain, but that variants were derived from this source, displaying different stem-pitting phenotypes. Two of these sub-isolates were characterised by full-genome sequence determination and biological evaluation on a citrus host range. Although sub-isolates GFMS12-1.3 and GFMS12-8 differed in stem-pitting severity in grapefruit,

sequence data suggests that minor sequence differences are responsible for the significant difference in symptom expression.

Results from this study support the notion that there was a segregation event in GFMS12 in 'Star Ruby' bud-wood source trees, which was probably effected by the host change. Over time, a severe variant, likely a minor component in the original source, became the dominant variant in the new host.

Further understanding of the host's response to CTV was investigated through transcriptome analyses and methodologies for virus diagnostics based on NGS were developed.

Future research

Identification of genetic differences between severe and mild CTV isolates of the same strain should be investigated further to identify genetic determinants of stem pitting.

Technology transfer

Post graduate qualification

Cook, G. (PhD). 2019. Characterization of citrus tristeza virus variants and their influence on symptom expression in grapefruit. University of Stellenbosch. <https://scholar.sun.ac.za/handle/10019.1/105724>

Scientific publications

Cook, G., van Vuuren, S. P., Breytenbach, J. H. J., Burger, J. T., and Maree, H. J. 2016a. Expanded Strain-Specific RT-PCR Assay for Differential Detection of Currently Known *Citrus Tristeza Virus* Strains: a Useful Screening Tool. *Journal of Phytopathology* 164:847-851.

Cook, G., van Vuuren, S. P., Breytenbach, J. H. J., Steyn, C., Burger, J. T., and Maree, H. J. 2016b. Characterization of Citrus tristeza virus Single-Variant Sources in Grapefruit in Greenhouse and Field Trials. *Plant Disease* 100:2251-2256.

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Visser, M., Burger, J. T., and Maree, H. J. 2016a. Targeted virus detection in next-generation sequencing data using an automated e-probe based approach. *Virology* 495:122-128.

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4.2.3 FINAL REPORT: Searching for a citrus tristeza virus source suitable for cross-protecting soft citrus

Project 968 (2004 - 2019) by J.H.J. Breytenbach, R. Clase and C. Steyn and G. Cook (CRI)

Summary

Citrus tristeza virus (CTV) was absent in some Clementine and mandarin propagation trees maintained at the Citrus Foundation Block (CFB). This inferred that the CTV source, applied for cross-protection, did not propagate or translocate well in a number of soft citrus cultivars. A glasshouse trial was conducted in 2006 to evaluate additional CTV sources in four soft citrus cultivars as potential cross-protection sources. Field trials were planned as a further evaluation of four CTV sources. Trees of two Clementine and two mandarin selections on Carrizo citrange rootstocks were prepared and pre-immunized with the CTV sources; CTVSC, SM47, SM48 and SM49. This project aimed to evaluate the influence of the CTV sources in two climatic regions. After the initial loss of both trials due to frost and poor drainage, the trials were redone and planted at Citrusdal in 2012 and at Burgersfort 2014. Tree canopy volumes were determined at the Citrusdal and Burgersfort trial sites, five and four years after planting, respectively. Tree canopy volumes at both sites differed greatly within treatments and numerous trees showed decline, which was not associated with the CTV treatments. Soil sample analysis from Burgersfort associated *Phytophthora* root-rot with declining trees. A similar random decline of trial trees was observed at the Citrusdal trial. The first harvests were poor and one mandarin cultivar dropped all fruit after splitting at one site, but this was cultivar related. CTV strain components of the sources were subsequently determined and the four sources were shown to be mixtures of various strains. This diminished the value of the trials as transmission of strain components to individual trial trees could not retrospectively be determined. These trials were terminated due to poor tree health at both sites, not associated with the CTV sources. Additionally, the uncertainty of the CTV strain components of each trial tree at planting negated further trial evaluation. CTV cross-protection trials will in future evaluate single-strain CTV sources to better understand the impact of individual strains.

Opsomming

CTV bronne wat gebruik was vir kruisbeskerming vir Clementine en mandarynbome het nie goed vermeerder of translokeer in 'n aantal kultivars wat in die Sitrusverbeteringsskema onderhou is nie en voortplantingsmateriaal van sekere kultivars is virusvry bevind. 'n Glashuis proef is gedurende 2006 gedoen om CTV bronne in sagte sitrus kultivars te evalueer as moontlike kruisbeskermings bronne. Die huidige veldproewe is 'n uitbreiding van die glashuis proef. Twee Clementine en twee mandaryn hibried seleksies is op Carrizo citrange onderstamme gekuleer en gepreïmmuniseer met die CTV bronne; CTVSC, SM47, SM48 en SM49. Dit was beoog om die invloed van die verskillende CTV bronne in twee klimaatstreke te evalueer. Na die aanvanklike verlies van beide proewe as gevolg van ryp en swak dreinerings, is hulle weer geplant. Bome is in Desember 2012 in Citrusdal hervestig en op die Burgersfort-perseel in 2014. Boomvolumes is onderskeidelik by Citrusdal en Burgersfort bepaal, vyf en vier jaar na plant. Boomvolumes by albei terreine het grootliks verskil binne behandelings en talle bome het agteruitgang getoon wat nie geassosieer was met die CTV-behandelings nie. Grondmonsteranalise by die Burgersfort perseel het die wortelvrot patogeen, *Phytophthora*, geassosieer met die swak bome. Hierdie lukraak agteruitgang van proefbome is ook in Citrusdal waargeneem. Die eerste oeste was swak en een mandarynkultivar by die Citrusdal perseel het al sy vrugte afgegooi nadat hulle gesplit het voor rypwording, maar dit was kultivar verwant. CTV-raskomponente van die bronne wat toegedien is, is bepaal nadat die proewe voorberei is. Daar is gevind dat die CTV bronne, mengsels van verskillende CTV rasse was. Dit verminder die waarde van die proewe aangesien die oordrag van raskomponente na individuele proefbome nie terugwerkend bepaal kan word nie en kan wissel. Hierdie proewe is beëindig weens swak boomgesondheid op beide terreine, wat nie met die CTV-bronne geassosieer was nie, asook die onsekerheid rondom die samestelling van die CTV komponente van die proefbome met plant. Enige verdere CTV-kruisbeskermingsproewe sal enkelras CTV bronne gebruik.

Introduction

Since the establishment of the South African citrus industry on tolerant rootstocks, it was generally accepted that citrus tristeza virus (CTV) has no effect on sweet oranges and mandarins. This situation can be partly ascribed to nurserymen who unwittingly applied cross-protection by selecting parent trees showing the best health and production. With the implementation of shoot-tip grafting, the virus-free material is vulnerable to infection by various

strains occurring in nature, which are transmitted by aphids. 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).

All citrus cultivars in the Southern African Citrus Improvement Scheme are rendered virus-free by shoot-tip grafting (de Lange, *et al.*, 1981). The abundance of the most effective aphid vector, *Toxoptera citricida* (Kirk.) will result in virus-free trees becoming naturally infected with various CTV strains (Schwarz, 1965) including virulent strains (Barkley, 1991; Calavan, *et al.*, 1980; Müller, *et al.*, 1968). It is therefore necessary to protect the virus-free plants from severe CTV strains by purposely infecting them with mild strains (de Lange, *et al.*, 1981; von Broembsen & Lee, 1988). The interaction of mild CTV sources with regard to cross-protection is specific with regard to biological activity (Müller & Costa, 1987; Van Vuuren, *et al.*, 1993) and therefore, mild CTV sources specifically for soft citrus cultivars should be identified. The LMS6 CTV source was approved as a pre-immunising source for all sweet oranges, Clementines and mandarins. During re-indexing of the Citrus Foundation Block mother trees in 2003 it was found that many of the Clementine and mandarin trees were free of CTV. This caused some concern as the budwood that was multiplied from these mother trees and supplied to the commercial nurseries, were virus-free and the trees would therefore be unprotected against natural CTV infection with severe strains. The virus-free condition was ascribed to the plastic screen that was used in the triozid protected house to protect the mother trees from the citrus black spot pathogen. It was speculated that the plastic raised the temperature inside the house and that the high temperature suppressed the CTV source. However, the grapefruit trees were still positive, but it was argued that grapefruit are grown in areas with high temperatures and therefore the sources that were used for pre-immunisation were adjusted to higher temperatures. This matter was further investigated by re-indexing the pre-immunised block in a tunnel at the ARC-ITSC Nelspruit. A large number of soft citrus cultivars tested negative and the thermotherapy theory was disproved. A glasshouse trial was initiated in 2006 to evaluate additional CTV sources. This trial was terminated at the end of the 2008/09 financial year where after a field trial was initiated for further evaluation of promising CTV sources. At the CIS Advisory Committee meeting in July 2006, it was decided that a change should be made to another CTV pre-immunizing source, compatible with mandarin types. Until a suitable CTV pre-immunizing source for soft citrus has been identified, the GFMS12 CTV source would be used for pre-immunization.

Stated objective

To evaluate various CTV sources in two different climatic regions, suitable for soft citrus production, as potential cross-protecting sources for soft citrus varieties.

Materials and methods

Two clementine selections, 'A' and 'B', and two mandarin hybrid selections, 'C' and 'D', on Carrizo citrange rootstock were grown according to normal nursery practices under aphid-free conditions. When the scions developed to approximately 5 mm in diameter, they were inoculated with the relevant CTV sources derived from sweet orange, that were previously evaluated in a glasshouse trial. The sources included CTVSC, SM47, SM48 and SM49. Tree performance with the various sources were compared to trees pre-immunised with GFMS12 (standard) and trees planted virus-free.

After confirmation of pre-immunisation by ELISA, the trees were planted at two localities in different climatic regions, both suitable for the production of soft citrus *viz.* Groblersdal in Mpumalanga and Citrusdal in the Western Cape. Trees at both trial sites were lost due to frost and poor drainage one year after planting. New trees had to be prepared and the trials re-planted at two new sites. The first trial was re-planted in the Citrusdal area during December 2012 and the second re-planted during spring 2014 at Burgersfort in Limpopo.

The effect of the CTV sources on growth, production and tree health was evaluated. 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).

Results and discussion

Citrusdal trial site:

The tree canopy volumes of trial trees at Citrusdal were determined five years after planting as presented in Table 4.2.3.1. No significant differences in growth were observed between treatments for any of the cultivars. However, tree volumes differed greatly within treatments and numerous trees in the trial showed decline, not associated with the CTV treatments.

The first fruit was harvested in 2017 at Citrusdal as presented in Table 4.2.3.3. Clementine 'A' trees with the SM48 CTV source had no fruit, but no differences were observed between other treatments. Mandarin hybrid 'C' dropped all fruit after splitting just before ripening. This phenomenon was reported previously for this cultivar.

Burgersfort trial site:

The tree canopy volumes of the trees at Burgersfort were determined four years after planting (Table 4.2.3.2). Similar to the trial in Citrusdal, tree volumes differed greatly within treatments and numerous trees showed decline, not associated with the CTV treatments. Trees showing decline were sampled and tested for CTV strain components. No correlation was found with the strains detected and the decline observed. Soil samples were taken to determine whether phytophthora root-rot could account for the decline. *Phytophthora spp.* were detected in most, but not all samples taken from soil surrounding the declining trees. This significantly impacted the evaluation of the effect of CTV treatments for this trial.

The first fruit was harvest in 2018 at the Burgersfort trial. None of the clementine 'A' trees fruited. No significant differences between treatments were obtained for clementine 'B' and mandarin hybrid 'C'. However, trees of mandarin hybrid 'D', planted virus free, yielded more fruit on average than the CTV treatments and the yield was significantly greater compared to treatment SM47, which had the lowest yield of all treatments. Trees of treatment SM47 were smaller on average, than other treatments, but this was most likely due to the phytophthora root-rot infections.

Table 4.2.3.1. Average tree canopy volumes of four soft citrus cultivars pre-immunised with different CTV sources five years after planting in Citrusdal, Western Cape.

Treatment	Canopy volume of scions (m ³)			
	Clementine A	Clementine B	Mandarin hybrid C	Mandarin hybrid D
CTVSC	6.4 a*	7.7 a	8.8 a	9.35 a
SM 47	5.4 a	6.9 a	8.5 a	11.5 a
SM 48	8.5 a	7.6 a	9.4 a	10.2 a
SM 49	6.9 a	9.3 a	9.4 a	11.5 a
GFMS 12	7.4 a	7.7 a	10.8 a	12.1 a
Virus-free	7.9 a	8.5 a	8.5 a	12.5 a

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

Table 4.2.3.2. Average tree canopy volumes of four soft citrus cultivars pre-immunised with different CTV sources 4 years after planting at Burgersfort, Limpopo.

Treatment	Canopy volume of scions (m ³)			
	Clementine A	Clementine B	Mandarin hybrid C	Mandarin hybrid D
CTVSC	5.4 a*	5.5 b	7.7 a	7.6 ab
SM 47	5.5 a	4.1 ab	7.8 a	4.9 a
SM 48	5.9 a	3.4 a	6.5 a	9.4 b
SM 49	5.7 a	4.4 ab	6.3 a	5.0 a
GFMS 12	5.9 a	5.1 ab	7.1 a	6.5 ab

Virus-free	4.6 a	4.2 ab	7.6 a	8.6 ab
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* Figures in each column followed by the same letter do not differ significantly at the 95% confidence level (Fisher's LSD).

Table 4.2.3.3. Average yield (kg per tree), of four soft citrus cultivars pre-immunised with different CTV sources 5 years after planting in Citrusdal, Western Cape.

Treatment	Yield kg/tree			
	Clementine A	Clementine B	Mandarin hybrid C	Mandarin hybrid D
CTVSC	29.6 b ^x	47.0 b	0 ^y	21.6 a
SM 47	30.3 b	39.3 ab	0	31.1 a
SM 48	0 a	30.0 a	0	24.8 a
SM 49	22.6 b	38.5 ab	0	22.3 a
GFMS 12	24.6 b	37.1 ab	0	26.0 a
Virus-free	35.0 b	33.0 ab	0	22.8 a

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

^y Mandarin hybrid 'C' dropped all fruit after splitting just before ripening.

Table 4.2.3.4. Average yield (kg per tree) of four soft citrus cultivars pre-immunised with different CTV sources 4 years after planting in Burgersfort, Limpopo.

Treatment	Yield (kg/tree)			
	Clementine A	Clementine B	Mandarin hybrid C	Mandarin hybrid D
CTVSC	0 ^x	26.1 a ^y	25.6 a	22.8 ab
SM 47	0	27.6 a	25.8 a	12.2 a
SM 48	0	19.7 a	17.6 a	14.3 ab
SM 49	0	28.7 a	27.3 a	14.6 ab
GFMS 12	0	29.0 a	16.3 a	17.2 ab
Virus-free	0	24.4 a	23.5 a	23.6 b

^x no fruit on Clementine 'A' trees

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

The CTV strain components of the original sources, used to inoculate the trial, were determined in project 1056, after commencement of this project. All the sources comprise mixtures of different CTV strains. This understanding complicated further evaluation of the field trials as transmission of the individual components to each trial tree was undermined. Additionally, tree decline at both sites, not associated with CTV treatments necessitated the termination of the trial.

Conclusion

This project aimed to test the influence of various CTV sources on soft citrus cultivars in two different climatic regions. After the initial loss of both trials due to frost and poor drainage, they were re-planted. Tree volumes at both sites differed greatly within treatments and numerous trees showed decline, not associated with the CTV treatments. Soil samples indicated phytophthora root-rot associated with declining trees. CTV strain components of the sources were determined after the trials commenced and it was determined that the CTV sources used were comprised of various strain components. This diminished the value of the trials as transmission of the various strain components to individual trial trees could not be retrospectively determined. The trials were terminated, primarily due to poor tree health at both sites, not associated with the CTV sources.

Future research

Field evaluation of single-strain CTV sources, in two soft citrus cultivars, are currently underway in project 1173.

Technology transfer

None

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4.2.4 FINAL REPORT: Training of dogs for the detection of African greening and Huanglongbing

Project 1184 (2017/8 – 2018/9) by G. Cook (CRI), J.H.J. Breytenbach (CRI), C. Steyn (CRI) and R. Clase (CRI).

Summary

A project to train a sniffer dog for early detection of infection of the African Greening pathogen, '*Candidatus* Liberibacter africanus (Laf) in citrus followed the use of sniffer dogs for detection of '*Candidatus* Liberibacter asiaticus (Las) infection in the USA. A dog was successfully imprinted to Laf positive plants and could differentiate between infected and uninfected plants with reasonable, but not sufficient accuracy. Indications of earlier detection capability compared to molecular tests was observed, but verification would require further trials. However, due to the decline in the working ability of the dog and the inability of the dog trainer to suitably address the problem, the project was discontinued.

Opsomming

'n Poging was aangewend om 'n snuffelhond op te lei vir vroeë opsporing van die Vergroeningspatogeen, '*Candidatus* Liberibacter africanus (Laf) in sitrus, wat gebaseer was op 'n benadering in die VSA waar honde vir die opsporing van 'Ca.' L. asiaticus (Las) gebruik is. 'n Hond is suksesvol opgelei om die reuk van Laf-

besmette sitrusplante uit te ken en kon onderskei tussen besmette en onbesmette plante met redelike, maar nie voldoende akkuraatheid nie. Vroeë opsporinge vermoë, in vergelyking met molekulêre toetse, is waargeneem, maar bevestiging sou verdere toetse vereis. As gevolg van die afname in die werksvermoë van die hond en die onvermoë van die honde-afrigter om die probleme reg te stel, is die projek gestaak.

Introduction

The potential incursion of '*Candidatus* Liberibacter asiaticus (Las), the causative agent of Huanglongbing (HLB), into southern Africa is an event that requires vigilance and strategic planning due to the destructive nature of this disease. Early pathogen detection and eradication is vital to prevent further dissemination by psyllid vectors. The presence of this pathogen and its associated vector, *Diaphorina citri*, (Liviidae) have both been reported on the African continent (Saponari et al. 2010; Shimwela et al. 2016), which significantly raises the threat level. Las can also be vectored by *Trioza erytaea*, the African citrus psyllid, although the simultaneous occurrence of this pest and HLB is less frequent due to their differing climatic adaptations. Apart from the natural spread of the pest and pathogen on the African continent, incursions through illegal smuggling of infected plant material from countries where the disease and pest are prevalent, is another pathway of entry. The Californian Department of Food and Agriculture implemented an intensive detection and eradication programme that has intercepted positive HLB trees in residential areas and has managed to contain the spread of HLB to commercial citrus. The success of such a programme relies on early detection before further dissemination.

Following the effective use of sniffer dogs for early detection of citrus canker in orchards in Florida, USA, researchers embarked on the use of sniffer dogs for early detection of HLB. The dogs were successfully imprinted to differentiate HLB-infected trees from healthy trees (Berger 2014). Tim Gottwald (USDA-ARS) leads this programme and reported that the dogs are able to detect positive trees, 2 weeks after HLB-inoculation, before detection is possible with PCR (personal communication). The dogs could however not differentiate Las from other citrus Liberibacter spp. viz. '*Ca.* L. americanus (Lam) and '*Ca.* L. africanus (Laf). Sub-species of Laf have additionally been identified in Rutaceous hosts in South Africa (Roberts et al. 2015) and one of these sub-species, '*Ca.* Liberibacter africanus subsp. clausenae' (LafCl), was recently detected on citrus in East Africa (Roberts et al. 2017). Despite this potential limitation, early detection of HLB using the dogs in hotter areas, not associated with African greening will be valuable. The dogs will also be useful for screening nurseries to ensure greening-free compliance.

The use of sniffer dogs for early detection of HLB is an important diagnostic tool to develop for screening orchards, nurseries and even residential areas. However, supportive diagnostics is required for Liberibacter identification given the diversity of the Liberibacters identified on the African continent and the dogs' reported inability to differentiate various Liberibacter species. CRI project 1157 is tasked to develop Liberibacter specific tests.

Stated objectives

- To train a dog(s) to detect infections of '*Candidatus* Liberibacter africanus' (Laf) and '*Ca.* L. asiaticus' (Las) in citrus.
- Sensitivity and specificity testing of the dog's capability for early detection compared to molecular diagnostics.

Materials and methods

Imprinting and initial training

The training technique used to imprint the dog with scents of Laf positive plants are proprietary and therefore not detailed. Imprinting was followed by training the dog to indicate infected material by sitting at positive samples. This was done by introducing the dog to both infected and un-infected material, first in an indoor environment in order to eliminate outdoor distractions. Once it was determined that the dog understood what was required, training progressed to outdoor environments, exposing the dog to distractions and differing environmental factors such as

wind. For the initial process ground scenting was done by placing infected and uninfected citrus leaves in sterile containers. These were placed randomly on a grassy area and then later in long veld grass and bushy areas.

Pot trial evaluations

Pot trials were used to evaluate the dog's detection capability and performance. For this, various citrus plants were graft-inoculated using a bark patch from a Laf positive source plant, originally obtained by single triozid transmission. Seedlings of 'Madam vinous' sweet orange, sour orange, 'Rough' lemon, 'Carrizo' citrange and 'Mexican' lime were inoculated. Approximately a month after inoculation the plants were tested for transmission success by conventional PCR using primers A2 and J5 of Hocquellet et al. (1999) and GoTaq G2 Hot Start Green Master Mix (Promega Corp.). Plants with positive graft-take that tested negative for Laf in the initial test were retested every few weeks thereafter to identify positive plants over time.

For the dog trials, each plant was allocated a number. Positive and un-inoculated plants were placed in a row consisting of 6-8 plants, approximately 2m apart. The dog was guided to scent each potted plant in a row which was recorded as a run. A positive indication was noted when the dog sat at the plant. A score sheet was used to record the plants used in each run and plant indicated as positive by the dog. Blind testing was done and the dog trainer was unaware of placement of positive samples. Plants were moved around to randomise the placement of samples. Every few runs different plants were introduced with the same approach. The percentage accuracy was determined by recording the number of indications on positive plants as well as indications on un-inoculated plants in a session.

Initially various citrus types were included in a single run, but this was seemingly a distraction to the dog. A change was made in the evaluation and only a single citrus type was included in a run. The training was also adapted to focus the training on a single citrus cultivar, Rough lemon.

Early detection trial

The early detection ability of the dog compared to molecular testing was done using 'Rough' lemon potted plants which were inoculated and comparatively tested over time. Molecular analysis was done by sampling three leaves from each inoculated plant for DNA extraction and real-time detection of Laf as described by Li et al. (2006) with amendments according to Roberts et al. (2015). Plants were tested three months after inoculation and plants that tested negative were retested at various intervals thereafter. The plants were tested a week prior to the dog trial.

Field trials

Initial transitioning to field trials included spiking a young, uninfected orchard by planting positive plants in the orchard rows. It however became obvious that the dog was detecting the different citrus types planted within the existing orchard rather than scenting for the pathogen. To bridge this problem, existing grapefruit plants, maintained in the glasshouse, were inoculated with Laf. Some plants were left un-inoculated as negative controls. These plants were approximately 1-2 m tall, maintained in 3L planting bags and were used to simulate a field trial. They were planted in prepared orchard rows in the bags. The plants were again removed from the field after the trial.

Results and discussion

Imprinting and training to indicate infected plants

A young female German shepherd cross was acquired and imprinting to African Greening positive plants was achieved.

Pot trial evaluations

The dog's accuracy to indicate Laf positive plants was tested in pot trials. The initial trials from February to May of 2018 are recorded in Table 4.2.4.1. The accuracy was inconsistent. Initially, various citrus types were included in a run, but this was seemingly a distraction to the dog. A change was made in the evaluation and only a single citrus type was used in a run. The rationale was that an orchard normally comprises a uniform citrus type and the dog would be exposed to a single cultivar in an orchard.

The additional change to use only 'Rough' lemon in the training did improve the accuracy as recorded in Table 4.2.4.2. These results are however not a true reflection on the dog's ability to differentiate infected from uninfected plants as indications on un-infected plants were often due to ill-discipline of the dog rather than an incorrect scenting. The discipline remained a problem and was not corrected over time. This influenced the reliability of the use of the dog for detection. Accuracy varied from 82-100% with false alerts ranging from 0-19%. The Scientific Working Group of Dogs and Orthogonal Detector Guidelines (SWGDOG) specify an acceptable norm as 90% accuracy for positive indications with a maximum false alert rate of 10% (Mendel et al. 2018).

Early detection trial

The first early detection trial was conducted in October 2018 to test the dog's ability to indicate on 42 'Rough' lemon plants inoculated with Laf. Eight un-inoculated plants were used as negative controls. Molecular tests, conducted a week prior, only detected Laf in 2 plants using real-time detection. The dog indicated on all inoculated plants, but also indicated on un-inoculated plants. There were 68 positive indications on Laf inoculated plants out of a total of 81 placements and 57 indications on un-inoculated plants out of 308 placements. Assuming all inoculated plants would develop systemic infections, the dog was 95% accurate and detected positive samples earlier than molecular tests. In December 2018 a further 23 plants tested positive with real-time PCR, confirming the dog's early indication on these plants.

A second trial was done in December using the same plants. The dog again indicated on all inoculated plants, but also indicated on a few un-inoculated plants. This related to a 90% accuracy if all plants developed systemic infections over time. In February 2019 another four plants tested positive with molecular tests, confirming the dog's early indication on these plants. However, at the end of February, 13 plants, positively indicated on by the dog, were still unconfirmed by molecular tests. Indications of earlier detection capability compared to molecular testing were therefore observed, but verification would require further trials.

Field trials

Initial attempts to transition the dog to the field work was done by planting positive and negative plants in in existing young orchards. It however became obvious that the dog was indicating on the different citrus types, planted within the existing orchard, rather than the greening infected plants.

To bridge this problem, existing, grapefruit plants were inoculated with Laf and were used to simulate a field trial. They were planted in prepared orchard rows in the planting bags and removed again after the trial. A preliminary trial using these trees showed promise with an 81% detection accuracy. Subsequent trials with these plants in December 2018 and January, February and April of 2019 were unsuccessful.

The dog's working ability declined over time and it was the opinion of the CRI team that erratic handling changes brought in by the trainer was confusing the dog. Concerns regarding handling were discussed at numerous meetings. The trainer did not appropriately address problems and the dog's working ability did not improve.

Table 4.2.4.1. Results for the first evaluations of the dog's detection accuracy using pot trials. Runs included positive and negative plants of either a single citrus type or a mix of citrus types. The percentage accuracy was determined by the number of indications on positive plants as well as indications on negative plants in a session.

Date	Citrus type	No. of runs	Indications on Laf positive plants	Indications on un-inoculated (Laf negative plants)	Percentage accuracy (%)
06-02-2018	Various ^x	11	28/36	28/57	61
07-02-2018	Various	67	91/101	59/376	86
07-02-2018	Various	32	27/34	29/170	82
08-02-2018	Various	26	40/54	10/130	87
27-03-2018	Various	20	37/56	21/111	76
28-03-2018	Various	40	86/92	19/107	87

28-03-2018	Rough Lemon	13	15/16	1/12	93
28-03-2018	Carrizo citrange	6	23/24	0/18	98
28-03-2018	Rough Lemon	9	34/36	9/27	83
28-03-2018	Carrizo citrange	4	16/16	1/12	96
28-03-2018	Various	9	21/23	6/23	83
29-03-2018	Carrizo citrange	10	22/24	6/30	85
29-03-2018	Rough Lemon	14	36/46	8/38	79
29-03-2018	Sour Orange	6	11/12	3/12	83
29-03-2018	Sweet orange	11	23/38	4/22	68
12-04-2018	Various	20	65/65	8/95	95
19-04-2018	Various	20	50/61	6/99	89
15-05-2018	Various	22	45/48	28/84	77
16-05-2018	Rough Lemon	26	54/60	18/78	83

^x various combinations of citrus types used in runs including lemon, trifoliate orange, sour orange and sweet orange.

Table 4.2.4.2. Results for accuracy pot trials using only 'Rough' lemon inoculated and un-inoculated plants after training adjustments.

Date	Citrus type	No. of runs	Indications on Laf positive plants	Indications on un-inoculated (Laf negative plants)	Percentage accuracy (%)
09-07-2019	Rough Lemon	5	10/10	0/30	100
09-07-2019	Carrizo citrange	5	4/4	1/22	96
16-10-2018	Rough Lemon	20	41/49	7/111	90
17-10-2018	Rough Lemon	57	68/81	57/308	82
17-10-2018	Rough Lemon	34	95/98	12/175	95
11-12-2018	Rough Lemon	10	18/20	6/59	90
12-12-2018	Rough Lemon	23	58/62	15/124	90
16-04-2018	Rough Lemon	30	77/84	11/126	91

The possibility of imprinting a second dog on '*Candidatus* Liberibacter asiaticus (Las) was also investigated and a request to import infected material to be maintained in a quarantine facility was submitted to DAFF. The request was declined and the acquisition and training of a second dog did not proceed.

Conclusion

A dog was successfully imprinted to African Greening positive plants and could differentiate between infected and uninfected plants with reasonable, but not sufficient accuracy. Indications of earlier detection capability compared to molecular tests was observed, but verifications required further trials. Due to the decline in the working ability of the dog the project was discontinued.

Future research

No further research is planned for sniffer dog detection of African Greening.

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4.2.5 **PROGRESS REPORT: Field evaluation of three single-strain CTV isolates on Navel and Soft Citrus cultivars**

Project 1173 (2017/8-2022/3) by G. Cook, J.H.J. Breytenbach, C. Steyn and R. Clase (CRI)

Summary

Single-strain citrus tristeza virus (CTV) isolates were characterized and evaluated in various industry cultivars in a glasshouse trial (project 1056). No detrimental symptoms were associated with these isolates. Selected cultivars and treatments of the trial were planted at various sites to evaluate field performance and to monitor the CTV translocation to new growth of the trees. This is done with the aim of testing the suitability of these isolates for use in CTV clone construction (project 1160), in addition to evaluating them as potential cross-protection sources. Previous grapefruit field trials indicated that single-strain CTV sources were associated with better horticultural performance compared to the multi-strain sources (project 742). Grapefruit and Valencia trees were planted in the Northern Cape, Navels in Mpumalanga and a Clementine and a Mandarin hybrid were planted in Limpopo Province. The Northern Cape trials were terminated as numerous trial trees were lost due to a lack of water shortly after planting. Canopy volumes of the Navels, Clementine and Mandarin hybrid trial trees were determined, one year after planting. No significant differences were observed between treatments of each cultivar. Translocation of the CTV isolates in various cultivars was tested by sampling the flush or young growth of the trial trees and testing for the presence of the various CTV strains. Good translocation of the three CTV isolates was observed in all the cultivars. The first fruits will be harvested in 2019.

Opsomming

Enkel-ras Citrus tristeza virus (CTV) isolate is gekarakteriseer en geëvalueer in verskeie bedryfskultivars in 'n glashuis proef (projek 1056). Geen nadelige simptome was geassosieer met hierdie isolate nie. Geselekteerde kultivars en behandelings van hierdie proef is in verskeie proefpersele geplant om veldprestasie en CTV-translokasie in die plante te evalueer. Die doel hiermee is om die verskeie isolate te evalueer as kandidate vir gebruik in CTV-kloonkonstruksie in projek 1160, asook om hulle te evalueer as moontlike kruisbeskermingsbronne. Vorige pomelo proewe het aangedui dat enkelras CTV bronne beter presteer as CTV bronne bestaande uit ras mengsels (projek 742). Dit is dus van waarde om die enkel-ras CTV bronne as potensiële kruisbeskermingsbronne te evalueer. Die pomelo en Valencia-bome is in die Noord-Kaap geplant en Navels in Mpumalanga. 'n Clementine- en 'n Mandaryn proef is in Limpopo geplant. Die Kakamas-proef is beëindig

aangesien talle proefbome gevrek het weens 'n tekort aan water kort na plant. Boomvolumes van die Navels-, Clementine- en Mandaryn-proefbome is bepaal, een jaar na plant. Geen beduidende verskille is waargeneem tussen behandelings van elke kultivar nie. Jong groei van proefbome is getoets vir die teenwoordigheid van die verskillende CTV-rasse met die doel om die translokasie vermoë van elke isolaat te bepaal. Goeie translokasie van die drie isolate is waargeneem in al die kultivars. Die eerste vrugte word in 2019 geoes.

4.2.6 **PROGRESS REPORT: Comparison of shoot tip grafted citrus with field-cut (old clone) material** Project 1074 (2013 - 2023) by G. Cook, J.H.J. Breytenbach, R. Clase and C. Steyn (CRI)

Summary

Sectors in the citrus industry assert that some cultivars are more profitable when trees are made from field-cut material compared to that supplied by the Citrus Improvement Scheme (CIS). This is investigated in a trial using two navels and one Valencia cultivar for which such claims were made. Graft transmissible pathogens, including viroids and viruses, are removed by shoot tip grafting from accessions submitted to the CIS. Thereafter an approved citrus tristeza virus (CTV) source is introduced to each accession within the cross-protection programme. Field trees can however acquire a range of graft transmissible pathogens over time, either by means of insect vector transmission or mechanically during routine orchard practices. The objective of this study is to compare tree health, fruit characteristics and production of CIS material with that of field-cut material. Budwood was collected from original field 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. Field sources were shown to contain various populations of CTV strains and citrus viroids. A field trial was planted at Burgersfort in 2016 and a second set of trial trees was kept in an insect-protected tunnel as a duplicate trial, as a second trial site could not be found. Each trial tree was tested to determine pathogen status and transmission efficiency of the various components of each source. Severe stem-pitting on sweet orange is uncommon in South African orchards due to the mild CTV sources applied in the cross-protection programme, however, field-derived sources used in the trial showed mild to severe stem-pitting on the young trees. After two and a half years in the field, significant differences in tree growth were observed between treatments. Reduced rootstock and scion diameters were associated with field-cut material of all three cultivars. These initial trial results demonstrate increased growth of CFB-derived material compared to field-cut material. The first fruit will be harvested from this trial in 2019.

Opsomming

Sektore binne die sitrusbedryf beweer dat sommige kultivars meer winsgewend is wanneer bome gemaak word van veld-gesnyde materiaal in vergelyking met dié wat deur die Sitrus Verbeteringskema (SVS) verskaf word. Die aanname word ondersoek in 'n proef wat twee navel en een Valencia-kultivar insluit, waarvoor sulke eise gemaak word. Oordraagbare patogene, insluitend viroïede en virusse, word verwyder deur middel van groeipuntenting en is daarna geïnkuleer met 'n goedgekeurde citrus tristeza virus (CTV) bron vir kruisbeskerming binne die SVS. Veldbome kan egter oor 'n tydperk 'n verskeidenheid ent-oordraagbare patogene optel, deur middel van insekvektoroordraging of meganies tydens roetine-boordpraktyke. Die doel van hierdie studie is om boomgesondheid, vrugteienskappe en produksie van SVS materiaal te vergelyk met dié van veld-gesnyde materiaal. 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. Dit was bewys dat veldbronne verskeie CTV-rasse en sitrusviroïede bevat. 'n Veldproef is in 2016 by Burgersfort geplant en 'n tweede stel proefbome is in 'n insekbeskermdede tunnel aangehou as 'n glashuisproef omdat 'n tweede proefperseel nie gevind kon word nie. Elke proefboom is getoets om die patoogeenstatus asook die transmissiesukses van die verskillende komponente van elke bron te bepaal. Stamgleuf op soet lemoene word nie algemeen in Suid-Afrika waargeneem nie as gevolg van die matige CTV-preïmmuniseringsbron wat in die kruisbeskerminsprogram gebruik word, maar die veldbronne bevat CTV-komponente wat ligte tot strawwe stamgleuf veroorsaak het op die jong proefbome. Die proefbome is al twee en

'n half jaar lank in die veld en alreeds is daar beduidende verskille in boomgroei waargeneem. Verminderde onderstam- en bostam- deursnee is geassosieer met veld-gesnyde materiaal van al drie kultivars. Hierdie aanvanklike proefresultate toon verbeterde groei met SVS-afkomstige materiaal in vergelyking met veld gesnyde materiaal. Die eerste oes word in 2019 verwag.

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

Project 1155 (2016/7 – 2024/5) by G. Cook, J.H.J. Breytenbach, C. Steyn, R. Clase and J. Joubert (CRI)

Summary

The choice of rootstock is an important consideration for the establishment of a citrus orchard. Apart from climate and soil suitability, rootstock selection should include considerations for resistance or tolerance to diseases and pests. Viroids are graft-transmissible agents which can induce a range of symptoms dependent on the sensitivity of the rootstock and scion. They are also mechanically transmitted by contaminated cutting tools and can unintentionally be introduced to and spread in nurseries and orchards. Viroids are seldom problematic if disease-free, certified bud-wood is used. Apart from the diseases, Exocortis and Cachexia, viroids can also induce symptoms such as bark cracking and stunting. There is limited experience regarding the effect of viroids on hybrid rootstocks introduced in the past two decades, including new selections from the US. Plant preparation for a field trial is underway to test the sensitivity of these newer commercial or potentially commercial rootstocks to citrus dwarfing viroid and hop stunt viroid-IIa. The trial is behind schedule due to seed acquisition delays. The trial trees were inoculated and the transmission success to each trial plant was tested by RT-PCR. Despite successful graft take, transmission was not optimal and the plants were re-inoculated. Australian trifoliolate rootstock seedlings were also budded with the Midnight Valencia scion, but bud growth was poor on this rootstock and sufficient plants are not available for inclusion of this rootstock in the trial. Each trial plant will be re-tested to confirm transmission, prior to planting in the spring of 2019.

Opsomming

Die keuse van onderstam is belangrik in die vestiging van 'n sitrus boord. Benewens klimaats- en grondgeskiktheid moet hierdie oorwegings weerstand of verdraagsaamheid teenoor siektes en plaë insluit. Viroïede is oordraagbare entiteite wat verskeie simptome kan veroorsaak op sensitiewe bo- en onderstamme. Hulle is selde problematies as siektevrye, gesertifiseerde enthout gebruik word, maar weens maklike meganiese oordraging deur snygereedskap en besmette enthout, word viroïdes soms, per ongeluk, in kwekerye en boorde versprei. Afgesien van die sieketoestande, 'Exocortis' en 'Cachexia', kan viroïede ook simptome soos baskraak en verdwering veroorsaak. Daar is beperkte ervaring met betrekking tot die effek van viroïede op onderstamme wat die afgelope twee dekades bekendgestel is, insluitende nuwe onderstamme afkomsitg uit die VSA. '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. Die proefvoorbereiding was vertraag as gevolg van saadvrystelling uit kwarantyn. Die proefbome was geinokuleer met die viroïed-behandelings en die oordragsukses na elke proefplant was deur RT-PCR getoets. Viroïed oordrag was nie optimaal nie en die plante was weer geinokuleer. Die Australiese trifoliaat onderstam saailinge is ook met die Midnight Valencia bostam geokuleer, maar groei was sleg op hierdie onderstam en voldoende plante van hierdie onderstam is nie beskikbaar om in te sluit in die proef nie. Elke proefplant sal weer getoets word om viroïed oordraging te bevestiging voor proef plant in die lente van 2019.

4.2.8 **PROGRESS REPORT: Validation of primer regions used for the differentiation of Asian HLB, African greening and its subspecies.**

Project 1200 (1 April 2018 – 31 March 2019) by Ronel Roberts (ARC-PHP) and Glynnis Cook (CRI)

Summary

Three *Liberibacter* species and two psyllid vectors are associated with citrus on the African continent. In East and Southern Africa citrus is affected by the African Greening disease, associated with the bacterial pathogen '*Candidatus Liberibacter africanus*' (Laf). However, '*Ca. L. asiaticus*' (Las), the bacterium associated with Huanglongbing (HLB), was identified in northern Ethiopia in 2010 and later erroneously reported from Uganda and Tanzania. '*Ca. L. africanus* subsp. *clausenae*' (LafCl), a bacterium first described on an indigenous Rutaceae species in South Africa, was misdiagnosed as Las in citrus in Uganda and Tanzania due to a non-specific diagnostic assay. *Trioza erytreae*, the vector of Laf, is present in East and Southern Africa. Additionally, in 2016, *Diaphorina citri*, the natural vector of Las, was reported in Tanzania and later also in Kenya and Zanzibar. The presence of both Las and *D. citri* in East Africa and the probable dispersal on the continent is of concern for southern African citrus production. Early detection of Las is critical for rapid response to an incursion. However, the presence of three *Liberibacter* species occurring in citrus in Africa requires a diagnostic assay capable of differentiating the species, preferably in a single assay. The ribonucleotide reductase β -subunit (*nrdB*) gene region is being investigated as a target region for development of a diagnostic test to detect and differentiate the *Liberibacter* species. This genome region was sequenced from Laf isolates originating from different geographical regions. A single sample showed a three base pair difference from the consensus sequence of the other Laf samples. This sequence variation within Laf in the *nrdB* region will be confirmed by a re-analysis of more samples. Nucleotide sequences of the *nrdB* gene region were determined for three Laf subspecies. The LafC, LafV and LafCl sequences share 89%, 88% and 89% sequence identity with Laf, 75%, 75% and 76% with Las and 79%, 78% and 80% with Lam, respectively. A Laf-specific High Resolution Melt (HRM) assay, targeting the *nrdB* region, was developed and tested. Laf and two Laf subspecies were amplified, but not Las and Lam. HRM assays evaluated for the development of a single assay to detect and differentiate Laf and Las show promise and further optimisation is underway.

Opsomming

Drie *Liberibacter* spesies en twee bladvloei vektore word geassosieër met sitrus op die Afrika kontinent. In Oos en Suider Afrika word sitrus geaffekteer deur Vergroening siekte, wat geassosieër word met die bakteriële patogeen, '*Candidatus Liberibacter africanus*' (Laf). '*Ca. L. asiaticus*' (Las), die bakteriële geassosieër met Huanglongbing (HLB), was geïdentifiseer in die noorde van Ethiopia in 2010 en later verkeerdelik gerapporteer in Uganda en Tanzania. '*Ca. L. africanus* subsp. *clausenae*' (LafCl), 'n bakteriële oorspronklik geïdentifiseer vanaf 'n inheemse Rutaceae spesie in Suid Afrika, was verkeerdelik gediagnoseer as Las in sitrus in Uganda en Tanzania as gevolg van 'n nie-spesifieke diagnostiese toets. *Trioza erytreae*, die vektor van Laf, is teenwoordig in Oos en Suider Afrika. In 2016 is *Diaphorina citri*, die natuurlike vektor van Las gerapporteer in Tanzania en later ook in Kenya en Zanzibar. Die teenwoordigheid van beide Las en *D. citri* in Oos Afrika, en die moontlike verspreiding op die kontinent, is 'n bedreiging vir sitrus produksie in suider Afrika. Vroeë opsporing van Las is krities vir spoedige reaksie om verspreiding van die patogeen te verhoed. Die teenwoordigheid van drie *Liberibacter* spesies op sitrus in Afrika beteken dat 'n diagnostiese toets in plek moet wees wat die verskillende *Liberibacter* spesies kan onderskei, verkieslik in 'n enkele toets. Die 'ribonucleotide reductase β -subunit' (*nrdB*) geen word tans ondersoek as 'n teiken vir die ontwikkeling van so 'n diagnostiese toets. Hierdie genoom area se nukleotied volgorde was bepaal vir Laf isolate afkomstig van verskillende geografiese streke. 'n Enkele monster het 'n drie nukleotied basispaar verskil getoon in vergelyking met ander isolate. Hierdie nukleotied variasie binne die *nrdB* geen van Laf sal bevestig word deur die analise van verdere monsters. Nukleotied volgordes van die *nrdB* geen was bepaal vir die Laf subspecies. Die LafC, LafV en LafCl nukleotied volgorde deel 89%, 88% en 89% nukleotied identiteit met Laf, 75%, 75% en 76% met Las en 79%, 78% en 80% met Lam, onderskeidelik. 'n Laf-spesifieke 'Hoë Resolusie Smelt' (HRS) toets, geteiken op die *nrdB* geen, was ontwikkel en geëvalueer. Laf en twee Laf subspecies was hiermee opgespoor, maar nie Las en Lam nie. Hierdie HRS toets lyk belowend as deel van die ontwikkelings proses vir die uiteindelijke doel vir Laf en Las opsporing en onderskeiding met 'n enkele diagnostiese toets. Verdere ontwikkeling word gedoen.

4.2.9 PROGRESS REPORT: Application of CTV infectious clones to combat HLB

Project 1160 (2016/17 – 2019/2020) by R Bester (SU), D Aldrich (SU), G. Cook (CRI), Kobus Breytenbach (CRI), Prof Johan T Burger (SU), Prof William O Dawson (University of Florida, USA), H.J. Maree (SU)

Summary

The confirmed presence of both '*Candidatus*' Liberibacter asiaticus (CLAs), and *Diaphorina citri* in East Africa, requires a proactive approach from the South African citrus industry to prepare for the eventual incursion of HLB. The aim of this project is to establish a suite of citrus tristeza virus (CTV) infectious clones with a range of 'payloads' that would form part of a management strategy to contain HLB and limit its impact. CTV infectious clones of genotype T36 were imported from collaborator Prof W.O. Dawson (Florida University) at the start of the project. Protocols for infiltration of these clones into *N. benthamiana* were optimised. Recombinant virions were then purified from systemically infected *N. benthamiana* plants by ultracentrifugation (at University of Cape Town) and citrus seedlings infected with the partially purified virions. A strategy was designed to convert these clones from a T36 genotype into the local RB (asymptomatic) and T3 (symptomatic) genotypes. The RB genotype based infectious clone will form the basis for a delivery vector whereas the T3 based infectious clone will be used for research purposes only. A cloning strategy was designed that uses intermediate plasmids that contain large portions of the respective CTV genotype. Some of these portions have been transferred to the clone and intermediate hybrids are replication competent, which provides promise that the final clone will be infectious. This task is ongoing. Additionally, a dual reporter infectious clone based on the T36 clone (Dawson) that expresses GFP and also contains a PDS silencing cassette was constructed and is currently being evaluated in citrus (silencing is already visible). The purpose of this clone is to evaluate the efficacy of a CTV-based vector as an expression and/or silencing vector. This clone will be used as a test platform to screen different payloads while the RB clone is still under construction. Several deletion mutant and hybrid clones have also been constructed to study the underlying mechanisms in the formation of stem piths, these clones are currently being evaluated in citrus to determine if they can replicate and spread systemically, before they will be used in various experiments.

Opsomming

Die bevestigde voorkoms van '*Candidatus*' Liberibacter asiaticus (CLAs), en *Diaphorina citri* in Oos-Afrika vereis 'n pro-aktiewe strategie van die Suid-Afrikaanse sitrusbedryf om voor te berei vir die uiteindelijke inval van Asiatiese vergroening (HLB). Die doel van hierdie projek is om 'n paneel van CTV infektiewe klone te vestig met 'n verskeidenheid 'payloads' wat sal deel uitmaak van 'n beheerstrategie om die impak van HLB te beperk. Infektiewe klone van die T36 genotipe is ingevoer vanaf ons medewerker, Prof W.O. Dawson (Florida University). Protokolle vir die infiltrasie van hierdie klone in *N. benthamiana* is geoptimeer. Rekombinante viruspartikels is daarna gesuiwer vanuit sistemies-geïnfekteerde *N. benthamiana* plante deur middel van ultra-sentrifugasie (by die Universiteit van Kaapstad). Sitrusaailinge is intussen suksesvol besmet met die deels-gesuiwerde viruspartikels. Strategieë is ontwerp om die T36-klone om te skakel na plaaslike RB (asimptomaties) en T3 (simptomaties) genotipes. Die RB-gebaseerde infektiewe kloon sal die basis vorm van die aflewingsvektor, terwyl die T3-gebaseerde infektiewe kloon slegs gebruik sal word vir navorsingsdoeleindes. Die kloneringsstrategie wat ontwerp is maak gebruik van intermediêre plasmiede om groot gedeeltes van die betrokke CTV genotipe se genoom te bevat. Van hierdie genoomdele is reeds vervang in die T36-templaatkloon en die intermediêre hibriede kon steeds repliseer, wat 'n belowende aanduiding is dat die finale kloon infektief sal wees. Daar is ook 'n dubbel-rapporteurder infektiewe kloon gebou, gebaseer op die T36-kloon (Dawson), wat beide GFP uitdruk asook 'n PDS 'silencing cassette' bevat. Hierdie kloon word tans geëvalueer in sitrus ("silencing" kan reeds gesien word). Die doel van die kloon is om die doeltreffendheid van 'n CTV-gebaseerde vektor te bepaal in terme van proteïen uitdrukking en/of 'silencing'. Hierdie kloon sal kan dien as 'n toetsplatform om die verskillende 'payloads' te sif terwyl die RB-kloon gefinaliseer word. Verskeie ORF-uitwissings- en ORF-vervangingsklone is ook gebou om die onderliggende meganismes van die vorming van stamgleuf te ondersoek. Hierdie klone word tans in sitrus

geëvalueer om hul repliserings- en verspreidingsvermoëns te bevestig, voordat hulle toegepas sal word in verskeie eksperimente.

4.3 **PROGRAMME: PREHARVEST DISEASES**

Programme coordinator: J Van Niekerk (CRI)

4.3.1 **Programme summary**

Within the preharvest disease programme the research focus is soilborne diseases of citrus and fruit and foliar diseases, including Citrus Blackspot, of citrus. The research focusses on finding alternative, softer management options, studies into the epidemiology of the different pathogens and optimizing the control of these pathogens through better application technology or better chemical control programmes.

Projects 762 and 1030 are specifically aimed at finding alternative means of control for *Phytophthora* and citrus nematode. Data have been recorded in project 762 since 2011. It is becoming clear that the different pre-plant soil fumigation treatments, specifically the 1,3 dichloropropene and metham sodium treatments, have caused the trees in these treatments to be taller with thicker trunks compared to the other treatments and the untreated control. Within project 1030, a glasshouse trial was done to test an algaecide for its ability to eliminate or reduce *Phytophthora nicotianae* root infections in rough lemon seedlings. Results indicated that, although not significantly, seedling growth was improved when 0.5 or 1.0 ppm of the algaecide was applied weekly for the duration of the trial. The seedlings from these treatments were bigger with bigger root systems compared to the untreated, uninoculated and treated, inoculated controls. In the fumigation trial monitored it was seen that just more than 1 year after fumigation and planting, there was already a significant difference between the fumigated and non-fumigated treatments. The trees growing in the fumigated rows were significantly bigger than trees in the non-fumigated rows. It was furthermore seen that in the fumigated soil there were no juvenile citrus nematodes present while in the non-fumigated soil a low number was present. In terms of *P. nicotianae* and *P. citrophthora* infested leaf discs the fumigated soils also had significantly lower levels of both pathogens compared to the non-fumigated soils. From these early results the beneficial effect of soil fumigation in a replant situation is already evident.

A decline and death of citrus trees have been reported from the Gamtoos and Sunday's River valleys for a number of years. Surveys of diseased trees were done as part of project 1068 in the Gamtoos and Sunday's River Valley production areas. Isolations from symptomatic trunk and root material yielded *Diaporthe* spp. isolates and isolates from genera within the Diatrypaceae family. These are pathogens that are known to infect woody hosts that are under stress. From the infected root tissue and surrounding soil, three different *Neocosmospora* (previously *Fusarium*) spp. were isolated. These included *N. solani*, a pathogen that is known to infect citrus trees when they are under stress. In order to evaluate some management options for this decline syndrome, two pot trials were established. In the first trial, Carrizo citrange rootstock seedlings were planted in soil from affected orchards. This soil was known to contain the abovementioned *Neocosmospora* spp. Four different chemical fungicide treatments were applied as soil drenches. Results indicated that seedlings receiving the benomyl drench had significantly bigger root systems with thicker stems compared to the other three chemical treatments. The rootstock pot trial, evaluating the growth performance of 12 different rootstocks in infected soil, did not yield conclusive results after one year. This could be due to the stress factors present in diseased orchards not being replicated in the pot trial. The rootstock trial will be repeated as an orchard planting in infected soil. The promising benomyl drench treatment were also shown to have appositive effect on small trees growing in a diseased orchard.

Project 1101 concluded in March 2019 and yielded some important results for better control of soilborne diseases in citrus nurseries. It was found that metham sodium is an effective method to treat different types of nursery potting media because it completely eliminates pathogen propagules from the media. Six phosphonate foliar applications done over winter and summer, significantly improved the nursery tree growth and also provided good protection against root infections by *Phytophthora nicotianae*. This practice can therefore be used as a good preventative management strategy in nurseries. The mefenoxam sensitivity of *P. nicotianae* and *P. citrophthora*

along with *Pythium* spp. were found to vary greatly between isolates within a species and between species. A low percentage of isolates were highly insensitive to mefenoxam but most of the isolates were still sensitive to this fungicide. However, practically the results do indicate that this fungicide must be used with caution in nurseries when insensitivity is expected. Similarly, it was shown for abovementioned soilborne pathogens that total elimination of pathogen propagules from irrigation water is only achieved if the water is treated with 6 ppm active chlorine for longer than 60 minutes. These results will lead to improved soilborne pathogen management in citrus nurseries. In terms of water treatment, the findings regarding chlorine sensitivity can be important for better preventative pathogen control.

Project 1215 is a new project that started in April 2019. Good progress in finding potential biological control agents for soilborne pathogens has been made. Components of valuable tools to be used in studying the interactions of the different pathogens have also been developed. Among several bacterial isolates obtained from the rhizosphere soil of diseased trees, five *Bacillus* and *Pseudomonas* isolates were obtained that showed very good *in vitro* inhibition of *Pythium irregulare*, *Phytophthora nicotianae* and *P. citrophthora*. Unfortunately, the inhibition of the *Neocosmospora* spp. associated with citrus was not that good. Twelve *Trichoderma* isolates were also obtained and these showed that they produce non-volatile compounds that completely inhibit the different oomycete pathogens. Again, the inhibition of the *Neocosmospora* spp. was not as good. Primers for the detection of abovementioned two *Phytophthora* spp. were developed successfully. In preliminary validation, the primers were shown to be specific to the species and did not detect any other closely related *Phytophthora* spp. or other soilborne pathogens. Further optimization and validation of the primers is in progress. Further DNA sequencing is needed to develop similar primers for the different *Neocosmospora* spp. regarded as citrus pathogens.

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. Research is therefore focused on the epidemiology and management of these pathogens. Project 750 focusses on the chemical management of ABS while project 970 focusses on CBS chemical management. In 750, it was found that the inclusion of boscalid and mineral oil at the beginning of a spray programme provided the best control, although the amount of control was not significantly different from that obtained with the programme consisting of mancozeb and copper applied at half of their registered rates, in tank mixtures. In project 970, some new fungicides were evaluated for CBS control with good results. Good CBS control ($\geq 90\%$ clean fruit) was achieved with several fungicides tested, but the best control (99.8% clean fruit) was achieved with the experimental fungicide, GF 3540, which was applied four times at 6-week intervals in a tank mixture with mineral oil. Dipotassium phosphate (RB1) did not perform well when applied alone but there was a marked improvement in the effectivity of the fungicide when applied in tank mixtures with strobilurins, where it replaced mancozeb.

Apart from evaluating different fungicides and spray programmes for CBS and ABS control, research is also done to improve spray application and spray calibration methods. In project 1132, different spray volumes were evaluated on different citrus types for the control of CBS, ABS and insect pests. Results showed that higher spray volumes led to better spray uniformity and better control of certain insect pests such as red scale and mealybug. Furthermore, the effect of canopy density and spray penetration was clear and had a direct effect on deposition parameters. Spray deposition parameters on the inner canopy positions were poorer than the outer canopy positions, which also correlated with biological efficacy. This study supports the importance of penetration of spray volumes into the tree canopy as well as the potential of reduced spray volumes. Project 1089 aimed at developing a non-destructive method to quantify canopy density so that it can be included as a factor in spray calibration. This was done by using LiDAR (Light Detection and Ranging) technology. Results indicated that LiDAR successfully observed the changes in tree canopy density after pruning, but the LiDAR parameter developed correlated poorly with manual measurements. At higher spray volumes, pruning had no to little effect on spray deposition parameters. However, when applying lower spray volumes, light pruning had a marked effect on spray deposition, improving it markedly in comparison to the results seen for unpruned or heavily pruned trees. If lower spray volumes are employed, tree canopy manipulation through pruning must be done to get adequate spray deposition.

Project RCE-6 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 different temperatures and alternate wetness interruption cycles on pycnidiospore germination and infection were investigated. The highest germination percentage for pycnidiospores was observed after an incubation period of 24 hours at 25°C and 30°C. Along with this, the highest average percentage pycnidiospore germination was observed when wetness was interrupted 8 and 12 hours after initial incubation (26.3% and 24.1%). These results will aid in improving the pycnidiospore infection prediction models for CBS. Project RCE-7 was aimed at developing a decision making tool to assist growers to make better decisions regarding fungicide applications for CBS control. Using various CBS epidemiological prediction models, a web-based software programme was developed called CRI-PhytRisk. This supports citrus growers in CBS areas to improve fungicide spray timing and choice of fungicides used as well as indicate CBS-risk following seasonal weather conditions. It also helps growers to spray when conditions are good to spray. This programme has been live since 2016 and a mobile application is also available. Further developments include the incorporation of on-farm weather station data as well as the addition of an *Alternaria* brown spot (ABS) model to provide growers with information about when conditions are favourable for this important disease of mandarins.

CBS models were developed to predict various processes in the epidemiology of the pathogen. Proper validation of these models was lacking and project RCE-8 aimed at validating these models. This would lead to improving disease prediction in different production areas and a better understanding of the pathogen epidemiology in these areas. Following further modelling work based on weather and ascospore trapping data, new pseudothecium maturation and ascospore release models for *P. citricarpa* were developed. These new models will be incorporated in CRI PhytRisk to provide a better disease management tool to growers.

With an increasing tendency to erect shade nets over high value citrus cultivars, there are fears that the change in microclimate under nets could affect the development of diseases. Consequently, a study aimed to determine how the use of shade nets could influence the development and spread of CBS within citrus orchards was initiated (Project 1187). Based on weather data from enclosed and open orchards, a higher risk of CBS infection (both ascospore and pycnidiospore infection) was predicted in the orchard under the net when compared to the orchard outside the net in Patensie in the 2017-18 season. In Nelspruit during the 2018-19 season, more days with pycnidiospore infections were predicted outside the net and slightly more days (only a day difference) with ascospore infections were predicted under the net. More days with ABS risks were predicted outside nets in both areas and this could be due to the high rainfall amounts recorded in these orchards. The increase of fruit resistance to CBS infections has never been conclusively quantified. In project 1186, the development of ontogenic resistance development to CBS infections of fruit is being studied in collaboration with researchers in Brazil. Results indicated that on inoculated fruit, disease incidence and severity decreases as fruit matures. Through the application of staggered spray trials, it was furthermore shown that protection of fruit after March is not needed.

Programopsomming

Die navorsingsfokus binne die voor-oes siekteprogram, is grondgedraagde siektes van sitrus, en vrug- en blaarsiektes van sitrus, insluitende Sitrus Swartvlek. Die navorsing fokus op die vind van alternatiewe, sagter bestuurs-opsies, studies op die epidemiologie van die verskillende patogene, en optimalisering van die beheer van hierdie patogene deur beter toedieningstechnologie of beter chemiese beheerprogramme.

Projekte 762 en 1030 is spesifiek daarop gemik om alternatiewe wyses van beheer vir *Phytophthora* en sitrus aalwurm te vind. Data in projek 762 word sedert 2011 aangeteken. Dit word duidelik dat die verskillende voorplant grondberokingsbehandelings, spesifiek die 1,3 dichloropropeen en metam-natrium handelings, veroorsaak dat die bome in hierdie handelings langer is en dikker stamme het, in vergelyking met die ander handelings en die onbehandelde kontrole. Binne projek 1030 is 'n glashuisproef uitgevoer ten einde 'n algdoder te toets vir sy vermoë om *Phytophthora nicotianae* wortel-infeksies in growwe suurlemoensaailinge uit te wis of te verminder. Resultate het aangetoon dat, hoewel nie betekenisvol nie, saailinggroei verbeter het wanneer 0.5 of

1.0 dpm van die algdoder weekliks vir die duur van die proef toegedien is. Die saailinge vanaf hierdie behandelings was groter met groter wortelsisteme in vergelyking met onbehandelde, nie-geïnkuleerde en behandelde, geïnkuleerde kontroles. In die berokingsproef wat gemonitor is, is gesien dat net meer as een jaar ná beroking en plant, daar alreeds 'n betekenisvolle verskil tussen berookte en nie-berookte behandelings was. Die bome wat in berookte rye gegroei het, was betekenisvol groter as die bome in die nie-berookte rye. Verder was daar in die berookte grond geen jong sitrus aalwurms teenwoordig nie, terwyl daar 'n lae getal in die nie-berookte grond teenwoordig was. In terme van *P. nicotianae* en *P. citrophthora* geïnfekteerde blaarskyfies, het die berookte gronde betekenisvol laer vlakke van beide patogene gehad, in vergelyking met die nie-berookte gronde. Die voordelige effek van grondberoking in 'n herplant situasie is alreeds vanuit hierdie vroeë resultate duidelik.

Agteruitgang en sterftes van sitrusbome is vir 'n aantal jare vanuit die Gamtoos- en Sondagsriviervallei aangeteken. Opnames van siek bome is as deel van projek 1068 in die Gamtoos- en Sondagsriviervallei produksie-areas gedoen. Isolاسies vanuit simptomatiesse stam- en wortelmateriaal, het *Diaporthe* spesies isolate en isolate van genera binne die Diatrypaceae familie opgelewer. Hierdie patogene is daarvoor bekend dat hul houtagtige gashere wat onder stres verkeer, infekteer. Drie verskillende *Neocosmospora* (voorheen *Fusarium*) spp. is vanuit geïnfekteerde wortelweefsel en omliggende grond geïsoleer. Dit sluit *N. solani* in, 'n patoogeen daarvoor bekend om sitrusbome te infekteer wat onder stres verkeer. Ten einde bestuurs-opsies vir hierdie agteruitgang sindroom te evalueer, is twee potproewe gevestig. In die eerste proef is Carrizo citrange onderstam saailinge in grond vanuit geïnfekteerde boorde geplant. Dit was bekend dat hierdie grond die bogenoemde *Neocosmospora* spp. bevat het. Vier verskillende chemiese fungisiedbehandelings is as grondrenkbehandelings toegedien. Resultate het aangetoon dat saailinge wat die benomyl drenkbehandeling ontvang het, betekenisvol groter wortelsisteme gehad het, met dikker stamme, in vergelyking met die ander drie chemiese behandelings. Die onderstam potproef wat die groeiprestasie van 12 verskillende onderstamme in geïnfekteerde grond geëvalueer het, het na een jaar nog geen afdoende resultate gelewer nie. Dit kan wees weens die strestoestand teenwoordig in die siek boorde, wat nie in die potproef herhaal kon word nie. Die onderstamproef gaan as 'n boordaanplanting in geïnfekteerde grond herhaal word. Die belowende benomyl drenkbehandeling het ook 'n positiewe effek getoon op klein boompies wat in 'n siek boord gegroei het.

Projek 1101 het in Maart 2019 tot 'n einde gekom en het belangrike resultate vir beter beheer van grondgedraagde siektes in sitruskwekerye opgelewer. Daar is gevind dat metam-natrium 'n effektiewe metode is vir die behandeling van verskillende tipes kwekery potmediums aangesien dit patoogeenpropagules heeltemal uit die medium verwyder. Ses fosfonaat blaartoedienings wat oor die winter en somer gedoen is, het die kwekery boomgroei betekenisvol verbeter en het ook goeie beskerming teen wortel-infeksies deur *Phytophthora nicotianae* gebied. Hierdie praktyk kan dus as 'n goeie voorkomende bestuursstrategie in kwekerye aangewend word. Daar is gevind dat die mfenoxam sensitiwiteit van *P. nicotianae* en *P. citrophthora*, tesame met *Pythium* spp., grootliks tussen isolate binne 'n spesie en tussen spesies varieer. 'n Hoë persentasie isolate was hoogs onsensitief teenoor mfenoxam, maar meeste van die isolate was steeds sensitief teenoor hierdie fungisied. Hierdie resultate dui egter prakties daarop dat hierdie fungisied versigtig in kwekerye gebruik moet word indien onsensitiwiteit verwag word. Soortgelyk is vir bogenoemde grondgedraagde patogene getoon dat totale uitwissing van patoogeenpropagules vanuit besproeiingswater slegs bereik word indien water met 6 dpm aktiewe chloor vir langer as 60 minute behandel word. Hierdie resultate sal tot verbeterde grondgedraagde patoogeenbestuur in sitruskwekerye lei. Die bevindinge rakende chloor sensitiwiteit kan in terme van waterbehandeling belangrik wees vir beter voorkomende patoogeenbeheer.

Projek 1215 is 'n nuwe projek wat in April 2019 begin is. Goeie vordering is gemaak rakende die vind van potensiële biologiese beheer-agente vir grondgedraagde patogene. Komponente van waardevolle hulpmiddels, wat in die studie van die interaksies van die verskillende patogene gebruik kan word, is ook ontwikkel. Tussen verskeie bakteriese isolate wat vanuit die risosfeer grond van siek bome verkry is, is vyf *Bacillus* en *Pseudomonas* isolate verkry wat baie goeie *in vitro* onderdrukking van *Pythium irregulare*, *Phytophthora nicotianae* en *P. citrophthora* toon. Ongelukkig was die onderdrukking van die *Neocosmospora* spp. wat met sitrus geassosieer word, nie so goed nie. Twaalf *Trichoderma* isolate is ook verkry en daar is getoon dat hulle nie-vlugtige verbindings

produseer wat die verskillende oömiseet patogene heeltemal onderdruk. Die onderdrukking van die *Neocosmospora* spp. was weereens nie goed nie. Inleiers vir die waarneming van bogenoemde twee *Phytophthora* spp. is suksesvol ontwikkel. In voorlopige validasie, het die inleiers getoon dat hulle spesifiek vir die spesie was en het nie enige ander naby-verwante *Phytophthora* spp. of ander grondgedraagde patogene waargeneem nie. Verdere optimalisering en validasie van die inleiers is onderweg. Verdere DNS volgordebepaling (“sequencing”) word benodig om soortgelyke inleiers vir die verskillende *Neocosmospora* spp. wat as sitrus patogene beskou word, te ontwikkel.

Sitrus Swartvlek (SSV) en *Alternaria* Bruinvlek (ABV) is twee belangrike vrug- en blaarsiektes wat die uitvoer van sitrusvrugte deur Suid-Afrikaanse produsente na vars markte belemmer. Navorsing fokus dus op die epidemiologie en bestuur van hierdie patogene. Projek 750 fokus op die chemiese bestuur van ABV, terwyl projek 970 op SSV chemiese bestuur fokus. In 750 is gevind dat die insluit van boscalid en minerale olie, aan die begin van die spuitprogram, die beste beheer verskaf het, hoewel die hoeveelheid beheer nie betekenisvol verskil het van dit wat verkry is met die program bestaande uit mankoseb en koper, toegedien in tenkmengsels, teen die helfte van hul geregistreerde dosisse nie. In projek 970 is ‘n paar nuwe fungisiedes met goeie resultate vir SSV beheer geëvalueer. Goeie SSV beheer ($\geq 90\%$ skoon vrugte) is met verskeie fungisiedes verkry wat getoets is, maar die beste beheer (99.8% skoon vrugte) is met die eksperimentele fungisied, GF 3540, verkry wat vier keer teen 6-week intervalle in ‘n tenkmengsel met minerale olie toegedien is. Di-kaliumfosfaat (RB1) het nie goed gedoen wanneer alleen toegedien is nie, maar daar was ‘n merkbare verbetering in die effektiwiteit van die fungisied wanneer in tenkmengsels met strobilurine toegedien is, waar dit mankoseb vervang het.

Behalwe vir die evaluering van verskillende fungisiedes en spuitprogramme vir SSV en ABV beheer, word navorsing ook gedoen om spuittoediening en spuitkalibrasie metodes te verbeter. In projek 1132, is verskillende spuitvolumes op verskillende sitrus tipes vir die beheer van SSV, ABV en insekplae geëvalueer. Resultate het getoon dat hoër spuitvolumes tot beter spuit uniformiteit en beter beheer van sekere insekplae soos rooidopluis en witluis lei. Verder was die effek van lowerdigtheid en spuitpenetrasie duidelik en dit het ‘n direkte effek op neerleggingsparameters gehad. Spuitneerleggingsparameters was swakker op die binneste lowerposisies as op die buitenste lowerposisies, wat ook met biologiese effektiwiteit gekorreleer het. Hierdie studie ondersteun die belang van penetrasie van spuitvolumes in die boomlower in, asook die potensiaal van verminderde spuitvolumes. Projek 1089 het ten doel gehad om ‘n nie-vernietigende metode te ontwikkel om die lowerdigtheid te kwantifiseer sodat dit as ‘n faktor in spuitkalibrasie ingesluit kan word. Dit is gedoen deur die gebruik van LiDAR (“Light Detection and Ranging”) tegnologie. Resultate het getoon dat LiDAR die veranderinge in boomlowerdigtheid suksesvol ná snoei waarneem, maar die LiDAR parameter wat ontwikkel is, korreleer swak met handmetings. Snoei het teen hoër spuitvolumes geen tot min effek op spuitneerleggingsparameters gehad. Wanneer laer spuitvolumes toegedien is, het ligte snoei egter ‘n merkbare effek op spuitneerlegging gehad. Dit is merkbaar verbeter in vergelyking met resultate vir ongesnoeide of hewig gesnoeide bome. Indien laer spuitvolumes toegepas word, moet boomlower manipulasie deur snoei gedoen word ten einde voldoende spuitneerlegging te verkry.

Projek RCE-6 beoog om die kennisgaping wat bestaan betreffende die ryppword van vrugstrukture en spoorontkiemingsvereistes van beide die geslagtelike en ongeslagtelike fases van die SSV patogeen, te oorbrug. Die effek van verskillende temperature en alternerende benutting onderbrekingsiklusse op piknidiospor-ontkieming en -infeksie, is ondersoek. Die hoogste ontkiemingspersentasie vir piknidiospore is na ‘n inkubasieperiode van 24 ure teen 25°C en 30°C waargeneem. Tesame hiermee, is die hoogste gemiddelde persentasie piknidiospor-ontkieming waargeneem wanneer benutting 8 en 12 ure ná aanvanklike inkubasie (26.3% en 24.1%) onderbreek is. Hierdie resultate sal help om die piknidiospor-infeksie voorspellingsmodelle vir SSV te verbeter. Projek RCE-7 was ten doel om ‘n besluitnemingshulpmiddel te ontwikkel om produsente by te staan om beter besluite rakende fungisiedtoedienings vir SSV beheer te maak. Deur gebruik te maak van verskeie SSV epidemiologiese voorspellingsmodelle, is ‘n web-gebaseerde sagteware program ontwikkel, genaamd CRI-PhytRisk. Dit ondersteun sitrusprodusente in SSV areas om fungisiedspuit tydsberekening en keuse van fungisiedes te verbeter, en dui ook SSV risiko aan volgende op seisoenale weerstoestande. Dit help ook produsente om te spuit

wanneer toestande goed is om te spuit. Hierdie program is sedert 2016 lewendig en 'n mobiele *app* is ook beskikbaar. Verdere ontwikkelings sluit die insluit van op-plaas weerstasie data in, asook die byvoeg van 'n *Alternaria* Bruinvlek (ABV) model om produsente van inligting te verskaf van wanneer toestande gunstig is vir hierdie belangrike siekte van mandaryne.

SSV modelle is ontwikkel om verskeie prosesse in die epidemiologie van die patogeen te voorspel. Behoorlike validasie van hierdie modelle het gekort en projek RCE-8 het ten doel gehad om hierdie modelle te valideer. Dit sal tot verbeterde siektevoorspelling in verskillende produksie-areas lei en die patogeen-epidemiologie in hierdie areas beter verstaan. Verdere modelleringswerk, gebaseer op weer- en askospoor lokvaldata, het tot die ontwikkeling van nuwe pseudotesium rypwordings- en askospoorvystellingsmodelle vir *P. citricarpa* gelei. Hierdie nuwe modelle sal in CRI PhytRisk geïnkorporeer word om 'n beter siektebestuur hulpmiddel vir produsente te verskaf.

Met 'n toenemende neiging om skadunette oor hoë-waarde sitruskultivars te span, is daar kommer dat die verandering in mikroklimaat onder nette die ontwikkeling van siekte kan affekteer. 'n Studie wat ten doel het om te bepaal hoe die gebruik van skadunette die ontwikkeling en verspreiding van SSV binne sitrusboorde kan beïnvloed, is gevolglik geïnisieer (Projek 1187). Gebaseer op weerdata vanaf bedekte en oop boorde, is gevind dat 'n hoër risiko van SSV infeksie (beide askospoor- en piknidiospoor-infeksie) in die boord onder net voorspel is, wanneer vergelyk word met boorde buite die net in Patensie in die 2017-18 seisoen. In Nelspruit gedurende die 2018-19 seisoen, is meer dae met piknidiospoor-infeksies buite die net voorspel en effens meer dae (slegs een dag verskil) met askospoor-infeksies is onder die net voorspel. Meer dae met ABV risiko's is buite die nette in beide areas voorspel, en dit kan weens die hoë reënvalhoeveelhede wees wat in hierdie boorde aangeteken is. Die toename van vrugweerstand teen SSV infeksies is nog nooit onweerlegbaar gekwantifiseer nie. In projek 1186 word die ontwikkeling van ontogeniese weerstandsontwikkeling teen SSV infeksies van vrugte in samewerking met navorsers in Brasilië bestudeer. Resultate het aangetoon dat siektevoorkoms en siekte-intensiteit op geïnkuleerde vrugte afneem soos wat die vrugte ryp word. Deur die toedien van tragsgewyse ("staggered") spuitproewe, is verder aangedui dat beskerming van vrugte ná Maart nie nodig is nie.

4.3.2 **FINAL REPORT: Preventative and curative management of soilborne pathogens in citrus nurseries.**

Project 1101 (RCE 5) (Dec 2014 – Mar 2019) by Jan van Niekerk, Elaine Basson and Charmaine Olivier (CRI)

Summary

In this project the different preventative and curative *Phytophthora* and *Pythium* management strategies were evaluated for efficacy. It was found that metham sodium is an effective method to treat different types of nursery potting media because it completely eliminates pathogen propagules from the media. It was furthermore found that captan is a good fungicide to apply as a drench in cases where the nursery plants do get infected with *Phytophthora*. It worked well in sand and media with more organic media. Mefenoxam did not work well in sand but well in more organic media. Further optimization for these applications in the different media is needed. Six phosphonate foliar applications done over winter and summer, significantly improved the nursery tree growth and also provided good protection against root infections by *Phytophthora nicotianae*. Characterization of the *Pythium* species occurring in South African citrus nurseries identified 10 different species with *P. irregulare* that was found to be the most prominent species and that in combination with *P. nicotianae* it causes more severe root rot than the two pathogens in isolation. The mefenoxam sensitivity of *P. nicotianae* and *P. citrophthora* along with *Pythium* spp. was found to vary greatly between isolates within a species and also between species. A low percentage of isolates were found to be highly insensitive to mefenoxam but most of the isolates were still sensitive to this fungicide. However, practically the results do indicate that this fungicide should be used with caution in nurseries when insensitivity is expected. Similarly, it was shown for abovementioned soilborne pathogens that total elimination of pathogen propagules from irrigation water is only achieved if the water is treated with 6 ppm active

chlorine for longer than 60 minutes. These results will lead to improved soilborne pathogen management in citrus nurseries.

Opsomming

In hierdie projek is die verskillende voorkomende en uitwissende *Phytophthora* en *Pythium* bestuursstrategieë vir hul effektiwiteit geëvalueer. Daar is gevind dat metam-natrium 'n effektiewe metode is om verskillende tipes kwekery potmedium te behandel, aangesien dit patogeenpropagules heeltemal uit die medium verwyder. Daar is verder gevind dat Kaptan 'n goeie fungisied is om as 'n drenkbehandeling toe te dien in gevalle waar die kwekeryplante met *Phytophthora* geïnfekteer is. Dit het goed gewerk in sand en medium met meer organiese medium. Mefenoxam het nie goed in sand gewerk nie, maar goed in meer organiese medium. Verdere optimalisering vir hierdie toedienings in die verskillende mediums word benodig. Ses fosfonaat blaartoedienings wat oor die winter en somer gedoen is, het die kwekeryboomgroei betekenisvol verbeter en het ook goeie beskerming teen wortel-infeksies deur *Phytophthora nicotianae* gebied. Karakterisering van *Pythium* spesies wat in Suid-Afrikaanse sitruskwekerye voorkom, het 10 verskillende spesies geïdentifiseer waarvan *P. irregulare* die mees prominente spesie was, en in kombinasie met *P. nicotianae*, het dit ernstiger wortelvrot veroorsaak as die twee patogene in isolasie. Die mefenoxam sensitiwiteit van *P. nicotianae* en *P. citrophthora*, tesame met *Pythium* spp. het grootliks tussen isolate binne 'n spesie en ook tussen spesies gevarieer. 'n Lae persentasie isolate was hoogs onsensitief teenoor mefenoxam, maar meeste van die isolate was steeds sensitief teenoor hierdie fungisied. Prakties dui hierdie resultate egter daarop dat hierdie fungisied versigtig gebruik moet word in kwekerye wanneer onsensitiwiteit vermag word. Soortgelyk is getoon dat vir bogenoemde grondgedraagde patogene, algehele uitwissing van patogeenpropagules vanuit besproeiingswater slegs bereik word indien die water met 6 dpm aktiewe chloor vir langer as 60 minute behandel word. Hierdie resultate sal tot verbeterde grondgedraagde patogeenbestuur in sitruskwekerye lei.

Introduction

South Africa is one of the largest exporters of citrus in the world. In total more than 60 000 ha are planted to citrus. This brings about that continuous demand is placed on citrus nurseries to provide vast numbers of high quality nursery trees for the replacement of old orchards or the establishment of new orchards.

Soilborne pathogens, especially *Phytophthora citrophthora* and *P. nicotianae*, have been shown to cause enormous losses in citrus worldwide (Meitz-Hopkins et al. 2013). *Phytophthora nicotianae* have been reported from citrus nurseries in South Africa (Wehner et al. 1986) and other parts of the world (Ahmed et al. 2012). Along with *P. nicotianae*, *Phytophthora* test results from the CRI Diagnostic Centre (DC) in Nelspruit have also indicated *Phytophthora citrophthora* to be sporadically present in some South African citrus nurseries.

The presence of these two important pathogens in South African citrus nurseries is of enormous importance as it has been shown that infected nursery trees are often the cause of orchards being infected by these two *Phytophthora* species (Ippolito et al. 2004). One of the most important factors for the successful establishment of a citrus orchard is the use of disease free nursery trees (Wehner et al. 1986). In South Africa, nursery trees need to be without any *Phytophthora* infection to be certified by the South African Citrus Improvement Scheme (Fourie et al. 2013).

A previous survey of soilborne pathogens in South African citrus nurseries listed *Pythium* spp. as occurring in five of the seven nurseries tested (Wehner et al. 1986). It was also reported as part of the same study that *Pythium* spp. reduced root growth of citrus seedlings, but not shoot growth. In an earlier study by Thompson et al. (1995) it was found that *Pythium* spp. along with *P. nicotianae* are associated with feeder root rot in citrus orchards of the old Transvaal province of South Africa. Kean et al. (2010) furthermore reported that *Pythium ultimum* was, along with *P. nicotianae*, regarded as the causal organisms of citrus root rot of citrus in Cambodia.

Along with the occurrence of abovementioned *Phytophthora* spp. in citrus nurseries, the results of the CRI DC also indicate that *Pythium* spp. occurs at significant levels in the irrigation water of nurseries as well as in the potting media. At this stage the presence of *Pythium* spp. is seen more as a warning sign for the nursery and only the presence of *Phytophthora* spp. leads to control measures being employed in the nursery. However, based on the previous reports of *Pythium* spp. being involved in citrus root rot, the high level of *Pythium* spp. present in samples received by the CRI DC could possibly indicate a more serious problem.

Phytophthora nicotianae, *P. citrophthora* and *Pythium* spp. can be soil or water borne (Grech and Rijkenberg, 1992; Ippolito et al. 2004). Preventative control measures are therefore employed by citrus nurseries to ensure that the potting medium and irrigation water is free from these pathogens. Chlorination of irrigation water is routinely used in citrus nurseries in South Africa to eradicate any pathogen propagules that might be present in the water. This practice is also employed by nurseries in other industries (Hong et al. 2003; Ghimire et al. 2011). For the disinfection of nursery potting medium methyl bromide (MeBr) is still widely used. However, the continued use of this chemical is under threat as it is regarded as one of the major contributing factors in the depletion of the ozone layer. Numerous studies have therefore focused on finding alternative soil or potting medium treatments to replace MeBr (Weiland et al. 2013).

Several products with the potential to replace MeBr as treatment for potting medium are present in South Africa and have been subjected to field trials where they showed good potential to control *Phytophthora* spp. in the soil. However, their effect as treatments for nursery medium has remained untested; treatment of nursery potting medium also needs to be 100% effective. New types of potting mediums have also come to the fore recently. One of these is coco peat or coir that is increasingly being used in citrus nurseries. It is currently unknown how effective conventional and novel preventative and curative measures are to control soilborne pathogen infestations in these different substrates.

The curative and preventative use of systemic fungicides including metalaxyl, fosetyl-AI and phosphorous acid in the management of *Phytophthora* spp. in various plants, including citrus has been the subject of numerous studies (Farih et al. 1981; Davis, 1982; Matheron and Matejka, 1988; Matheron et al. 1997). In the event of *Phytophthora* spp. being detected in any South African citrus nursery, the current practice entails the drench application of metalaxyl or captan to infected trees, with a follow-up treatment if needed.

Despite the various preventative and curative control measures employed in South African citrus nurseries, CRI DC results still indicate that *Phytophthora* spp. are often present in nurseries along with high numbers of *Pythium* spp. This could indicate that the current preventative treatment of chlorination of irrigation water is not 100% effective. Hong et al. (2003) reported this specific situation with regards to *Phytophthora* spp. control on ornamental crop nurseries in Virginia, USA. Repeated *Phytophthora* positive tests that are recorded by the CRI DC for some citrus nurseries also indicate that there are also some problems with the curative measures that are employed. Metalaxyl or mefenoxam resistance could be a factor here as it has been reported to occur in *P. nicotianae* on citrus and ornamental hosts in the USA (Ferrin and Kabashima, 1991; Timmer et al. 1998).

Another systemic chemical that has been tested for the control of *Phytophthora* infections in various crops is phosphorous acid. Studies have shown that phosphorous acid has direct and indirect effects on *Phytophthora* spp. (Fenn and Coffey, 1984; Coffey and Joseph, 1985; Fenn and Coffey, 1985). Direct effects include inhibition of sporangium development, zoospore release and mycelium growth (Fenn and Coffey, 1984; Coffey and Joseph, 1985; Fenn and Coffey, 1985). Scoparone is a phytoalexin associated with citrus resistance to infection by *P. citrophthora*. Afek and Szejnberg (1989) found that when citrus trees are treated with phosphorous acid the levels of scoparone is two to four times higher in treated trees compared to untreated trees. It was furthermore shown by these authors that when trees were inoculated with *P. citrophthora* and then treated with phosphorous acid the severity of infection was significantly less than inoculated, untreated trees.

Phosphorous acid containing products in South Africa are registered exclusively for use on mature, bearing citrus trees for the control of root and collar rot and postharvest brown rot. The registered application methods for these products are limited to trunk paints or foliar sprays. No mention is made on the label of any of these products as to use on nursery trees; what dosages to use and the application intervals or the method of application.

The first aim of this project would be to characterize *Phytophthora* and *Pythium* spp. present in South African citrus nurseries. This would include molecular and morphological characterization of the pathogens, pathogenicity to nursery trees of the different pathogen species, testing for possible mefenoxam resistance and chlorine insensitivity and possible synergies between *Phytophthora* and *Pythium* spp. In the event of mefenoxam resistance being identified, a management strategy for these resistant isolates will be developed.

A second aim would be the development of a technique to test fumigants for the replacement of MeBr in the treatment of potting medium. Once the technique has been established, promising fumigants from field trials conducted at CRI will be tested. Treatment protocols will subsequently be developed for using the new fumigants in the citrus nursery environment to treat potting medium. The aim would thirdly be to determine if currently used preventative and curative measures employed to control soilborne pathogens are effective in new types of potting medium being used, especially coir and mixes with coir.

The last aim would be to test the effect of different phosphonates to be used as a preventative or curative soilborne disease management tool in citrus nurseries. Once effective products along with dosages, treatment intervals and application methods have been identified, protocols for using these products in the nurseries will be developed.

Stated objectives

Year 1:

1. Collection of *Pythium* and *Phytophthora* isolates from citrus nurseries.
2. Developing a technique to test potting medium fumigants.
3. Testing of alternative fumigants to replace MeBr for the treatment of potting medium.
4. Evaluating currently used mefenoxam and captan applications for effective control of *Phytophthora* infestations in coir and coir mixtures.
5. Evaluate phosphonates as foliar applications for their preventative and curative action against *Phytophthora* infestations of nursery trees.

Year 2

1. Morphological and molecular characterization of *Pythium* and *Phytophthora* isolates obtained from citrus nurseries.
2. Determine the level of mefenoxam resistance present in *Phytophthora* isolates obtained from nurseries.
3. Testing the level of chlorine sensitivity of *Pythium* and *Phytophthora* isolates obtained from citrus nurseries.
4. Evaluating currently used mefenoxam and captan applications for effective control of *Phytophthora* infestations in coir and coir mixtures.
5. Evaluate phosphonates as foliar applications for their preventative and curative action against *Phytophthora* infestations of nursery trees.

Year 3

1. Testing the pathogenicity of the identified *Pythium* spp. towards citrus nursery seedlings.

2. Investigate possible synergies between *Phytophthora* and *Pythium* spp. in dual inoculation trials using citrus seedlings.
3. Developing a strategy to manage mefenoxam resistant *Phytophthora* isolates in a citrus nursery.

Materials and methods

1. *Collection of Pythium and Phytophthora isolates from citrus nurseries.*

Isolates of *Phytophthora nicotianae*, *P. citrophthora* and *Pythium* spp. were collected from South African citrus nurseries by taking samples of the growing medium in pots containing small citrus trees in the different nurseries. These samples were divided into the different compartments of ice trays, one ice tray per sample. The growing medium in each compartment was covered with distilled water before placing two citrus leaf discs, 5 mm in diameter, in each compartment. Before cutting the leaf discs from the citrus leaves they were washed thoroughly with distilled water. Leaves were collected from trees not subjected to any fungicide treatment. The ice trays were covered to prevent light infiltration and incubated at ambient temperature on the laboratory bench for 48 h. After incubation, leaf discs were removed from the water surface, blotted on absorbent paper towel, and plated onto 90 mm Petri dishes containing PARPH medium (Kannwischer and Mitchell, 1978). The plates were incubated in the dark at 29 °C before being inspected for *Phytophthora* and *Pythium* spp. colonies. From the plates isolates were selected and transferred to water agar (WA, Biological agar, Biolab, Wadeville, South Africa) and incubated further at 29 °C for 48 h. Colonies were purified from the WA by hyphal tipping onto 90 mm Petri dishes containing V8 agar (Galindo and Gallegly, 1960). Isolates were stored in molecular grade water in 2 ml Eppendorf tubes at 25 °C.

2. *Testing of alternative fumigants to replace MeBr for the treatment of potting medium.*

Two hundred litres each of composted pine bark (CPB) and river sand (RS) were steam sterilized before being split into 4 batches of 50L each in plastic boxes with lids. These boxes, containing either CPB or RS, were inoculated with a *Phytophthora nicotianae* broth for a period of 2 weeks. During this time, the boxes with media was incubated at 29 °C. After inoculation and incubation of 2 weeks, the boxes were tested for the presence of *Phytophthora nicotianae* using the standard soil baiting technique. Upon testing positive for *Phytophthora nicotianae* presence, boxes were treated with a metham sodium solution at a concentration of 0.9 ml/L. For each medium, two boxes were left untreated as a control while the other box was drenched with 5 liters of abovementioned metham sodium solution. After drenching, boxed was closed and left for 4 days after which lids was removed to aerate boxes for a further 3 days before testing again for the presence of *Phytophthora nicotianae* in the medium.

3. *Evaluating currently used mefenoxam and captan applications for effective control of Phytophthora infestations in coir and coir mixtures.*

Rough lemon (RL) seedlings were planted in 5 L planting bags containing four different potting mediums. The mediums were composted pine bark (CPB), CPB plus coir (50:50) mixture, 100% coir and 100% river sand. Before planting the CPB and river sand were steam sterilized twice to eradicate any possible infestations by soilborne pathogens. After transplant the seedlings were grown for eight weeks before the trial commenced. After eight weeks, the medium in which the seedlings were growing were inoculated with a *Phytophthora nicotianae* inoculum mixture. The inoculation was repeated twice weekly for a period of four weeks. After the inoculation period, samples were drawn from the potting media to determine *Phytophthora* infestation levels through soil baiting with unsprayed citrus leaves. Treatments applied were the standard captan (Thor 500 WP, Ag-Chem Africa) and mefenoxam (Ridomil Gold, Syngenta) drench treatments. Dosages were for captan 5 g/1L water and mefenoxam 0.1 ml/planting bag which is the recommended dosages to the nurseries. Volumes applied to the planting bags were adjusted according to the medium in the bag. Ammonium phosphite (Brilliant, Arysta Life Science) and potassium phosphite (Fighter, Ag-Chem Africa) were also applied as drenches to determine if they have the same

effect as mefenoxam and captan. All treatments were applied once. Four weeks after treatment, samples were taken to determine the *Phytophthora* levels in the medium through soil baiting. Seedlings were destructively evaluated at termination. Tree and root mass were measured for the different treatments.

4. *Evaluate phosphonates as foliar applications for their preventative and curative action against Phytophthora infestations of nursery trees.*

Carrizo citrange seedlings were transplanted into 5 L plastic planting bags containing a 50:50 mixture of sterilized river sand and composted pine bark. Seedlings were grown for a period of 6 months and watered as needed before the trial commenced.

Ammonium phosphite (Brilliant, Arysta Life Science), potassium phosphite (Fighter, Ag-Chem Africa) or potassium phosphonate (Phytex 200 SL, Villa Crop Protection) were applied to the seedlings as either a foliar spray or a drench. Dosages applied were according to the label recommendations for mature bearing citrus trees and application intervals were approximately 6 weeks. The respective dosages were 666 ml/100 L water for potassium phosphite, 570 ml/100 L water for potassium phosphite and 1 L/100 L water for potassium phosphonate. Foliar applications were done using a handheld spray applicator and seedlings were sprayed to just before run off. Prior to drench applications, irrigation was withheld for 1 day to ensure that the potting mixture will absorb the total drench volume. Drench volume applied by hand was 540 ml per 5 L bag. After drench application, irrigation was again withheld for 1 day to prevent the applied product from being washed from the potting mixture and allow for optimal uptake by the seedling roots.

The foliar sprays and drench applications were applied according to three different application regimes, with each regime applied to a separate set of seedlings. In winter applications were done in July, end of August and mid-2015, while summer applications were done at end of November, mid-January and the end of February. The last regime was a combination of the winter and summer applications that gave a total of 6 applications.

Phytophthora nicotianae inoculum was prepared by making a sterile soil and water mixture that consisted of 2 kg of sterilized soil mixed with 20 L of double distilled water. This mixture was inoculated by putting colonized agar blocks taken from a 7-day old *P. nicotianae* culture into the mixture, 50 blocks per batch of 20 L with a total of 80 L that was made up. The inoculated mixture was then incubated in the dark at 29 °C for 5 days before the water and soil mixture was used as inoculum. Seedlings was inoculated by pouring 250 ml of the inoculum mixture evenly over the potting mixture surface in the pot. After inoculation irrigation was again withheld for 1 day to prevent inoculum being washed out of the pots. The inoculation was done twice weekly for a period of 4 weeks that occurred during October.

Four weeks after the final applications the trial was evaluated destructively. Tree height, root mass, shoot mass and tree mass were determined for each treatment combination. *Phytophthora* infestation levels in roots were also determined by doing isolations from the roots. Isolations were done by rinsing the root systems under running tap water and blotting it dry on sterilized paper towel. From each root system 20 root pieces were cut at random and plated onto PARPH medium in 90 mm Petri dishes, 4 plates with 5 root pieces each. After isolation, plates were incubated at 29 °C for 48 hours before counting the number of root pieces yielding *Phytophthora nicotianae*. For each treatment combination the number of positive root pieces were noted. All recorded data were statistically analyzed using ANOVA and Fisher's LSD test at 95% confidence level in XLSTAT (Addinsoft, New York, USA).

Each chemical, inoculation/non-inoculated, application regime and application type combination was repeated four times on four seedlings that were placed in a randomized block design in the glasshouse. The whole trial was repeated twice at the same time.

5. *Morphological and molecular characterization of Pythium and Phytophthora isolates obtained from citrus nurseries.*

Phytophthora species identification

Selected isolates were grown on V8 agar at 29 °C for 7 d before mycelium were harvested for genomic DNA extraction. Genomic DNA was extracted from the mycelium using a modified CTAB based extraction protocol (Allen et al., 2006).

The ITS region of the isolates was amplified using the primers ITS 6 (Cooke and Duncan, 1997) and ITS 4 (White et al., 1990). The PCR reaction consisted of 20.0 µl GoTaq® G2 Hot Start Green Master Mix (Promega Corporation, Madison WI, USA), 1.0 µl of each primer (concentration of 10 µM), 16 µl PCR grade water and 2 µl genomic DNA, for a total volume of 40 µl. Amplifications were conducted in a 2720 Applied Biosystems (Applied Biosystems, Foster City, California, USA) thermal cycler. Initial denaturation was at 94 °C for 5 min, followed by 32 cycles of 94°C for 30 s, annealing for 30 s at 55 °C, extension at 72 °C for 30 s, with a final extension step at 72 °C for 5 min. PCR products were resolved in a 1% agarose gel and DNA fragments were visualized by staining with an ethidium bromide solution. The resulting PCR products were restriction digested with enzymes *Hinfl* and *HhaI* in a single reaction, according to the manufacturer's instructions (Fermentas Inc, Burlington, Ontario Canada). The PCR-RFLP products were run on a 3% agarose gel and isolates with the same RFLP banding pattern were assigned to the same RFLP group.

The ITS regions of at least two isolates of each PCR-RFLP group were sequenced and double stranded consensus sequences were obtained. The consensus sequences were subjected to BLAST analyses in Genbank (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and identified to species level based on a similarity of at least 99% to existing *P. nicotianae* or *P. citrophthora* ITS sequences on Genbank (<https://www.ncbi.nlm.nih.gov/genbank/>).

Pythium species identification

Pythium isolates were plated onto corn meal agar (CMA) medium and grown for 7 days at 25°C. DNA extraction was done using a standard cetyl trimethyl ammonium bromide (CTAB) based extraction protocol (Doyle and Doyle, 1987). A 25 µl PCR reaction for one isolate consisted of 2 µl diluted genomic DNA, 12.5 µl Promega G2 GoTaq Master Mix, 0.75 µl of both primers (10uM) ITS 4 and ITS 6 and 9 µl nuclease free water. Amplifications were conducted in a 2720 Applied Biosystems thermal cycler. This reaction amplifies a portion of the ITS region of the nuclear ribosomal RNA. The reaction parameters were as follows: 94°C, 5 min; 32 cycles of 94°C, 30 s; 58°C for 30 s, 72°C for 30 s and completed with 72°C for 5 min and followed a final step at 4°C. PCR products were resolved in a 1.5 % agarose gel and DNA fragments were visualized by staining with an ethidium bromide solution.

The resulting PCR products were restriction digested with enzymes *Hinfl* and *HhaI* in a single reaction, according to the manufacturer's instructions (Fermentas Inc, Burlington, Ontario Canada). The multi enzyme reaction mixture of one isolate consists of 1 µl of each enzyme, 10 µl enzyme buffer (X10), 8 µl distilled water and 8 µl PCR product. The 20 µl tubes were incubated at 37 °C for 5-15 min. PCR-RFLP products were run on a 3% agarose gel and isolates with the same RFLP banding pattern were assigned to the same RFLP group. Some isolates could not be separated based on the dual restriction enzyme PCR-RFLP. Therefore, with these isolates a single enzyme digest was done, using only the enzyme *HhaI* and 9 µl of distilled water in the reaction mixture.

The ITS regions of at least two isolates of each PCR-RFLP group were sequenced and double stranded consensus sequences were obtained. The consensus sequences were subjected to BLAST analyses in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) and preliminary identified to species level based on a similarity of at least 99% to existing *Pythium* spp. The ITS region, for certain isolates did not exhibit conclusive distinction between species that are closely related and therefore the *cox2* gene sequence data was generated in order to get a more definite species identity. The PCR reaction mixture for the *cox2* gene amplification and visualization was the same except the forward primer, *cox2-F*, and the reverse primer, *cox2-RC4*, was used. The amplification parameters were: 95°C, 4 min; 36 cycles of 95°C, 40 sec; 50°C for 40 sec, 72°C for 60 sec and completed with 72°C for 5 min and followed by 4°C.

From Hyde et al. (2014), 104 representative sequences were selected for inclusion in the phylogenetic analyses of the ITS and cox2 regions. The two representative isolates from the closely related genus, *Lagenidium*, were used as outgroups. Sequences of the two gene regions were aligned separately using the G-INS-i and L-INS-i algorithms respectively for the cox2 and ITS regions in the MAFFT plugin of Geneious R9 (Kato and Standley., 2013), and concatenated in Geneious R9. Maximum likelihood analysis was performed in PhyML-mpi (Guindon et al., 2010). Branch support was calculated from 100 bootstrap replicates for the concatenated dataset only. FigTree v. 1.3.1 (<http://tree.bio.ed.ac.uk/software/figtree/>) was used to view and edit the Maximum likelihood phylogenetic tree and branch values of less than 60% was left out.

6. Determine the level of mefenoxam resistance present in *Phytophthora* and *Pythium* isolates obtained from nurseries.

Phytophthora sensitivity testing

The sensitivity testing was conducted according to the slightly amended protocol described in Timmer et al., (1998). A total of 54 *P. citrophthora* and 59 *P. nicotianae* isolates from different nurseries in different citrus production areas, in South Africa, were selected and plated out on 90 mm Petri dishes containing corn meal agar (Sigma-Aldrich, St. Louis, Missouri, USA) and incubated at 29 °C for a period of 5 d. After incubation, 5 mm plugs were cut from the edge of the actively growing cultures and plated onto 90 mm Petri dishes containing CMA amended with mefenoxam (Ridomil Gold 450 EC, Syngenta, Basel, Switzerland) at 0, 1.0, 2.0, 5.0, 10.0, 25.0, 50.0 or 100.0 ppm. These plates were then incubated at 29 °C for 2 d.

Each isolate concentration combination was repeated using two plates while the whole trial was repeated twice at the same time. Colony diameter was measured in two directions and the average colony diameter was calculated for each isolate at each concentration. The percentage inhibition for each plate at each concentration for all the isolates were calculated and subjected to statistical analyses to group isolates and determine an EC₅₀, -80 and -90 value for each isolate. The percentage inhibition was calculated using the following equation:

$$\% \text{ inhibition} = \frac{\text{control (0 ppm)} - \text{measurement}}{\text{control (0 ppm)}} \times 100$$

The Mitscherlich function [$y = a(1-e^{-bx})$] or % Inhibition = Maximum Inhibition [1-e-(Rate)(Concentration)] fitted the data well and was used throughout the study. For the rest of this study % inhibition will be referred to as %Inhb and maximum inhibition, MaxInhb. The function was fitted for the two Petri dishes representing each isolate concentration combination within each of the two trials.

EC₅₀, -80, -90 values were calculated from the estimated regression parameters (MaxInhb and Rate of inhibition) for each isolate. Wherever MaxInhb < EC, these respective values could not be calculated according to the appropriate equations (EC₅₀ = (-log(1-(50/a)))/b; EC₈₀ = (-log(1-(80/a)))/b; EC₉₀ = (-log(1-(90/a)))/b). MaxInhb did not always give realistic values, especially where the Rate of inhibition was very slow, because MaxInhb is a value somewhere at a theoretical concentration. An additional value was therefore calculated: PInhbConc100 = a [1-e^{-b(100)}]. This value represents the %Inhb at a fungicide concentration of 100 ppm which gave a more realistic interpretation within the boundaries of the data than just MaxInhb. Regression parameters and EC₅₀, -80, -90 values were subjected to analysis of variance (ANOVA; ANOVA not shown) and cluster analysis using Ward's clustering method to cluster isolates. Principle component analysis (PCA) was also done with the 113 isolates and cluster numbers as labels, to see if the grouping or clustering obtained from the cluster analysis made sense.

Pythium species sensitivity testing

Purified *Pythium* isolates were plated out on 90 mm Petri dishes CMA and incubated for 7 days at 25°C. After incubation, 5 mm plugs were cut from the edge of the actively growing cultures and plated onto 90 mm Petri dishes amended with mefenoxam, an isomer of metlaxyl, fungicide from a 1000 ppm stock solution that was made up with 0.208 µl mefenoxam suspended in 100 ml water and added at appropriate rates to the molten agar to give 0, 1.0, 2.0, 5.0, 10.0, 25.0, 50.0 and 100 parts per million (ppm). Each isolate concentration combination was repeated using two plates while the whole trial was repeated twice at the same time. The resulting four plates per fungicide concentration per isolate were incubated for 48 hours at 25°C. Colony diameter was measured in two directions and the average colony diameter was calculated for each isolate at each concentration. The percentage inhibition for each plate at each concentration for all the isolates were calculated and subjected to statistical analyses to group isolates and determine an EC₅₀, -₈₀ and -₉₀ value for each isolate. The percentage inhibition was calculated per isolate using the following equation:

$$\% \text{ inhibition} = \frac{\text{control (0 ppm)} - \text{measurement}}{\text{control (0 ppm)}} \times 100$$

The Mitscherlich function ($y=a(1-e)^{-bx}$) or $[\% \text{Inhibition} = \text{Maximum Inhibition}(1-e)^{-(\text{Rate})(\text{Concentration})}]$ fitted the data well and used throughout the study. For the rest of this study % inhibition will be referred to as %Inhb and maximum inhibition, MaxInhb. The function was fitted for the two Petri dishes representing each isolate concentration combination within each of the two trials. Only isolate 58 had a different pattern and no convergence with this feature could be obtained. It was therefore omitted from further analyses.

EC₅₀, -₈₀, -₉₀ values were calculated from the estimated regression parameters (MaxInhb and Rate) for each isolate. Wherever MaxInhb < EC, these respective values could not be calculated according to the appropriate equations ($EC_{50} = (-\log(1-(50/a)))/b$; $EC_{80} = (-\log(1-(80/a)))/b$; $EC_{90} = (-\log(1-(90/a)))/b$). MaxInhb did not always give realistic values, especially where the Rate is very slow, because MaxInhb is the value somewhere at a theoretical concentration. An additional value was therefore calculated: $[\text{PInhbConc}100 = a(1-e)^{-b(100)}]$. This value represents the %Inhb at a fungicide concentration of 100 ppm which gave a more realistic interpretation within the boundaries of the data than just the MaxInhb. Regression parameters and EC₅₀, -₈₀, -₉₀ values were subjected to analysis of variance (ANOVA; ANOVA not shown) and cluster analysis using Ward's clustering method to cluster isolates. Principle component analysis (PCA) was also done with the 94 isolates and cluster numbers as labels, to see if the grouping or clustering obtained from the cluster analysis made sense. At first, seven clusters were specified only to ensure that all possible groupings were distinguished. On the PCA with the full data set there were only three clear variables. However, for a group of four isolates no EC₅₀ value could be calculated and therefore, for these isolates the EC₅₀ was set with % Inhibition < 50% = 100, which is very artificial. These four isolates were therefore omitted from further analysis. This resulted in the remaining isolates being grouped into six clearly distinct groups. The six groups were rated from 1-6 where 1 was very insensitive to the fungicide tested and 6 very sensitive. The groups were further compared with the help of ANOVA and the means were compared using Student's t-test.

7. *Testing the level of chlorine sensitivity of Pythium and Phytophthora isolates obtained from citrus nurseries.*

Phytophthora species sensitivity

Hong et al., (2003) found that *Phytophthora* spp. mycelium fragments are more insensitive to chlorine than zoospores. A mycelium fragment suspension was therefore used in the chlorine sensitivity trials. Ten percent V8 broth (V8 Original Vegetable Juice, Campbell Soup Company, USA) was prepared by adding 0.5 g CaCO₃ (Calcium carbonate; Merck, Kenilworth, New Jersey, USA) and 50 mL V8 juice to a Schott bottle containing 450 mL filtered water and autoclaved at 121°C for 15 min. *Phytophthora* isolates of the two species (32 *P. nicotianae* and 30 *P. citrophthora*) were plated out onto 90 mm Petri dishes containing CMA medium, and incubated for 7 -

10 d at 29 °C. After sufficient growth, the agar from one 90 mm Petri dish was divided into smaller pieces using a scalpel, and placed into the prepared 10% V8 broth. The inoculated V8 broth was then placed on an orbital shaker (100 rpm; FHM electronics, SHKO 20, South Africa) at 29 °C for 21 d in the dark.

To prepare the mycelium broth, autoclaved filtered water was adjusted to pH 6.5, using a pH meter (Combo pH & EC, HI 98129, Hanna Instruments, Woonsocket, Rhode Island, USA). Adjustment of pH was done using sodium hydroxide (NaOH 40 g mol⁻¹, Merck; 2 g in 250 mL autoclaved, filtered water) and hydrochloric acid (HCl 32%, Merck; 5 mL in 250 mL autoclaved, filtered water). The mycelium mass harvested from the 10% V8 broth was drained using a 180-µm sieve before being washed twice with 100 mL autoclaved filtered water. Excess water was then pressed out of the remaining fungal mycelium mass using two sterile stainless steel teaspoons. For trial purposes a measured *Phytophthora* suspension was prepared by blending 1 g (wet mass) mycelium in 100 mL filtered water (pH 6.5) for 30 s, followed by filtration (1000 µm sieve) into 500 mL deionized water (pH 6.5).

Trial variables included chlorine concentration (0, 1.5, 3 and 6 ppm) and different chlorine exposure times (0, 5, 10, 30 and 60 min). A chlorine stock solution (SS) was prepared by adding 0.15 g chlorine granules (HTH®, Kempton Park, Johannesburg, South Africa) to 100 mL filtered, autoclaved water (pH 6.5). From this chlorine SS, 0, 0.75, 1.5 or 3.0 mL chlorine was added to different Schott bottles containing 500 mL water to make solutions of 0, 1.5, 3 and 6 ppm active chlorine respectively. As a positive control, a 1.5 and 6 ppm active chlorine solution was tested using a chlorine photometer (Total Chlorine Ultra High Range Portable Photometer, HI 96771, Hanna Instruments Inc.) before the commencement of each trial set. The aforementioned chlorine solutions were also de-activated to ensure that the sodium thiosulfate (Na₂S₂O₃*5H₂O; 248.21 g/mol, Merck) stock solution (1.47 g in 1000 mL filtered, autoclaved water) was still functional.

The prepared mycelium suspension was mixed on a magnetic stirrer plate for 10 min before being used to inoculate two PARPH plates as a positive control. Each mycelium suspension (two separate suspensions per *Phytophthora* isolate) was amended (treated) with a specified active chlorine suspension at one of abovementioned concentrations and mixed for a further 30 s. Following each exposure time, 40 mL of solution was dispensed into two containers and de-activated using the sodium thiosulfate SS. For deactivation 0, 0.3, 0.6 or 1.2 mL sodium thiosulfate SS was required to de-activate 0, 1.5, 3 and 6 ppm active chlorine, respectively. De-activated solution from each container was used to inoculate two PARPH plates (1 mL) and subsequently spread using a hockey stick and incubated for 2 days at 29°C; Insta-Test® low range [0 – 10 ppm] free chlorine test strips (LaMotte) were used to confirm de-activation.

Following incubation, *Phytophthora* spp. colonies were counted and percentage mortality determined using the following formula: $[(C_n - T_n) / C_n] * 100$ where C_n is the number of colonies on control plates and T_n the number of colonies on treated plates. The percentage mortality data was subjected to statistical analyses using SAS (SAS Institute Inc. NC, USA). Fisher's LSD was furthermore calculated at the 5% level to compare means.

Pythium species sensitivity

The effect of chlorine concentration and different exposure times on the mortality of nine *Pythium* species (19 isolates) was evaluated. Isolates were first grown on CMA for seven days, then 10 plugs of pure mycelium (6 mm diameter) were transferred to petri dishes and flooded with just enough V8 broth (Galindo and Gallegly, 1960) to completely cover all the plugs. Isolates were cultured in the V8 broth for seven days at 25°C. The V8 broth was prepared by mixing 175 mL V8 juice into 800-900 mL deionized water with 3.5 g CaCO₃ to make up 1 L. V8 broth were then autoclaved for 15 min at 121°C.

One-week-old cultures, grown and incubated at 25°C were used to make the mycelium suspension for the chlorine sensitivity tests. After seven days, the V8 mycelium broth from each petri dish was filtered through a 150 µm sterile metal sieve, washed twice with 100 mL autoclaved, deionized water to remove nutrients and blotted dry with paper toweling. One gram of mycelium was blended for 30 s in deionized water (pH 6.5). The blended mycelium was

poured through a 1000 µm sterile metal sieve into a 500 mL Schott bottle and deionized water (pH 6.5) was added to make final volume up to 500 mL before mixing well.

The mycelia of the isolates were exposed to four different chlorine concentrations (0, 1.5, 3 and 6 ppm) at five different exposure times (0, 5, 10, 30 and 60 min), plus an untreated positive control. The active chlorine concentrations were measured with chlorine strips. Chlorine stock solution was prepared from 1.15 g HTH (Arch Chemicals) powder with 100 mL deionized water adjusted to pH 6.5. Sodium thiosulfate stock solution was prepared by adding 0.147 g sodium thiosulfate into 100 mL water. Chlorine treatments were achieved by mixing a relevant amount of chlorine stock solution (0, 0.75, 1.5 and 3 mL) with 500 mL of mycelium suspension respectively on a magnetic stirrer for 30 s. For each chlorine concentration 0, 0.3, 0.6 and 1.2 mL sodium thiosulfate stock solution were added to de-activate 0, 1.5, 3 and 6 ppm chlorine, respectively, in 40 mL solution. Chlorine strips were used to determine whether the solution is de-activated completely.

At each contact time, an aliquot of 500 µL was removed from each 40 mL container and spread onto two petri dishes containing PARP medium (Timmer et al., 1998). These petri dishes were then incubated for two days at 25 °C and the total number of *Pythium* colonies in each petri dish was recorded. The test was repeated twice for each chlorine concentration x exposure time x isolate combination and the percentage mortality at each concentration and exposure time was calculated using the formula $[(C_n - T_n)/C_n] * 100$ where C_n is the number of colonies on control plates and T_n the number of colonies on treated plates. The percentage mortality data was subjected to statistical analyses using SAS (SAS Institute Inc. NC, USA). Fisher's LSD was furthermore calculated at the 5% level to compare means.

8. *Testing the pathogenicity of the identified Pythium spp. towards citrus nursery seedlings.*

Troyer citrange rootstock seedlings were used in the pathogenicity tests. Pathogenicity tests were conducted in a glasshouse with controlled temperatures of 25°C. The rootstock seeds were sown in 1L plastic bags filled with sterile soil and grown until the seedlings reached a height of 10-30 cm. Prior to planting, the potting medium was sterilized at 80°C for 180 min.

In preparing the sand bran inoculum, each *Pythium* isolate was cultured on corn meal agar (CMA, Sigma-Aldrich) for seven days at 25°C. The sand-bran inoculum was prepared by mixing 400 g washed river sand with 40 g wheat bran and 60 mL distilled water in 1000 mL Schott bottles before autoclaving for 15 min at 121°C for two consecutive days. Ten (6 mm diameter) CMA plugs, containing *Pythium* mycelium was used to inoculate the sand-bran mixture (one isolate per bottle). The control bottles were inoculated with sterile, uncolonized CMA agar plugs only. Additionally, a combination of *Pythium irregulare* and *Phytophthora nicotianae* were included as positive control inoculation, along with a *P. nicotianae* alone control inoculation. The inoculum was incubated for 14 days at 25°C without direct light exposure. It was shaken every second day to ensure even growth of the mycelium throughout the inoculum mixture.

For inoculation, a concentration of 50 g/L - inoculum/planting bag was used. Inoculation was done by placing the inoculum into four holes made around seedlings in the pots. After inoculation the seedlings were watered every second day until the trial were terminated. Seedlings were arranged in a randomized block design and each isolate was replicated on eight seedlings. Pathogenicity of the isolates were determined by evaluating and measuring the shoot length before and after inoculation, seedling weight, root weight and volume, and the percent survival rate after 11 weeks of incubation. Results were analyzed statistically using SAS (SAS Institute Inc. NC, USA). Re-isolations were made from seedling roots to fulfill Kock's postulates. Roots were washed under tap water to remove the soil. Samples were surface-sterilized with 70% ethanol for 30 s and placed on sterile filter paper to dry. Four small root pieces were plated onto each PARP and PARPH petri-dish and incubated in the dark at 25°C. Pure fungal growth emerging from the plated tissue were transferred to V8 medium to study the morphological characteristics. After two days, 10 V8-agar plugs (6 mm diameter) were transferred to petri dishes and flooded with just enough non-sterile soil extract (NSSE) to completely cover all the plugs. Plates were placed under

fluorescent lights for 24 h to induce sporangium formation. Isolates were identified morphologically to genus level based on the morphological features of *Pythium* and *Phytophthora* (Santoso et al., 2015; Puglisi et al., 2017).

Results and discussion

1. Collection of *Pythium* and *Phytophthora* isolates from citrus nurseries.

Upon completion of isolate collection, 54 *Phytophthora nicotianae*, 59 *P. citrophthora* and 103 *Pythium* spp. isolates were collected. These isolates were used in the rest of the study.

2. Testing of alternative fumigants to replace MeBr for the treatment of potting medium.

Results of *Phytophthora* testing after treatment of CPB or RS with metham sodium indicated a clear effect of the treatment (Table 4.3.2.1). In the untreated CPB 4.94% of leaf discs was infested with *Phytophthora* spp. and 37.06% discs was infested with *Pythium* spp. From the RS 0.93% of leaf discs was infested with *Phytophthora* spp. and 25.15% with *Pythium* spp (Table 4.3.2.1). After drench treatment with a 0.9 ml/L metham sodium solution, soil baiting results indicated that the treatment was effective in totally eliminating *Phytophthora* and *Pythium* infestation from the CPB and RS (Table 4.3.2.1).

Table 4.3.2.1. Mean percentage *Phytophthora* or *Pythium* infested leaf discs in untreated and metham sodium treated composted pine bark or river sand.

Treatment	Medium	Mean % <i>Phytophthora</i> infested leaf discs	Mean % <i>Pythium</i> infested leaf discs
Untreated	Composted pine bark	4.94a ¹	37.06a
Untreated	Sand	0.93b	25.15b
Metham sodium	Composted pine bark	0.00b	0.00c
Metham sodium	Sand	0.00b	0.00c
P-value		0.08	<0.0001

¹Means followed by the same letter are not significantly different at a 90% confidence level.

3. Evaluating currently used mefenoxam and captan applications for effective control of *Phytophthora* infestations in coir and coir mixtures.

The statistical analysis of the *Phytophthora* soil baiting done on treated media four weeks post treatment revealed a significant potting medium and treatment interaction ($P = 0.0491$). The effectiveness of the various treatments was markedly different in the different potting mediums (Table 4.3.2.2). In coir the best two treatments were captan and mefenoxam that had mean percentages of *Phytophthora* infested leaf discs of 8.57% and 6.43% respectively. This was significantly lower than the untreated control with a mean of 70.71% infested leaf discs (Table 4.3.2.2). In the composted pine bark (CPB) abovementioned two treatments were again the best performing, both being statistically better than the untreated control. The captan was again the best performing treatment. However, in this case the two treatments had mean percentages of *Phytophthora* infested leaf discs of 17.14% and 19.29% respectively, which were higher than seen in the coir. In the CPB:Coir mixture captan was effective, having only 2.14% infested leaf discs compared to the untreated control that had 92.14%. In this case the mefenoxam was the second best treatment. However, it had a mean of 33.57% infested leaf discs which indicates poor control of *Phytophthora* although it was significantly better than the untreated control (Table 4.3.2.2). Captan in sand was 100% effective in that it had a mean of 0.0% *Phytophthora* infested leaf discs, indicating that the single treatment eradicated the *Phytophthora* infestation in the inoculated sand. The other three treatments, including the mefenoxam treatment were not effective, not performing significantly better than the untreated control (Table 4.3.2.2).

From the results it is evident that the captan treatment were the most effective in the different media. However, some loss in efficacy was seen in the media with higher organic matter content. This could be due to the captan, a contact fungicide, binding to the organic matter, making it ineffective to kill the *Phytophthora* propagules in the potting media. The mefenoxam on the other hand was more effective in the organic media such as the Coir and CPB mixtures. Gondar et al. (2013) found that with increase in organic matter in a soil, the adsorption levels of this fungicide increases. Combined with this Monkiedje and Spiteller (2005) found that the half-life of mefenoxam increases in soil as the organic matter content increases. Our results could therefore indicate that the mefenoxam if for longer active and therefore effective in the media with high organic matter compared to the sand where it might readily leach out of the media. The lack of total control of the *Phytophthora* by the different fungicides could also be due to some level of fungicide insensitivity of the *Phytophthora* isolate mixture used.

Future work should therefore investigate the optimal dosage of captan and mefenoxam in different growth media differing in organic matter content. With this can be combined treatment of *Phytophthora* isolates with different levels of fungicide sensitivity.

Table 4.3.2.2. Mean percentage *Phytophthora* infested leaf discs recorded from soil baiting from four different potting mediums four weeks post treatment with four different chemicals.

Potting medium	Treatment	Mean % <i>Phytophthora</i> infested leaf discs
Coir	ammonium phosphite	17,14eg ¹
	captan	8,57f-h
	potassium phosphate	25,71d-h
	mefenoxam	6,43f-h
	Untreated control	70,71ab
Composted pine bark (CPB)	ammonium phosphite	50,17b-d
	captan	17,14e-h
	potassium phosphate	37,86c-e
	mefenoxam	19,29e-h
	Untreated control	62,86bc
CPB:Coir	ammonium phosphite	42,14c-e
	captan	2,14g-h
	potassium phosphate	49,29b-d
	mefenoxam	33,57d-f
	Untreated control	92,14a
Sand	ammonium phosphite	24,29d-h
	captan	0,00h
	potassium phosphate	37,86c-e
	mefenoxam	28,57d-g
	Untreated control	30,00d-g
P-value		0,0491

¹ Means followed by the same letter are not significantly different at a 95% confidence level ($P = 0.05$).

4. Evaluate phosphonates as foliar applications for their preventative and curative action against *Phytophthora* infestations of nursery trees.

Analyses of the results revealed several significant interactions. In the first, a chemical x application type interaction ($P = 0.0001$) was observed for plant biomass. These results showed that for ammonium and potassium phosphite trees that received foliar sprays, regardless of application regime, had significantly higher mean plant biomass than the untreated inoculated control (Table 4.3.2.3). Mean plant biomass of inoculated seedlings receiving ammonium phosphite as a foliar spray was 126.60 g compared to inoculated seedlings receiving potassium

phosphite as foliar sprays that had a mean mass of 118.83 g. Both these were significantly higher than the mean plant mass of seedlings that received these respective chemicals as drench applications as well as the untreated inoculated control (99.93 g; Table 4.3.2.3). For potassium phosphonate the pattern was reversed with the inoculated seedlings where a potting medium drench was applied having the highest plant biomass (128.43 g), again significantly higher than the untreated control (99.93 g; Table 4.3.2.3). Another significant interaction was the chemical x application type interaction ($P = 0.0073$) observed for mean shoot length (Table 4.3.2.3). For ammonium and potassium phosphite, the best application method in terms of mean shoot length was foliar applications. The mean shoot length was respectively 1610.60 mm and 1665.73 mm that were both significantly more than the untreated inoculated control (1290.61 mm). Drench application of potassium phosphonate again gave the best results and had a mean shoot length of 1651.45 mm that was also significantly more than the untreated, inoculated control (Table 4.3.2.3).

A chemical x application regime interaction ($P = 0.0229$) was also observed for mean plant biomass (Table 4.3.2.4). For ammonium phosphite applications, the best application regimes were regimes 1 and 2 that both performed significantly better than the untreated control. In regime 1 the chemical was applied only during the winter period of July to October which led to a mean plant biomass of 124.07 g which was not significantly better than regime 2 (123.16 g) where the applications were done from July until February the following year. However, both chemical x application regime interactions were statistically better than the untreated inoculated control (99.93g; Table 4.3.2.2).

Ammonium phosphite applications according to regime 3 (October to February sprays) were the worst performing regime and did not result in mean seedling biomass that was significantly higher than the untreated control (Table 4.3.2.4). In the case of potassium phosphite applications, none of the regimes led to mean seedling biomass significantly more than the untreated control. However, regimes 2 and 3 led to between 10 g and 14 g heavier seedlings compared to the untreated control (Table 4.3.2.4). This was much better than regime 1 where the difference in mass was only 2.51 g (Table 4.3.2.4). Regimes 2 and 3 were also for potassium phosphonate applications the best regimes with regime 3 having a slightly higher mean seedling biomass than regime 2. Both were, however, significantly better than the untreated inoculated control (Table 4.3.2.4). Regime 1 were not significantly poorer than the other two regimes or the untreated control. It did, however, have a mean seedling biomass 10 g to 15 g lower than the other two regimes (Table 4.3.2.4).

Table 4.3.2.3. Mean plant biomass and shoot length of *Phytophthora nicotianae* inoculated citrus rootstock seedlings subjected to ammonium phosphite, potassium phosphite or potassium phosphonate drench or foliar applications in comparison to an untreated control.

Chemical	Application type	Mean plant biomass (g) ¹	Mean shoot length (mm) ¹
Ammonium phosphite	Drench	105,10c-e	1482,38ab
	Foliar spray	126,60b	1610,60ab
Potassium phosphite	Drench	98,94e	1424,33bc
	Foliar spray	118,83a-c	1665,73a
Potassium phosphonate	Drench	128,43a	1651,45a
	Foliar spray	114,29b-d	1517,84ab
Untreated control	—	99,93de	1290,61c
P-value		0.0001	0.0073

¹ Means followed by the same letter are not significantly different at a 95% confidence level ($P = 0.05$).

Table 4.3.2.4. Mean plant biomass of *Phytophthora nicotianae* inoculated citrus rootstock seedlings subjected to ammonium phosphite, potassium phosphite or potassium phosphonate applications according to three different application regimes in comparison to an untreated control.

Chemical	Application regime	Mean plant biomass (g) ¹
Ammonium phosphite	1	124,07ab
	2	123,16ab
	3	100,33c
Potassium phosphite	1	102,44c
	2	113,44a-c
	3	110,78bc
Potassium phosphonate	1	112,78a-c
	2	123,42ab
	3	127,86a
Untreated control	—	99,93c
P-value		0.0229

¹ Means followed by the same letter are not significantly different at a 95% confidence level ($P = 0.05$).

The application type and regime did not show any interaction with the mean percentage *Phytophthora* infested root pieces. The different chemical treatments did, however have a significant ($P < 0.0001$) effect on the mean percentage *Phytophthora* infested root pieces, with all performing significantly better than the untreated control (Table 4.3.2.5). The best treatment was potassium phosphonate which had a mean percentage infested root pieces of 0.08% versus the 0.89% and 0.30% of potassium phosphite and ammonium phosphite respectively (Table 4.3.2.5). As mentioned above, all treatments had significantly lower percentage of infested root pieces than the untreated inoculated control (16.07%; Table 4.3.2.5).

Table 4.3.2.5. Mean percentage *Phytophthora nicotianae* infested root pieces in inoculated citrus rootstock seedlings following treatment with ammonium phosphite, potassium phosphite or potassium phosphonate.

Treatment	Mean % <i>Phytophthora</i> infested root pieces ¹
Ammonium phosphite	0,30a
Potassium phosphite	0,89a
Potassium phosphonate	0,08a
Untreated control	16,07b
P-value	< 0.0001

¹ Means followed by the same letter are not significantly different at a 95% confidence level ($P = 0.05$).

These results clearly showed that the application of phosphonates to nursery seedlings is highly beneficial. It not only increases plant size significantly but also gives significant protection of seedling roots against *Phytophthora nicotianae* infection. It was furthermore shown that the best results were achieved by foliar applications and that six applications, spread out over the summer and winter growth periods, gave the best results. These results are in agreement of the findings of Oren and Yogeve (2002) and Walker (1988) who indicated that foliar or drench applications reduced the level of root infections by *Phytophthora nicotianae* while increasing seedling size in comparison to the untreated inoculated control.

5. *Morphological and molecular characterization of Pythium and Phytophthora isolates obtained from citrus nurseries.*

Phytophthora characterization

Based on the ITS-RFLP analysis, the 113 isolates were divided into two distinct groups. ITS sequence analyses of representative isolates from each group identified 54 of the isolates obtained from citrus nurseries as *P. nicotianae*. The remaining 59 isolates were identified as *P. citrophthora*. The species identity was based on a 100% nucleotide homogeneity with *P. nicotianae* and *P. citrophthora* isolates lodged from previous studies on Genbank (<https://www.ncbi.nlm.nih.gov/genbank/>).

Pythium characterization

A maximum likelihood phylogeny was constructed of *Pythium sensu lato* (s.l.) based on the concatenated ITS and cox2 sequence data (Figure 4.3.2.1). Both regions were used because some species are indistinguishable with just the use of ITS (Robideau et al., 2011). Due to uncertain regions, genetically diverse species complexes also arise. PCR amplification yielded 862-1000 bp (base pairs) for the ITS region and the cox2 yielded gene regions of 581 bp. Strains in bold (CONS) are the isolates used in this study and the genus *Lagenidium* was used as the outgroup. The results for this section all refers to Figure 1.

The first clade (99% bootstrap support) consists of the *P. irregulare* complex that includes *P. irregulare*, *P. cryptoirregulare*, *P. cylindrosporium* and *P. regulare* (Spies et al., 2011) and is taxonomically challenging. *P. irregulare sensu stricto* (s.s) and *P. cryptoirregulare* are morphologically similar whereas *P. cylindrosporium* and *P. regulare* are morphologically distinct. Isolates 81, 76, 106, 131, 174, 149, 59, 80, 117, 42, 110, 84, 52, 9, 39, 79, 127 and 222 are clustered with the ex-type strain *P. irregulare* s.s. (CBS 250.28) and are therefore considered to be *P. irregulare*. Isolates 82, 4, 2, 19, 24 and 198 are similar to the ex-type strain of *P. cryptoirregulare* (CBS 118731) and isolates 144, 18, 40, 28, 152, 172, 97, 113, 108 and 175 are similar to the ex-type strain of *P. cylindrosporium* (CBS 218.94) and these isolates are respectively considered to be *P. cryptoirregulare* and *P. cylindrosporium* in this study. The *P. irregulare* s.s. clade has acceptable bootstrap support with 89%; whereas *P. cylindrosporium* has support of 100%. However, the *P. cryptoirregulare* clade had <60% bootstrap support.

Phytopythium is a new genus that was introduced to accommodate *Pythium* clade K, where the clade was first described in Lévesque and De Cock (2004), that forms an intermediate genus between *Pythium* and *Phytophthora* (De Cock et al., 2015). The *Phytopythium* clade has a bootstrap support of 87%. Isolates 91 and 203 formed a well-supported clade (100% bootstrap support) within the *Phytopythium* clade and is a possible new species, yet to be described, which is closely related to the ex-type strain *Phytopythium mercuriale* (CBS 122443). Isolates 137 and 111 are clustered within the *Phytopythium vexans* species complex clade. Isolate 137 grouped with the representative ex-type strain *Phytopythium vexans* s.s. (CBS 119.80) and therefore considered to be *Phytopythium vexans* s.s and 111 is *Phytopythium vexans* s.l. The next clade with isolates 197, 105, 193, 167 and 16 have varying bootstrap values that ranges from a moderate 64- to a well-supported 99%. The data grouped relatively well in this clade that included reference sequences of ex-type strains *P. nunn* (CBS 808.96) and *P. orthogonon* (CBS 376.72), but within the clade there was not good support to distinguish between *P. nunn* and *P. orthogonon*. These isolates were therefore from here on referred to as *P. nunn/orthogonon*. Isolate 85 clustered with the reference sequence of ex-type strain *P. splendens* (CBS 462.48) with good bootstrap support of 100% and is therefore *P. splendens*.

The clade that consists of ex-type strains *P. arrhenomanes* (CBS 324.62), *P. aristosporium* (CBS 263.38) and isolate 202 have 100% bootstrap support. However, these two *Pythium* spp. are indistinguishable using cox1 and ITS (Robideau et al, 2011). According to the Mycobank database, *P. arrhenomanes* is the oldest entry (Drechsler 1928), thus gets higher priority and, after conducting BLAST, a 99% identity was found and isolate 202 is considered to be *P. arrhenomanes*. Isolate 102 clustered with ex-type strains *P. periillum* (CBS 169.68), *P. graminicola* (CBS 327.62) and *P. tardicrescens* (Lev1534) and the clade has a 100% bootstrap support. This isolate is considered to be *P. graminicola* because, according to this study, this species can be distinguished from *P. periillum* with cox2 using BLAST with a 99% similarity and is the oldest entry in Mycobank (Subramaniam, 1928).

Isolates 161 and 160 is considered to be *P. torulosum* with a branch support of 99% and the reference sequence is CBS 316.33. Isolate 155 is considered to be *P. myriotylum* with a bootstrap support of 99% and identified with *cox2* and BLAST (99% similarity) to this particular species. The clade containing isolates 104 and 65 have a bootstrap support of 100% and consists of the *P. coloratum* species group that involves ex-type strains namely, *P. coloratum* (CBS 154.64), *P. dissotocum* (CBS 166.68), *P. marinum* (CBS 750.96) and *P. lutarium* (CBS 222.88). These species have very similar ITS and *cox2* sequences, making it hard to distinguish between them and thus are these two isolates furthermore considered to belong to the *P. coloratum* group.

Thus, in short 10 species were identified in this study, namely *P. irregulare* complex, one possible novel species that has yet to be described, *Phytophthium vexans* complex, *P. nunn/ortogonon*, *P. splendens*, *P. arrhenomanes*, *P. graminicola*, *P. torulosum*, *P. myriotylum* and *P. coloratum* species group.

P. irregulare s.s were the species mostly isolated in this study (35%). The *P. irregulare* complex isolates consists of 84% identified in this study and the high incidence of *P. irregulare* was in accordance to Maseko and Countinho (2002) where *P. irregulare* was the most commonly isolated species in citrus nurseries. The results from this study confirm that there are overall several *Pythium* species in citrus nurseries and confirm results of Thompson et al., 1995 and Maseko and Countinho, 2002.

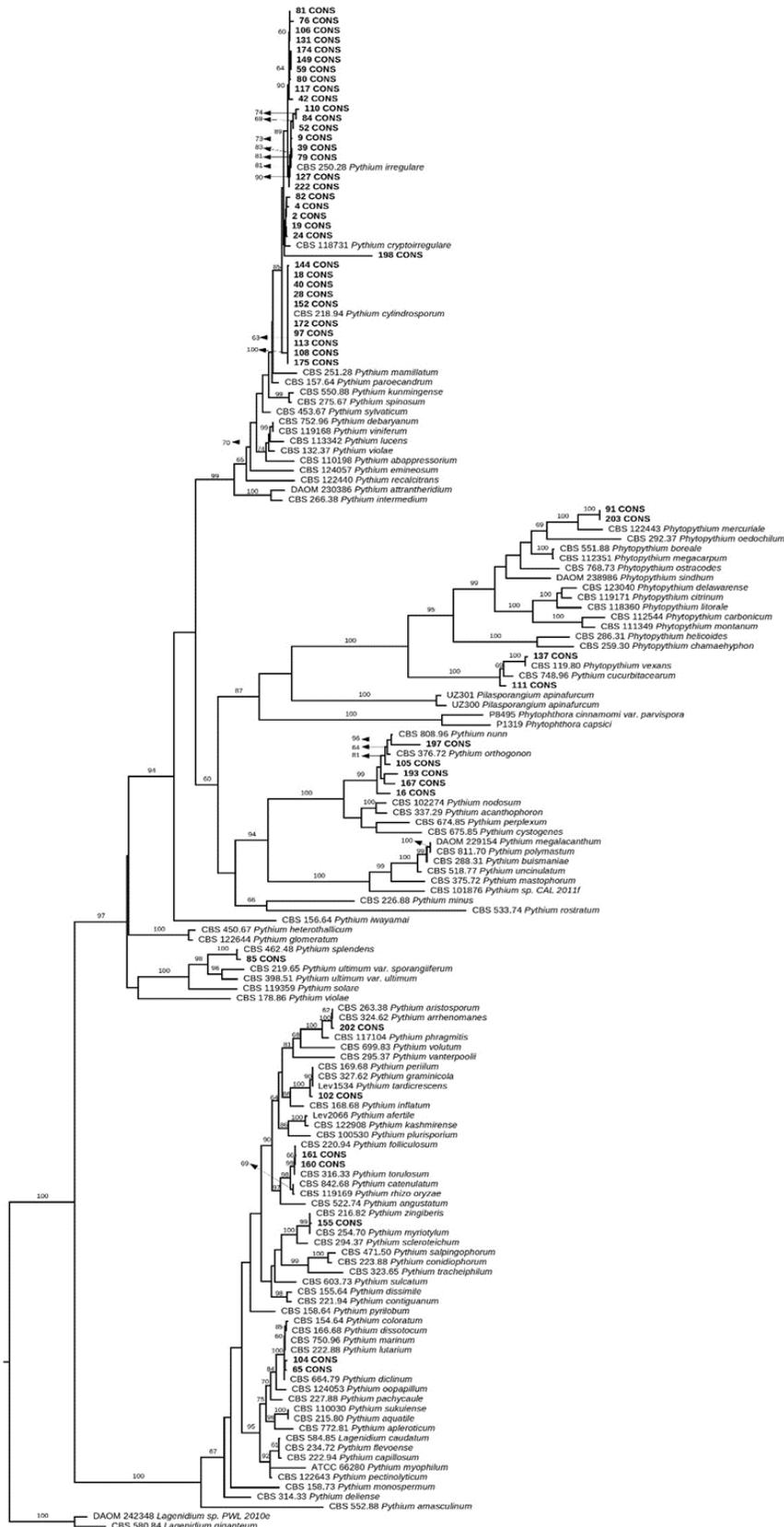


Figure 4.3.2.1. Maximum likelihood phylogeny of *Pythium* s.l. based on the concatenated ITS and cox2 regions. CONS represent isolates in this study. Related genus *Phytophthora* and *Lagenidium* are included. Bootstrap support values below 60% are not shown.

6. Determine the level of mefenoxam resistance present in *Phytophthora* and *Pythium* isolates obtained from nurseries.

Phytophthora sensitivity testing

Both the Ward's cluster analysis and PCA revealed that the *P. citrophthora* and *P. nicotianae* isolates could be divided into 6 sensitivity groups. The analysis of variance (ANOVA) comparing the groups using the regression parameters and EC₅₀, -₈₀ and -₉₀ data showed highly significant ($P < 0.0001$) sensitivity group effects for EC₅₀, EC₈₀ and EC₉₀ (ANOVA not shown). The ANOVA of the PlnhbCon100 and Rate of inhibition data showed a significant sensitivity group x species interaction ($P < 0.0001$ and $P = 0.0021$ respectively; ANOVA not shown).

Within group 6 the isolates of both *P. citrophthora* and *P. nicotianae* were 100% inhibited by a concentration of 100 ppm mefenoxam. Within group 5 the *P. nicotianae* isolates reached a mean of 96.02% inhibition that was significantly more compared to the *P. citrophthora* isolates in the same group (92.35% inhibition) (Table 4.3.2.6). Both these means of group 5 were significantly lower compared to those of group 6. Also, within group 4 the mean inhibition of *P. nicotianae* isolates was 80.94% that was statistically more than the mean inhibition (70.29%) of the *P. citrophthora* isolates in this group (Table 4.3.2.6). As before, these two means were lower than that observed for the two species in groups 5 and 6. Only *P. citrophthora* isolates were grouped into group 2 and 3. In these groups the mean inhibition of isolates were 54.88% (group 3) and 44.30% (group 2), significantly poorer than the means observed in the other groups (Table 4.3.2.6). Isolates of both species were placed in group 1 that had the lowest sensitivity to mefenoxam. In this group the *P. citrophthora* isolates were 39.26% inhibited compared to the *P. nicotianae* isolates that were only 30.74% inhibited. The mean percentage inhibition for both species in group 1 were then also significantly the lowest observed in any of the groups for either species (Table 4.3.2.6).

The rate of inhibition results for the group x species interaction showed similar trends as seen for the PlnhbCon100 results. The groups with the higher PlnhbCon100 means, also had the highest rates of inhibition (Table 4.3.2.6). Group 6 had statistically the highest mean rates of inhibition of all the groups. The rate of inhibition of *P. citrophthora* isolates were 17.75, significantly higher than the rate of the *P. nicotianae* isolates (15.82) in this group. The inhibition rate of the *P. citrophthora* isolates in group 5 was 2.62 that was significantly faster again than the rate of the *P. nicotianae* isolates (1.86). This latter mean rate was comparable to the rates observed for the two species in group 4 (Table 4.3.2.1). In this group the mean rate of inhibition for *P. citrophthora* was 1.81 compared to the mean for *P. nicotianae* that was 1.31. In groups 1 – 3 the mean rates of the two species were statistically the same and ranged between 0.002 and 0.068 (Table 4.3.2.6).

For the isolates of both species occurring in sensitivity group 1 it was shown that at 100 ppm mefenoxam, the maximum inhibition only reached a mean of 39.36% and that the rate of inhibition was only 0.068 (Table 4.3.2.6). Consequently, no EC₅₀, EC₈₀ or EC₉₀ values could be determined for this group (Table 4.3.2.7). In the case of group 2, the mean EC₅₀ value was 123.69 ppm that was statistically higher than the EC₅₀ mean of any other group. Similarly, the EC₈₀ (214.12 ppm) and EC₉₀ (250.25 ppm) values of this group was the highest of all the groups (Table 4.3.2.7). In the case of group 3, a mean EC₅₀ could be calculated which was 76.12 ppm, the second highest of all the groups. Again no EC₈₀ or EC₉₀ means could be determined, possibly also due to the slow rate of inhibition of this group (Tables 4.3.2.6 and 4.3.2.7). Group 4 had mean EC₅₀ (0.82 ppm) and EC₉₀ (2.86 ppm) values that were statistically similar to those of groups 5 and 6. However, an EC₉₀ could also not be calculated for this group (Table 4.3.2.7). Groups 5 and 6 had EC₅₀ (0.45 ppm), EC₈₀ (0.10 ppm) and EC₉₀ (1.68 ppm) means that were statistically similar to those of group 6. For the latter group the mean EC₅₀ (0.04 ppm), EC₈₀ (0.10 ppm) and EC₉₀ (1.15 ppm) values was the lowest of all the groups (Table 4.3.2.7). This indicated that the isolates falling in this group were the most sensitive to mefenoxam.

It was seen that within the different sensitivity groups, the number of isolates of *P. citrophthora* and *P. nicotianae* varied greatly. In the case of *P. citrophthora* most of the isolates (89.7%) fell in groups 1 – 4 where the percentage inhibition at 100 ppm mefenoxam ranged between 39.36% and 70.29% (Table 4.3.2.8). Based on the classification of Hu *et al.*, (2008) these will then be seen as intermediately insensitive or sensitive to mefenoxam. However, for *P. nicotianae* it was seen that only 5% of isolates fell in abovementioned groups while 95% fell in groups 5 and 6. These would therefore be classified as mefenoxam sensitive (Table 4.3.2.8).

In the study of Hwang and Benson (2005) isolates of *P. cryptogea*, *P. nicotianae* and *P. palmivora*, occurring on floriculture crops in North Carolina, was also divided into different mefenoxam sensitivity groups. In groups 1 – 3 the mean percentage inhibition at 100 ppm mefenoxam was below 60%, indicating according to the study of Hu *et al.*, (2008), that the isolates of *P. citrophthora* and *P. nicotianae* in these groups were insensitive to mefenoxam. Compared to this the isolates of these two species in groups 4 and 5 can be regarded as intermediately sensitive, while the isolates in group 6 would then be sensitive. It was furthermore seen that in the different groups with the lowest mefenoxam sensitivity that *P. citrophthora* had the highest number of isolates in these insensitive groups, compared to *P. nicotianae* isolates. Within the groups *P. citrophthora* often also had a lower percentage of inhibition compared to *P. nicotianae*, indicating lower mefenoxam sensitivity. This is in agreement with the findings of Farih *et al.*, (1981) and Coffey and Bower (1984) that found *P. citrophthora* isolates from citrus being less sensitive to mefenoxam compared to *P. nicotianae* isolates from the same host. Results of the mean EC₅₀, EC₈₀ and EC₉₀ values of the different groups indicated that these values could not be determined for the isolates in groups 1 - 4. This is probably due the fact that at 100 ppm mefenoxam, the highest concentration used in this study, the calculated percentage inhibition for *P. citrophthora* and *P. nicotianae* isolates in these groups was below 50 – 90%. It is therefore clear that for these isolates 100% inhibition will only be achieved at mefenoxam concentrations well above 100 ppm. This was illustrated by the results from group 2 where the EC₉₀ value was 250.25 ppm mefenoxam.

In a study by Timmer *et al.* (1998) similar results were obtained where they found that certain isolates of *P. nicotianae* from citrus had EC₅₀ values that were also above 100 ppm. Similarly, did Farih *et al.* (1981) find that for some isolates of *P. citrophthora* and *P. nicotianae* from citrus, 100% inhibition of mycelial growth was only observed at concentrations above 100 ppm. High levels of mefenoxam or metalaxyl insensitivity among isolates of these two species occurring on citrus is therefore not unknown. Even among *P. nicotianae* isolates from ornamental crops it was discovered by Ferrin and Kabashima (1991) that the highly insensitive isolates had EC₅₀ values above 100 ppm. In the remaining groups in the current study, the EC values were well below 2 ppm and in the case of group 6 even below 0.2 ppm which also corresponds to findings among isolates from citrus in abovementioned studies.

Within *P. infestans* the basis of mefenoxam or metalaxyl insensitivity and differences in sensitivity between isolates within a species, and between species, were found to be due to genotypic differences between the isolates and species (Goodwin *et al.*, 1996). The specific level of sensitivity within a genotype was determined by the insensitivity loci present in the specific genotype (Fabritius *et al.*, 1997). Childers *et al.*, (2015) furthermore discovered that sensitive isolates of *P. infestans* can acquire mefenoxam resistance upon repeated exposure *in vitro* to the fungicide. However, it was also seen that *in vitro* the isolates with acquired resistance did lose some of their resistance when they were repeatedly plated onto media not amended with mefenoxam. It is therefore possible that sensitive isolates of *P. nicotianae* and *P. citrophthora* can also acquire resistance to mefenoxam when repeatedly exposed to this fungicide in a nursery or orchard. Careful use of this fungicide in citrus nurseries are therefore very important to prevent the development of highly resistant isolates in the nursery that will find their way to a new orchard. This becomes even more important in light of the findings of Timmer *et al.*, (1998) who clearly showed that insensitive isolates from citrus nurseries can compete with sensitive isolates in their ability to cause root rot. Furthermore, these insensitive isolates maintain their insensitivity for a period of time even after use of the fungicide was stopped.

Table 4.3.2.6. Mean PlnhbConc100 and rate of inhibition values of *Phytophthora citrophthora* and *Phytophthora nicotianae* isolates grouped into mefenoxam sensitivity groups 1-6 following *in vitro* exposure to different mefenoxam concentrations.

Sensitivity group	Species	PlnhbConc100 (%)	Rate of inhibition
1	<i>P. citrophthora</i>	39.36 h ¹	0.068 e
	<i>P. nicotianae</i>	30.74 i	0.036 e
2	<i>P. citrophthora</i>	44.30 g	0.002 e
	<i>P. nicotianae</i>	---	---
3	<i>P. citrophthora</i>	54.88 f	0.027 e
	<i>P. nicotianae</i>	---	---
4	<i>P. citrophthora</i>	70.29 e	1.811 d
	<i>P. nicotianae</i>	80.94 d	1.307 d
5	<i>P. citrophthora</i>	92.35 c	2.616 c
	<i>P. nicotianae</i>	96.02 b	1.858 c
6	<i>P. citrophthora</i>	100.00 a	17.752 a
	<i>P. nicotianae</i>	100.00 a	15.820 a
LSD		3.430	0.7200
P – value		< 0.0001	0.0021

¹Means followed by the same letter are not statistically different at a 95% confidence level.

Table 4.3.2.7. Mean EC₅₀, EC₈₀ and EC₉₀ values of the different mefenoxam sensitivity groups identified after *in vitro* exposure of *Phytophthora citrophthora* and *Phytophthora nicotianae* isolates to different mefenoxam concentrations.

Sensitivity group	EC ₅₀	EC ₈₀	EC ₉₀
1	---	---	---
2	123.69 a ¹	214.12 a	250.25 a
3	76.11 b	---	---
4	0.82 c	2.86 b	---
5	0.45 c	1.11 b	1.68 b
6	0.04 c	0.10 b	0.15 b
LSD	5.101	8.545	12.608
P – value	< 0.0001	< 0.0001	< 0.0001

¹Means followed by the same letter are not statistically different at a 95% confidence level.

Table 4.3.2.8. Number of *Phytophthora citrophthora* and *Phytophthora nicotianae* isolates occurring in the different mefenoxam sensitivity groups.

Species	Mefenoxam sensitivity group	No of isolates in group
<i>Phytophthora citrophthora</i> (n= 58)	1	5 (8.6%)
	2	2 (3.4%)
	3	4 (6.9%)
	4	41 (70.7%)

	5	4 (6.9%)
	6	2 (3.4%)
<i>Phytophthora nicotianae</i> (n = 60)	1	1 (1.7%)
	2	0 (0.0%)
	3	0 (0.0%)
	4	2 (3.3%)
	5	35 (58.3%)
	6	22 (36.7%)

Pythium sensitivity

The initial Ward's cluster and PCA analyses indicated seven isolate groups (Figure 4.3.2.2 and 4.3.2.3). Within these seven groups rated from 0-6, group 0 consisted of four isolates for which an EC₅₀ could not be calculated due to its percentage inhibition not reaching 100% at any of the tested concentrations (Figure 4.3.2.2). At a mefenoxam concentration of 100 ppm, the theoretical level of inhibition was calculated to be 41.97% (Table 4.3.2.9). Omitting this group of isolates from further PCA analyses, led to isolates being grouped in six distinct groups based on the regression parameters of MaxInhb and Rate and EC₅₀ values (Figure 4.3.2.4). The six groups were consequently rated from 1-6 where 1 was very insensitive to the fungicide tested and 6 very sensitive. Group 0 was therefore seen as very resistant to mefenoxam.

The ANOVA of the mean regression parameters (%InhbCons100 and Rate) and EC₅₀, ₈₀, and ₉₀ values indicated significant ($P < 0.0001$) differences between isolates [IsolateNr (Group)] within the different groups. This indicated that variation existed between isolates within groups with regards to the different variables. Between the 6 groups it was furthermore seen that with regards to abovementioned variables, there were also significant ($P < 0.0001$) differences. As stated above, group 0 consisted of 4 isolates and no EC₅₀ could be calculated for this group as it never reached 50% inhibition at any of the tested concentrations (Figure 4.3.2.2). At a mefenoxam concentration of 100ppm this group's mean percentage inhibition (%InhbCons100) was furthermore calculated at 41.97% which was significantly lower than any of the other groups' values (Table 4.3.2.9). This group had a mean rate (0.781) that were significantly lower than that of groups 2, 4, 5 and 6, but higher than groups 1 and 3. This indicated that its percentage inhibition increased at a faster rate than the latter groups but that it never reached a mean percentage inhibition above 50% (Table 4.3.2.9; Figure 4.3.2.2).

The mean %InhbCons100 value for group 1 was calculated at 92.43% which was significantly higher compared to groups 0, 2 and 4 but significantly lower than groups 3, 5 and 6 (Table 4.3.2.9). Although statistically similar to group 3, the rate of group 1 was 0.022 that was the lowest of all the groups, indicating that the mean percentage inhibition within this group increased very slowly with an increase in mefenoxam concentration (Figure 4.3.2.2). This then also supports the fact that this group had significantly higher mean EC₅₀, ₈₀ and ₉₀ values (30.60 66.84 and 90.21 ppm, respectively; Table 4.3.2.9). Group 2 had a mean %InhbCons100 value of 62.07%, significantly lower than those of groups 1, 3, 4, 5 and 6 (Table 4.3.2.9). This group furthermore did not reach an inhibition level above 80% and therefore EC₈₀ and ₉₀ values could not be calculated for this group (Table 4.3.2.9, Figure 4.3.2.2). Its rate of inhibition development was 1.812 which was significantly higher than groups 0, 1 and 3 but significantly lower than the rest (Table 4.3.2.9). This rate then led to an EC₅₀ value of 1.158 ppm which was third highest of all the groups (Table 4.3.2.9). In the case of group 3, it had a mean %InhbCons100 value of 95.26% that was the third highest among all the groups (Table 4.3.2.9). This group's rate was at 0.124 significantly lower than the rates of the other groups, except for group 1. This led to this group having, behind group 1, the highest EC₅₀, ₈₀ and ₉₀ values (6.774, 17.395 and 25.897 ppm respectively). These were significantly higher than the corresponding values of the other groups, except group 1 (Table 4.3.2.9).

Due to group 4 never reaching 90% inhibition, an EC₉₀ value could not be calculated for this group (Table 4.3.2.9, Figure 4.3.2.2). Its mean %InhbCons100 value was 82.66% that was significantly higher than groups 0 and 2 but lower than those of the other groups (Table 4.3.2.9). This group furthermore had the third highest rate of 2.879, significantly lower than groups 5 and 6 but significantly higher compared to groups 0 – 3. This relative rapid increase in percentage inhibition caused this group to have a mean EC₅₀ value of 0.376 that was statistically amongst the three lowest values along with groups 5 and 6. Its EC₈₀ value (1.301) was furthermore statistically similar to group 5 but higher compared to group 6 and lower than groups 1 and 3 (Table 4.3.2.9). Again, due to the percentage inhibition never reaching 90% or more, an EC₉₀ value could not be calculated for this group (Table 4.3.2.9, Figure 4.3.2.2). Group 5 had statistically the third highest %InhbCons100 value of 94.42% and also the second highest rate (3.180) behind group 6 (Table 4.3.2.9). This high rate of increase in percentage inhibition led to group 5 having statistically the same mean EC₅₀ (0.280) than group 6 and statistically lower than the EC₅₀ values of the other groups, except group 4 (Table 4.3.2.9). Its EC₈₀ value was also statistically similar to groups 4 and 6 while its EC₉₀ value was significantly higher than group 6 but significantly lower than groups 1 and 3. Group 6 represented the most sensitive isolates and had significantly the highest %InhbCons100 value (99.85%) and rate (19.000) (Table 4.3.2.9). This in turn led this group having the lowest EC₅₀, ₈₀, and ₉₀ values that were in most cases statistically similar to significantly lower than the other groups of isolates (Table 4.3.2.9).

The ANOVA conducted in this study of the mean regression parameters (%InhbCons100 and Rate) and EC₅₀, ₈₀, and ₉₀ values indicated significant ($P < 0.0001$) differences between isolates within the different groups (0-6). This indicated that there was variation between isolates within groups. Between the seven main groups there were also significant ($P < 0.0001$) differences. This clearly shows that there is fungicide sensitivity variance within a *Pythium* population and even highly insensitive isolates (Group 0) where no EC₅₀ could be calculated for this isolate as it never reached 50% inhibition at any of the tested concentrations. Moorman *et al.* (2002) stated that an isolate is considered mefenoxam resistant if 100 µg of mefenoxam per ml does not slow an isolate's growth by 50% compared with growth where there no fungicide is present (EC₅₀ > 100 µg/ml). The species *P. cylindrosporium*, *P. dissotocum*, *P. heterothallicum*, *P. splendens*, and *P. ultimum* displayed resistance to mefenoxam and 38- and 37% of the *P. aphanidermatum* and *P. irregulare* isolates were resistant.

The range of mefenoxam sensitivity among *Pythium* isolates in this study was in accordance to those reported from other mefenoxam studies mentioned above. Maseko and Countinho (2002) stated that *Pythium* spp. are not a serious problem in citrus and does not play an important role, and the results in this study are very contradictory to this statement. It may be needed to routinely determine the fungicide sensitivity level of pathogens because of the continuously movement of plant material in nurseries. If the *Pythium* spp. can be identified to species level and the varying mefenoxam sensitivity for the isolates can be obtained, better control measures can be put in place and cultivators can rotate the use of fungicides.

Table 4.3.2.9. Mean Max% Inhibition, %InhbCons100, Rate, EC₅₀, ₈₀, and ₉₀ values of the 6 isolate groups identified based on Ward's cluster and PCA analyses.

Group	N	%InhbCons100	Rate	EC ₅₀	EC ₈₀	EC ₉₀
0	8	41.97 g	0.781 e	-	-	-
1	8	92.43 d	0.022 f	30.599 a	66.838 a	90.207 a
2	12	62.07 f	1.812 d	1.158 c	-	-
3	30	95.26 b	0.124 f	6.774 b	17.395 b	25.897 b
4	14	82.66 e	2.879 c	0.376 d	1.301 c	-
5	50	94.42 c	3.180 b	0.280 d	0.707 d c	1.239 c
6	65	99.85 a	19.000 a	0.037 d	0.085 d	0.122 d
LSD		0.590	0.203	0.350	0.691	0.974

¹Means followed by the same letter are not significantly different.

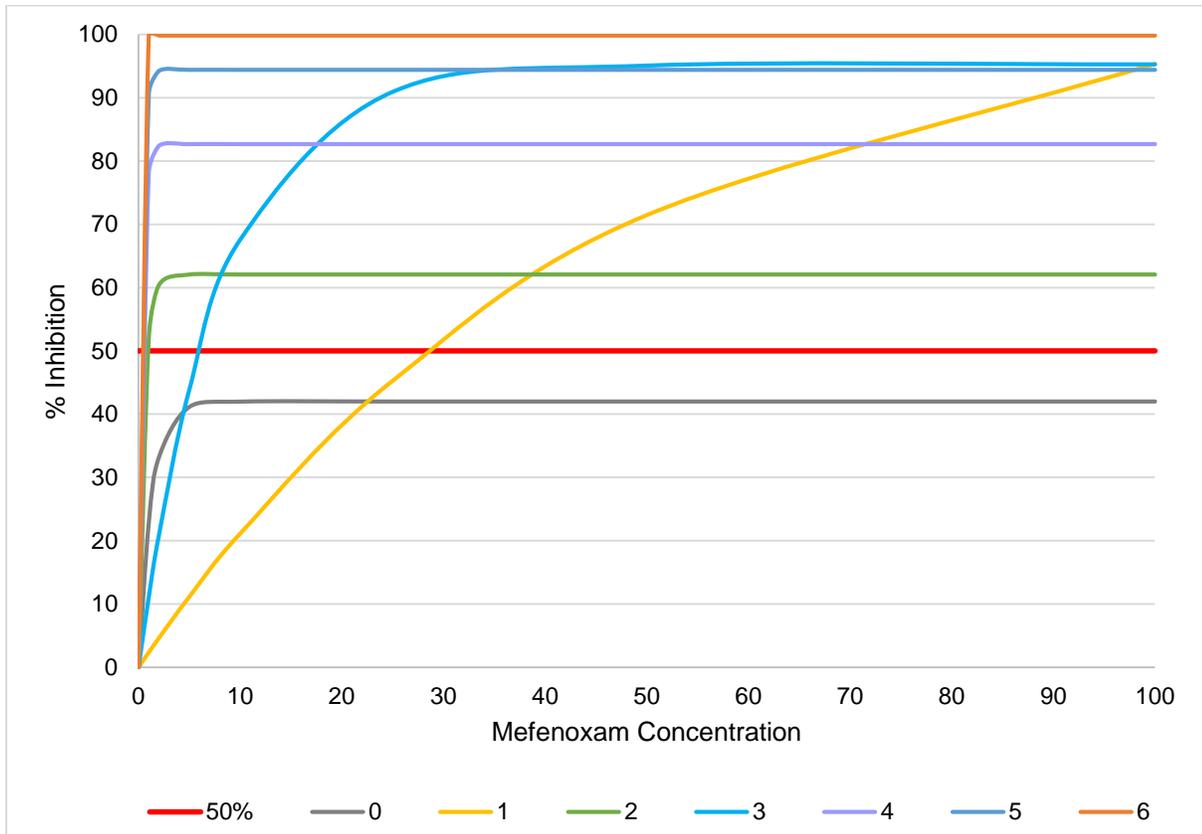


Figure 4.3.2.2. Mean percentage inhibition values for each isolate group plotted against mefenoxam concentration (ppm).

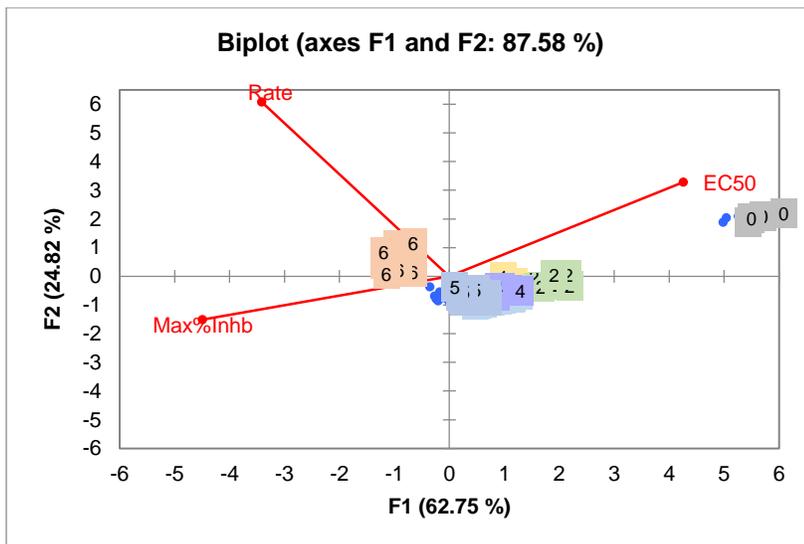


Figure 4.3.2.3. Principle component analysis (PCA) indicating 7 isolate groups based on the regression parameters (Max%Inh and Rate) and EC₅₀ values.

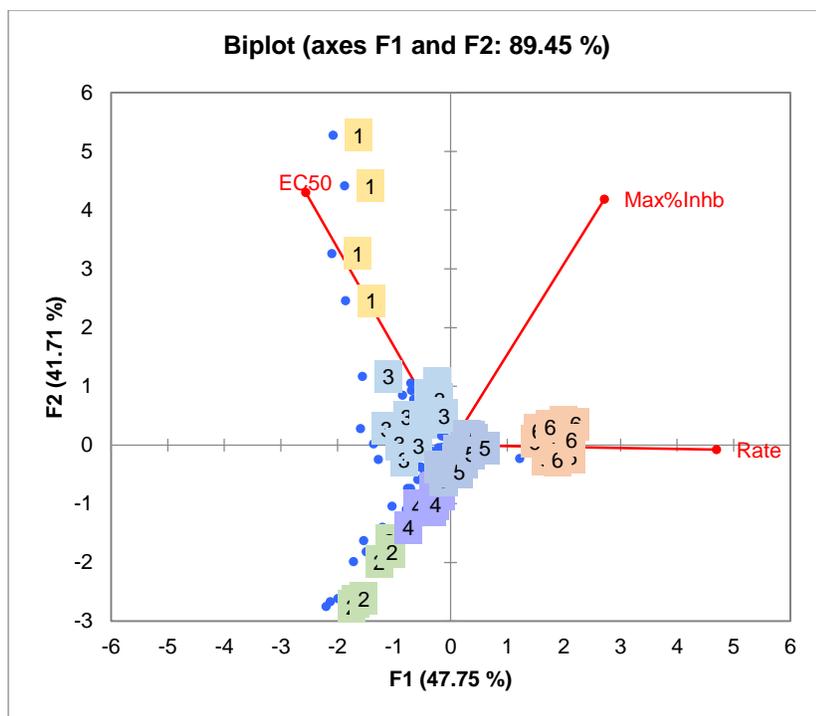


Figure 4.3.2.4. Principle component analysis (PCA), without group 0, indicating 6 distinct isolate groups based on the regression parameters (MaxInhb and Rate) and EC₅₀ values.

7. *Testing the level of chlorine sensitivity of Pythium and Phytophthora isolates obtained from citrus nurseries.*

Phytophthora species sensitivity

The analysis of variance (ANOVA) of the percentage mortality data indicated highly significant ($P < 0.0001$) experimental repetition x species x isolate x chlorine concentration interaction as well as an experimental repetition x chlorine concentration x exposure time interactions. These multifactor interactions are attributed to the significant ($P < 0.0001$) variation seen in the percentage inhibition between the two experimental repetitions, which could be due to the different mycelial suspensions used for each repetition, combined with the significant ($P < 0.0001$) variation seen in mean percentage inhibition between the different chlorine concentrations (results not shown). Between isolates within the two *Phytophthora* spp., the ANOVA furthermore indicated that there was statistical ($P < 0.0001$) differences between percentage inhibition reached. The inhibition of *P. citrophthora* isolates by chlorine ranged from 27.09% to 73.47%, whereas *P. nicotianae* isolates' inhibition values ranged from 19.69% to 62.30%. The results from the significant ($P < 0.0001$) chlorine concentration x exposure time interaction indicated, as expected, no mortality of the *Phytophthora* spp. recorded at 0 ppm chlorine. When the chlorine concentration was increased to 1.5 ppm, the percentage mortality at no exposure (chlorine deactivated immediately) was 9.20%. This percentage then increased with each subsequent increase in exposure time to reach a maximum of 17.18% after 60 min exposure to 6 ppm active chlorine (Table 4.3.2.10). When the 3 ppm chlorine was deactivated immediately, the mean mortality was still 46.64% which then increased significantly to end at 77.50% after 60 min exposure. At 6 ppm chlorine the initial mortality was 85.14% with immediate deactivation. Again, an increase in exposure time led to a higher mortality and reached 99.12% after a 60 min exposure time (Table 4.3.2.10).

Interesting results was also observed in regards to the sensitivity of *P. citrophthora* and *P. nicotianae* isolates towards different concentrations of active chlorine. No difference was observed between isolates of the two species. It was clearly shown that increasing exposure time at a specific chlorine concentration, increased percentage mortality. However, mean percentage mortality only came close to 100% when the isolates (32 *P.*

nicotianae and 30 *P. citrophthora*) were exposed to 6 ppm chlorine for 60 min. Hong *et al.* (2003) tested limited numbers of isolates, originating from irrigation water from ornamental nurseries, of *P. nicotianae*, *P. capsici*, *P. cinnamomi*, *P. citricola*, *P. citrophthora*, *P. cryptogea* and *P. megasperma* for their chlorine sensitivity. They also observed that with an increase in active chlorine concentration, the mean percentage mortality increased. However, they did not see a concentration and exposure time interaction such as seen in the current study. This could be due to the low numbers of isolates of the different species tested. Chlorination was introduced in South African citrus nurseries based on the study done by Grech and Rijkenberg (1992). They indicated that chlorination eliminates soilborne pathogen propagules from irrigation water, consequently reducing the level of *Phytophthora* infection in the roots of citrus rootstock seedlings irrigated with treated water. The current study was to our knowledge the first study focusing on chlorine sensitivity of multiple *P. citrophthora* and *P. nicotianae* isolates subjected to a range of chlorine concentrations and exposure times. Practically, the results of the current study indicate that for complete elimination of *Phytophthora* spp. propagules from citrus nursery irrigation water, treatment with 6 ppm active chlorine for 60 min or longer is required.

Table 4.3.2.10. The mean percentage mortality of *Phytophthora nicotianae* and *Phytophthora citrophthora* propagules exposed to 0, 1.5, 3.0 or 6.0 ppm active chlorine of exposure times of 0, 5, 10, 30 or 60 minutes.

Active chlorine concentration (ppm)	Exposure time (min)	Mean % mortality ¹
	0	0.00 n
	5	0.00 n
0	10	0.00 n
	30	0.00 n
	60	0.00 n
	0	9.20 m
	5	13.57 l
1.5	10	15.62 k
	30	15.96 jk
	60	17.18 j
	0	46.64 i
	5	58.87 h
3	10	63.97 g
	30	73.10 f
	60	77.50 e
	0	85.14 d
	5	94.48 c
6	10	96.27 b
	30	98.60 a
	60	99.12 a
LSD		1.238

¹Means followed by the same letter are not significantly different.

Pythium species sensitivity

Analysis of variance (ANOVA) of the percentage mortality data indicated a significant species x isolate x exposure time interaction ($P = 0.0630$) at the 90% confidence level. However, due to the aim of determining the optimal chlorine concentration and exposure time for the treatment of citrus nursery irrigation water, the non-significant ($P = 0.9846$) interaction will be discussed in detail. The mean percentage mortality results indicated that, across the

19 isolates from different species the mean percentage mortality increased depending on the exposure time at a specific chlorine concentration (Table 4.3.2.11). As expected at 0 ppm chlorine there was no mortality recorded. However, at 1.5 ppm chlorine, the mean percentage mortality ranged from 87.82% at 0 min exposure time to 92.67% at an exposure time of 60 min (Table 4.3.2.11). At 3 ppm chlorine the mean percentage mortality at 0 min was 93.06% that increased to 97.87% at 60 min exposure time. At the highest concentration of 6 ppm chlorine, the mean percentage mortality started at 94.16% (0 min) and increased to 98.75% at 60 min exposure (Table 4.3.2.11).

The mortality of all the *Pythium* species increased as the exposure time increased at a specific chlorine concentration. Unlike sporangia and zoospores, the mycelia and conidia of fungal pathogens are coated with thick cell walls and the chlorine may need more time to oxidize the mycelia and conidia (Cayanan *et al.*, 2009). This explains why the highest mean percentage mortality was observed at 60 min for each chlorine concentration. The percentage mortality of the mycelial fragments increased with exposure time regardless of the chlorine concentrations.

Table 4.3.2.11. Mean percentage mortality of different *Pythium* species subjected to different active chlorine concentrations and exposure times.

Concentration	Exposure Time	N	%Mortality ¹
0	0	38	0.00 g
0	5	38	0.00 g
0	10	38	0.00 g
0	30	38	0.00 g
0	60	38	0.00 g
1.5	0	38	87.82 f
1.5	5	37	89.80 def
1.5	10	38	89.56 ef
1.5	30	38	91.95 cdef
1.5	60	38	92.67 bcdef
3	0	38	93.06 bcdef
3	5	38	93.13 bcde
3	10	38	93.92 abcde
3	30	38	95.75 abc
3	60	38	97.87 ab
6	0	38	94.16 abcde
6	5	38	94.95 abcd
6	10	38	95.28 abc
6	30	38	98.57 a
6	60	38	98.75 a
LSD			5.297

¹ Means with the same letter do not differ significantly.

8. *Testing the pathogenicity of the identified Pythium spp. towards citrus nursery seedlings.*

Analysis of variance (ANOVA) indicated no significant interaction between *Pythium* species based on seedling length increase or seedling weight. However, at the 90% confidence level, a significant interaction between species was observed for the root weight ($P = 0.0767$) and root volume ($P = 0.0767$) data. In comparison to the

uninoculated control, *P. myriotylum*, *Phytophythium mercuriale* and *P. cryptoirregulare* all cause an increase in both mean root weight and volume. However, none of the increases observed were significant (Table 4.3.2.12). *Phytophythium vexans s.s.*, *Phytophthora nicotianae*, *Phytophythium vexans s.l.*, *P. cylindrosporium* inoculation caused mean root weights between 1.95 g and 1.99 g that were slightly lower than the mean root weight observed in the uninoculated seedlings (2.00 g; Table 4.3.2.12). This slight reduction in root weight was not shown by the root volume data. For above-mentioned species the mean root volumes were in some cases higher and in some cases lower in comparison to the control. However, again these differences were not significant. The group of species that included *P. graminicola*, *P. coloratum*, *P. torulosum* and *P. irregulare s.s* showed mean seedling root weights ranging between 1.83 g and 1.88 g. Although not significantly lower than the mean root weight of the control seedlings (2.00 g) these reductions were markedly. In terms of mean root volume, inoculations with these species led to mean root volumes between 1.99 cm³ and 2.03 cm³ that were, although not significant, again lower than the mean root volume of the control seedlings (2.13 cm³; Table 4.3.2.12). The combination inoculation of *Phytophthora nicotianae* and *P. irregulare s.s* were the only inoculation that caused a significant reduction in both mean root weight and volume. This inoculation led to a mean root weight of 1.61 g and mean root volume of 1.80 cm³ in comparison with the control seedlings with means of 2.00 g and 2.13 cm³ respectively (Table 4.3.2.12).

The results from this study shows that there are at least four *Pythium* species that do reduce seedling root weight and volume that will be detrimental to seedling growth in the nursery. It was furthermore shown that in combination specifically *P. irregulare* in combination with *Phytophthora nicotianae* causes the most severe reduction in root weight and volume. This is of concern as this species was found to be the most abundant in citrus nurseries and are often found in nurseries where *P. nicotianae* is also present. Both these therefore need to be regarded as important and need to be properly managed in the citrus nurseries.

Table 4.3.2.12. Mean root weight (g) and volume (cm³) of seedlings inoculated with different *Pythium* species, *Phytophthora nicotianae* and a control.

Species	N	Weight Root ¹	Volume Root ¹
Control	8	2.00 a	2.13 a
<i>Pythium myriotylum</i>	8	2.08 a	2.20 a
<i>Phytophythium mercuriale</i>	8	2.04 a	2.17 a
<i>Pythium cryptoirregulare</i>	8	2.02 a	2.14 a
<i>Phytophythium vexans s.s</i>	8	1.99 a	2.14 a
<i>Phytophthora nicotianae</i>	8	1.99 a	2.12 a
<i>Phytophythium vexans s.l</i>	8	1.96 a	2.10 a
<i>Pythium cylindrosporium</i>	24	1.95 a	2.09 a
<i>Pythium graminicola</i>	8	1.88 a	2.03 ab
<i>Pythium coloratum</i>	8	1.87 ab	2.02 ab
<i>Pythium torulosum</i>	16	1.83 ab	1.99 ab
<i>Pythium irregulare s.s</i>	56	1.83 ab	1.99 ab
<i>Pyth irreg + Phyt nicot</i>	8	1.61 b	1.80 b
LSD		0.277	0.239

¹ Means with the same letter do not differ significantly at the 90% confidence level.

Conclusion

1. Captan and mefenoxam vary in their efficacy as curative treatments for *Phytophthora* infestation of nursery potting media. Captan is effective in media with high organic matter and sand while mefenoxam is only effective in media with higher organic matter content.

2. Phosphonate foliar applications during winter and summer proved to be effective in protecting rootstock seedlings from *Phytophthora nicotianae* infection while also increasing plant size.
3. A small percentage of *P. nicotianae* and *P. citrophthora* isolates were found to be highly insensitive to mefenoxam. Nurseries should therefore use this fungicide with caution.
4. Some *Pythium* isolates were also found to be highly insensitive to mefenoxam. This could explain why potting media tests often still show *Pythium* to be present after mefenoxam treatments.
5. In cases where mefenoxam insensitivity is suspected, nurseries should rather use captan as a curative treatment.
6. Based on the chlorine sensitivity studies it was shown conclusively that in order to completely eliminate propagules from irrigation water, it should be treated with at least 6 ppm active chlorine for more than 60 minutes.
7. *Pythium irregulare* were found to have the largest negative effect on seedling root growth and in combination with *P. nicotianae* it caused more severe root rot symptoms than each pathogen on its own.

Future research

1. Development of a molecular marker based test to determine the level of mefenoxam sensitivity of *P. nicotianae* and *P. citrophthora* isolates.
2. Development of a molecular marker based test to determine the level of mefenoxam sensitivity of *Pythium irregulare* isolates.
3. Study the effect of different potting media on the efficacy of mefenoxam and the development of media specific dosage recommendations for mefenoxam use in nurseries.

Technology transfer

1. J.M. van Niekerk, E. Basson, and C. Olivier. 2018. **Chlorine sensitivity of *Phytophthora nicotianae* and *P. citrophthora* isolates from South African citrus nurseries.** 10th Citrus Research Symposium, 19 - 22 August 2018, Champagne Sports Resort, KwaZulu-Natal, South Africa
2. J.M. van Niekerk, E. Basson, C. Olivier, A. McLeod. 2017. **Mefenoxam and chlorine sensitivity of *Phytophthora citrophthora* and *P. nicotianae* isolates in South African citrus nurseries.** 50th Annual Congress of the Southern African Society of Plant Pathology, 15 - 18 January 2017, Champagne Sports Resort, KwaZulu-Natal, South Africa
3. Jan van Niekerk, Elaine Basson and Adele McLeod. 2016. **Identification of *Pythium* spp. occurring in South African citrus nurseries.** 9th Citrus Research Symposium, 21 – 25 August 2016, Champagne Sports Resort, KwaZulu-Natal, South Africa
4. Jan van Niekerk, Charmaine Olivier, Elaine Basson and Adele McLeod. 2016. **Variation in mefenoxam sensitivity among *Phytophthora* spp. isolates from South African citrus nurseries.** 9th Citrus Research Symposium, 21 – 25 August 2016, Champagne Sports Resort, KwaZulu-Natal, South Africa
5. Elizabeth van der Merwe and Jan M. van Niekerk. 2018. **Identification and mefenoxam sensitivity of *Pythium* spp. occurring in South African citrus nurseries.** 10th Citrus Research Symposium, 19 - 22 August 2018, Champagne Sports Resort, KwaZulu-Natal, South Africa
6. J.M. van Niekerk, Basson, E., Olivier, C. and McLeod, A. 2016. **Mefenoxam sensitivity of *Phytophthora citrophthora* and *P. nicotianae* isolates in South African citrus nurseries.** International Citrus Congress, 18 – 23 September 2016, Mabu Thermas & Resort, Foz do Iguaço, Brazil

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

Project 1030 (2008 – 2018/19) by JM van Niekerk, MC Pretorius & C Olivier (CRI)

Summary

The aim of this project is to continuously search for better methods to control soilborne pathogens and citrus nematodes. In the past season a glasshouse trial was done to evaluate an algaecide for its ability to eliminate *Phytophthora nicotianae* root infections in rough lemon seedlings. The algaecide was applied at different concentrations and according to different regimes for a period of 6 months. Results indicated that, although not significantly, seedling growth was improved when 0.5 or 1.0 ppm of the algaecide was applied weekly for the duration of the trial. The seedlings from these treatments were bigger with bigger root systems compared to the untreated, un-inoculated and treated, inoculated controls. In further testing the use of this product for treatment of irrigation water can also be investigated. In the fumigation trial monitored it was seen that just more than 1 year after fumigation and planting, there was already a significant difference between the fumigated and non-fumigated treatments. The trees growing in the fumigated rows were significantly bigger than trees in the non-fumigated rows. It was furthermore seen that in the fumigated soil there were no juvenile citrus nematodes present while in the non-fumigated soil a mean number of 745 was present. In terms of *P. nicotianae* and *P. citrophthora* infested leaf discs the fumigated soils also had significantly lower levels of both pathogens compared to the non-fumigated soils. From these early results the beneficial effect of soil fumigation in a replant situation is already evident.

Opsomming

Die doel van hierdie projek is om voortdurend na beter metodes te soek vir die beheer van grondgedraagde patogene en sitrus-aalwurms. Die afgelope seisoen is 'n glashuisproef uitgevoer om 'n algdoder te evalueer vir sy vermoë om *Phytophthora nicotianae* wortel-infeksies in growwe skil suurlemoensaailinge uit te wis. Die algdoder is teen verskillende konsentrasies en volgens verskillende regimes vir 'n periode van 6 maande toegedien. Resultate het getoon dat, hoewel nie betekenisvol nie, saailingegroei verbeter het wanneer 0.5 of 1.0 dpm van die algdoder weekliks vir die duur van die proef toegedien is. Die saailinge vanaf hierdie behandelings was groter met groter wortelsisteme in vergelyking met die onbehandelde, ongeïnkuleerde en behandelde, geïnkuleerde kontroles. In verdere toetse kan die gebruik van hierdie produk vir die behandeling van besproeiingswater ook ondersoek word. In die berokingsproewe wat gemonitor is, is gesien dat net meer as een jaar ná beroking en plant, daar reeds 'n betekenisvolle verskil tussen berookte en nie-berookte behandelings was. Die bome wat in die berookte rye gegroei het, was betekenisvol groter as die bome in die nie-berookte rye. Daar is verder gesien dat in die berookte grond, geen onvolwasse sitrus-aalwurms teenwoordig was nie, terwyl in die nie-berookte grond, 'n gemiddelde getal van 745 teenwoordig was. In terme van *P. nicotianae* en *P. citrophthora* besmette blaarskyfies,

het die beroekte gronde betekenisvol laer vlakke van beide patogene in vergelyking met die nie-beroekte gronde gehad. Die voordelige effek van grondberoking in 'n herplantsituasie is reeds uit hierdie vroeë resultate duidelik.

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 trifoliolate orange (*Poncirus trifoliolate* 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 al.* 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.

Objective 2018/2019

- The development and evaluation of new products or existing products for the control of soilborne pests and diseases in citrus orchards and nurseries.

Materials and methods

*Testing of an algaecide for its effect on rough lemon seedling growth and root infestation by *Phytophthora nicotianae**

The algaecide that was tested was a saturated complex ionic aqueous solution and will be referred to in the report as Product PP. This product was tested for the first time in the 2018/2019 season for efficacy against *P. nicotianae* infection of rough lemon citrus rootstock seedlings. Testing occurred in a glasshouse.

Rough lemon seedlings were planted in 5L potting bags containing a 50:50 mixture of steam sterilized composted pine bark and top soil. Seedlings were grown in the glass house for a period of 3 months before the trial commenced. Ten seedlings were used per treatment as set out in Table 1. Treatments 2 – 10 were all inoculated with a *Phytophthora nicotianae* mycelium and zoospore suspension for a period of 4 weeks. The inoculum suspension was applied weekly and with each application 350 ml suspension as applied per seedling. During the trial period seedlings were watered to maintain continuous moist conditions in the pots, promoting the pathogen colonization of the potting medium. The algaecide applications were done according to the schedule set out in Table 1 for treatments 3-10. Treatments 3-7 and 10 were applied once, 28 days after the last inoculum application. Treatments 8 and 9 were applied weekly for the duration of the trial.

One month after the last algaecide applications in treatments 8 and 9, the trial was terminated and evaluated. During the evaluation, seedling root weight, shoot weight, seedling weight and shoot length were determined for each of the seedlings subjected to the different treatments. Isolations from roots were also done to determine the level of *P. nicotianae* root colonization of seedlings subjected to the different treatments. Collected data were subjected to analysis of variance (ANOVA) and Fisher's LSD test at a 95% confidence level was used to separate means.

*Evaluation of pre-plant soil fumigation of a replant soil on *Phytophthora* spp. and citrus nematode levels in soil and growth of young citrus trees*

In September 2017 an old Midnight Valencia on Carrizo citrange rootstock was removed in the Kirkwood area, Eastern Cape. The aim was to replant immediately with Tango on Carrizo citrange trees. Prior to tree removal soil and root samples were taken at 40 sites in the old orchard. These were analyzed at the CRI Diagnostic Centre (DC) in Nelspruit. The analyses indicated that on the number of citrus nematode juveniles in the soil were on

average 2068 per 250 cc soil. In the root samples on average 1532 female nematodes were present. Both *Phytophthora citrophthora* and *P. nicotianae* were also shown to be present in the orchard soil.

Soil preparation was done and rows pegged out. Certain rows were fumigated with a 60:40 chloropicrin: 1.3 dichloropropene mixture in the product Tri-Form 60. The fumigation dosage was 60 g/m². In February 2019 the first round of trial evaluation was done. Ten trees were marked in the fumigated rows and 10 in the non-fumigated rows. At these trees soil samples were taken for nematode and Phytophthora analyses at the DC. Tree height and stem diameter was also measured. The recorded data were subjected to statistical analyses and are reported below.

Table 4.3.3.1: Application schedule followed in the evaluation of an algacide for its effect on rough lemon seedling growth and root infestation by *Phytophthora nicotianae*

Treatment	Product	Concentration (ppm)	Timing																							
			Jan				Feb				March				Apr				May				Jun			
			w1	w2	w3	w4	w1	w2	w3	w4	w1	w2	w3	w4	w1	w2	w3	w4	w1	w2	w3	w4	w1	w2	w3	
1	Untreated control (Negative control)	-																								
2	Untreated Inoculated control (Positive control)	-																								
3	Product PP	0.5					X																			
4	Product PP	1.0					X																			
5	Product PP	1.5					X																			
6	Product PP	2.0					X																			
7	Product PP	50					X																			
8	Product PP	0.5		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
9	Product PP	1.0		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
10	Mefenoxam	0.1ml/Bag					X																			

Results and discussion

Objective / Milestone	Achievement
Apr –Jun 2018 1. Annual report 2. Complete trial applications and evaluations.	1. Annual report was written and submitted. 2. Final trial applications were done as well as trial evaluations.
Jul – Sept 2018 1. Trial planning 2. First applications according to trial layout	1. Trial was planned and products obtained. 2. The applications were done according to the trial layout
Oct – Dec 2018 1. Do applications according to trial layout. 2. Collect soil and root samples.	1. Applications were done according to trial layout. 2. Soil and root samples were collected.
Jan – Mar 2019 1. Do applications according to trial layout. 2. Collect soil and root samples.	1. Applications were done according to trial layout. 2. Soil and root samples were collected.

Testing of an algaecide for its effect on rough lemon seedling growth and root infestation by Phytophthora nicotianae

Statistical analysis of the plant measurements done, indicated only in some cases significant differences between the different treatments within the different plant measurements. In terms of mean root mass, the mass recorded ranged between 44.0 g and 101.7 g. The best performing treatment was the continuous application of 0.5 ppm algaecide (treatment 9) with a mean root mass of 101.7 g. This was followed once-off application of 50 ppm (treatment 7; mean of 81.4 g) and the once-off application of 1.0 ppm (mean of 81.0; treatment 4). However, between these 3 treatments there were no significant differences. The mean root masses of these treatments were furthermore not significantly, but markedly, heavier than the means recorded for the two control treatments that were 69.9 g and 66.1 g respectively (Table 4.3.3.2).

In terms of mean shoot mass of the seedlings, there were again statistically very little difference between the treatments. It was only the mean (50.0 g) of treatment 6 that was significantly lower than all the other treatments. For this measurement the 2 best performing treatments were again treatment 9 with a mean shoot mass of 91.2 g followed by treatment 8 that had a mean of 88.1 g. Both these treatments were applied continuously throughout the trial period. Treatment 8 was applied at 1.0 ppm of the algaecide and treatment 9 at 0.5 ppm. These two treatments were also the best performing in terms of plant mass. Treatment 9 seedlings had a mean plant mass of 192.9 g followed by treatment 8 with mean seedling mass of 151.5 g (Table 4.3.3.2). The mean stem diameter of the seedlings from the different treatments was again not statistically different and ranged between 6.9 mm and 9.3 mm. Mean shoot length also showed that treatments 8 and 9 were the best performing. Treatment 8 had a mean shoot length of 1394.3 mm followed by treatment 9 with a mean shoot length of 1381.0 mm. However, between the different treatments there was no significant differences (Table 4.3.3.2).

Evaluation of pre-plant soil fumigation of a replant soil on Phytophthora spp. and citrus nematode levels in soil and growth of young citrus trees

One year after fumigation and planting of the trees, some clear differences were already visible between the trees growing in the fumigated soil versus trees growing in non-fumigated trees. Analysis of the data revealed that with regards to mean stem diameter and mean tree height there was significant differences between trees growing in fumigated soil and trees growing in non-fumigated soils. The fumigated trees had a mean stem diameter of 26.4 mm versus the non-fumigated trees' stem diameter of 22.6 mm. The mean tree height of fumigated trees was 120.7 cm and that of the non-fumigated trees 110.7 cm (Figure 4.3.3.1). With regards to citrus nematode counts in the soil, the results showed that in the non-fumigated soil there was a mean number of 745 juveniles present per 250 cc soil, while there were no juveniles present in the fumigated soil. At the start of the trial, prior to removal of the old orchard, 40 soil and root samples were collected at random in the old orchard. Analysis of these samples indicated that on average there was 2058 juveniles per 250 cc soil and 1532 females per 10 g roots. Soil baiting from these samples furthermore indicated the presence of *Phytophthora citrophthora* and *P. nicotianae* in the soil. Soil baiting results indicated that in terms of *P. citrophthora*, 62.5% leaf discs were infested with this pathogen, while in fumigated soil this mean was significantly lower with only 2.5% leaf discs being infested with this pathogen. In the case of *P. nicotianae* 79.4% leaf discs were infested when baiting was done from non-fumigated soil. This declined significantly to 25.4% in fumigated soil (Figure 4.3.3.3).

Table 4.3.3.2. Mean root mass, shoot mass, plant mass, stem diameter and stem length of rough lemon root stock seedlings treated with the evaluated algaeicide at different concentrations and application regimes.

No	Treatment	Root mass (g)	Shoot mass (g)	Plant mass (g)	Stem diameter (mm)	Shoot length (mm)
1	Untreated, un-inoculated control	69.9 ab	73.4 abc	143.3 ab	8.3 ab	1072.0 a
2	Untreated, <i>P. nicotianae</i> inoculated control	66.1 ab	75.4 abc	141.6 ab	8.3 ab	1311.4 a
3	Product PP 0.5 ppm	73.3 ab	79.3 abc	152.6 ab	9.3 a	1151.4 a
4	Product PP 1.0 ppm	81.0 ab	81.7 abc	162.7 ab	8.9 a	1337.1 a
5	Product PP 1.5 ppm	80.5 ab	60.5 bc	141.0 ab	7.9 ab	1086.3 a
6	Product PP 2.0 ppm	44.0 b	50.0 c	94.0 b	6.9 b	967.5 a
7	Product PP 50 ppm	81.4 ab	61.3 bc	142.7 ab	7.9 ab	1048.6 a
8	Product PP 1.0 ppm	80.2 ab	88.1 ab	168.3 ab	8.3 ab	1394.4 a
9	Product PP 0.5 ppm	101.7 a	91.2 a	192.9 a	8.3 ab	1381.0 a
10	Mefenoxam 0.1 ml	51.6 b	60.4 bc	112.0 b	7.2 b	1110.0 a
	LSD-value	35.71	27.11	59.12	1.31	450.89

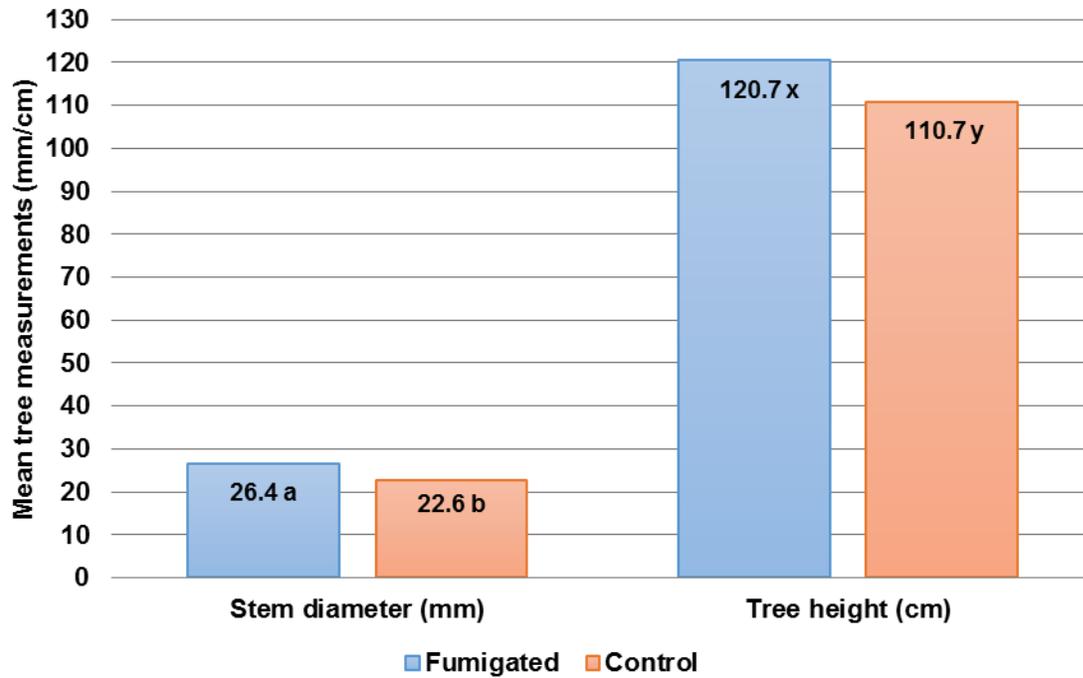


Figure 4.3.3.1. Mean stem diameter and tree height of trees growing in fumigated and non-fumigated soil.

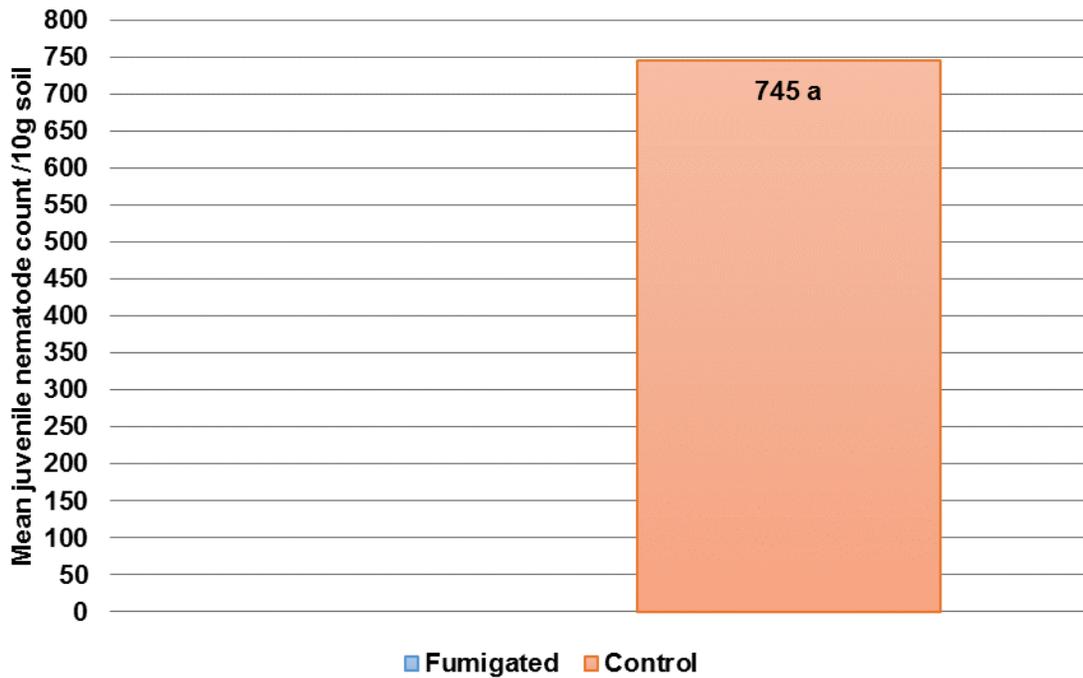


Figure 4.3.3.2. Mean number of citrus nematode juveniles present per 250 cc soil in fumigated and non-fumigated soil.

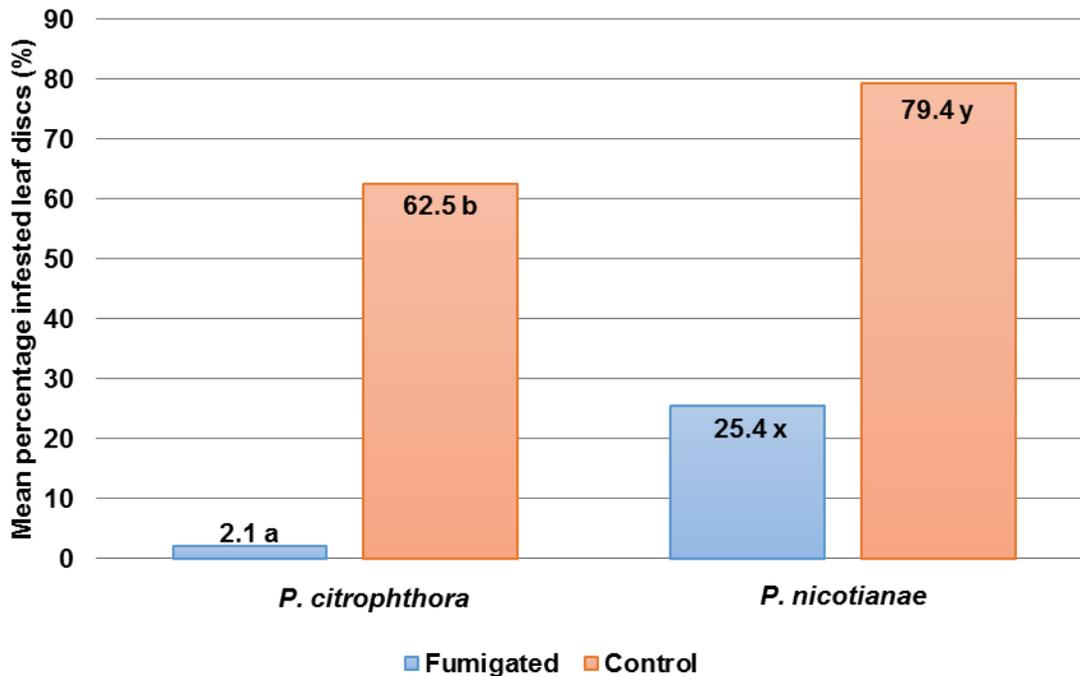


Figure 4.3.3.3. Mean percentage of *Phytophthora citrophthora* and *P. nicotianae* infested leaf discs following soil baiting analysis of fumigated and non-fumigated soil.

Conclusions to date

Results from the glasshouse evaluation of the algaecide indicated that there was definitely an effect on seedling growth. The rough lemon seedlings that received continuous weekly applications of either 1.0 or 0.5 ppm of the algaecide were markedly bigger with bigger root systems, compared to the control treatments. At this stage the results of *Phytophthora nicotianae* root infections is still outstanding. The effect of the algaecide on root infections are therefore unknown. However, the algaecide should be tested also for the ability to eliminate soilborne pathogen propagules from irrigation water.

Early results from the pre-plant fumigation trial indicated that after only one year, there was already significant differences between the growth of trees in the fumigated soil and the trees growing in the non-fumigated soil. This trial will be monitored further.

Technology transfer

Relevant results from previous work were presented at the 2018 CRI Citrus Research Symposium.

Further objectives and work plan

Continue to search for alternative products and methods 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.

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4.3.4 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 P. Moyo and B. Mabunda (CRI)

Summary

Different fungicide spray programmes, including the use of boscalid as part of a spray programme, were evaluated for the control of *Alternaria alternata* which causes Alternaria brown spot (ABS) on citrus. The

spray programmes were evaluated on 'Nova' mandarins in the Kirkwood area in the Eastern Cape Province. The inclusion of boscalid and mineral oil at the beginning of a spray programme provided the best control, although the amount of control was not significantly different from that obtained with the programme consisting of mancozeb and copper applied at half of their registered rates, in tank mixtures.

Opsomming

Verskillende fungisied spuitprogramme, insluitende die gebruik van Boscalid as deel van 'n spuitprogram, is vir die beheer van *Alternaria alternata*, wat Alternaria bruinvlek (ABV) op sitrus veroorsaak, geëvalueer. Die spuitprogramme is op 'Nova' mandaryne in die Kirkwood-area in die Oos-Kaapprovinsie geëvalueer. Die insluiting van Boscalid en minerale olie aan die begin van 'n spuitprogram, het die beste beheer verskaf, hoewel die hoeveelheid beheer nie betekenisvol verskil het van dit wat verkry is met die program wat uit mankoseb en koper, toegedien teen helfte van hul geregistreerde dosisse in tenkmengsels, bestaan het nie.

Introduction

Alternaria brown spot (ABS) is a serious disease of tangerines (*Citrus reticulata*) and their hybrids in all citrus producing regions of South Africa (Dalikilic *et al*, 2005; Peever *et al*, 2005). 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 (Dalikilic *et al*, 2005). The presence of ABS in South Africa is still a serious problem on all cultivars derived from crosses with 'Dancy' mandarin such as 'Nova', 'Minneola' and 'Mor'.

The ABS disease is caused by *Alternaria alternata*. This fungus attacks young leaves, twigs and fruit, causing small black necrotic spots after a 24 to 36 h incubation period. The ABS pathogen sporulates abundantly on lesions on mature leaves remaining in the canopy (Timmer *et al*, 1998, 2003; Reis *et al*, 2006). The pathogen produces a host-specific toxin that causes lesions to expand, often resulting in leaf and fruit drop as well as twig dieback (Pegg 1966; Peever *et al*, 2004, 2005). 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, fruits are only susceptible from petal fall until they reach about 5 cm in diameter.

Cultural measures, such as wider tree spacing and pruning to allow air movement and drying-off of trees, the elimination of overhead irrigation and avoidance of excess nitrogen fertilizer, can assist in reducing disease severity in some orchards (Dalikilic *et al*, 2005). However, fungicide applications are essential for disease control and production of blemish-free fruit (Schutte *et al*, 1992). 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.

Objective

To evaluate different spray programmes on a susceptible 'Nova' mandarin orchard.

Materials and methods

A susceptible 21-year-old 'Nova' mandarin orchard, located in Kirkwood in the Eastern Cape, was selected as a trial site for the 2017-2018 season. Spray programmes included the application of mancozeb and

copper in tank mixtures at half of their registered rates, as well as the inclusion of boscalid as part of a spray programme (Table 4.3.4.1). Each treatment consisted of five single data trees as replicates. Guard trees were located between plots within rows. Unsprayed trees served as controls. Fungicides were applied with a trailer-mounted, high-volume, high-pressure (2,500-3,000 kPa) sprayer with two hand-held spray guns. Spray volumes varied according to the size and canopy density of the tree but all trees were sprayed to the point of run-off.

At fruit maturity in May 2018, 100 fruit per data tree were evaluated 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). Fruit were evaluated for any sign of phytotoxicity by randomly harvesting 15 fruit from each treatment and visually inspecting them.

Results and discussion

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

All treatments, irrespective of spray programme, had significantly higher percentage of clean exportable fruit when compared to the untreated control, which yielded 58% clean fruit (Table 4.3.4.1). The highest percentage (87% clean fruit) of clean fruit was achieved with the spray programme that included the application of boscalid with oil at the beginning of the programme. This amount of control was, however, not significantly different from that achieved with the programme consisting of mancozeb and copper applied at half their registered rates (treatment 4, Table 4.3.4.1). The standard spray programme used by the farmer (treatment 5) yielded 74% clean fruit, which was significantly lower than the amount of control produced with treatments 2 and 4.

Table 4.3.4.1. Application dates, rates and evaluation of fungicides applied in tank mixtures for the control of *Alternaria* brown spot in Kirkwood, South Africa, for the period 28 September 2017 to 18 February 2018.

Treatment		Dosage (g/ml per 100L water tank mixture)	Percentage of fruit in each class		
			Lesions/fruit ^v		
			0	1-5	≥6
1	Untreated control		58.0c	18.4a	23.6a
2	Boscalid+ Oil/CuOCI/MZ + Strob + Oil/CuOCI + Strob + Oil /CuOCI ^w	25g + 250ml/ 200g/150g + 10ml + 250ml/150ml + 10ml + 250ml/200g	87.0a	10.0b	3.0b
3	Boscalid + Strob + Oil/CuOCI / MZ + Strob + Oil/CuOCI/CuOCI ^x	25g + 10ml + 250ml/200g/150g + 10ml + 250ml/200g/200g	73.6b	19.4a	7.4b
4	Mz + CuOCI/ Mz + CuOCI ^y	100g + 100g/100g + 100g/100g + 100g/100g + 100g/100g + 100g/100g + 100g	84.0a	12.8ab	3.2b

5	Grower's standard spray ^z		74.0b	17. ab	9.0b
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^vMeans in a column, based on 500 fruit (100 fruit/data tree), followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.

^wSpray dates were: 09 October 2017; 24 October 2017; 27 November 2017; 10 January 2018; 26 February 2018

^xSpray dates were: 09 October 2017; 24 October 2017; 27 November 2017; 10 January 2018; 14 February 2018

^ySpray dates were: 09 October 2017; 24 October 2017; 17 November 2017; 05 December 2017; 10 January 2018; 09 February 2018

^zSpray dates were: 28 September 2017; 24 October 2017; 05 December 2017; 10 January 2018; 26 February 2018; 28 March 2018; 18 April 2018; 05 May 2018

Conclusion to date

All the experimental programmes yielded statistically more clean fruit than the untreated control, but the addition of boscalid in the beginning of the spray programme resulted in the highest percentage of clean fruit.

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.

Future objectives and work plan

Research in future will focus on the inclusion of new chemistry into already existing spray programmes, with the ultimate goal of decreasing the amount of applications during the season. As mancozeb remains a problem in most market, alternatives will have to be identified.

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4.3.5 **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 Dr JM van Niekerk & MC Pretorius (CRI)

Summary

The aim of this project is to find pre-plant treatments that are effective in keeping orchard soils free from citrus nematode and *Phytophthora* spp. 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. To date no treatment has stood out in terms of nematode control results. However, based on tree height and trunk diameter measurements, the pre-plant fumigation treatments with 1,3 dichloropropene and metham sodium are starting to stand out. The trees in these treatments are taller with thicker trunks compared to the cadusafos and control treatments. It is therefore becoming clear that pre-plant soil fumigation in a replant situation does improve tree growth in comparison to no treatment or post-plant treatments.

Opsomming

Die doel van hierdie projek is om vóór-plant behandelings te vind wat effektief is om boordgronde vry van sitrus aalwurm en *Phytophthora* spp. te hou vir so lank as moontlik ná plant. Die proef is al sedert Januarie 2010 aan die gang. Die verskeie behandelings is vóór plant in Januarie 2010 toegedien, terwyl sommige behandelings steeds jaarliks in Januarie en November toegedien word. Boomstamdeursnit, boomhoogte, aalwurm grond- en wortel-analise, *Phytophthora* status in die grond, en 'n visuele boomgradering, is die parameters wat jaarliks sedert die begin van die proef gemonitor word. Tot op datum het geen behandeling in terme van aalwurmbeheerresultate uitgestaan nie. Gebaseer op boomhoogte en stamdeursnitmetings, begin die vóór-plant berokingsbehandelings met 1,3 dichloropropene en metam sodium uitstaan. Die bome in hierdie behandelings is langer met dikker stamme in vergelyking met die cadusafos en kontrole behandelings. Dit word dus duidelik dat vóór-plant grondberoking in 'n herplant situasie boomgroei verbeter in vergelyking met geen behandeling of ná-plant behandelings.

4.3.6 **PROGRESS REPORT: Characterization and management of Valley Bushveld citrus decline** Project 1068 (2012/3 – 2017/18) by JM van Niekerk and MC Pretorius (CRI)

Summary

The 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. Surveys of diseased trees were done in the Gamtoos and Sunday's River Valley production areas. Isolations from symptomatic trunk and root material yielded *Diaporthe* spp. isolates and isolates from genera within the Diatrypaceae family. These are pathogens that are known to infect woody hosts that are under stress. From the infected root tissue and surrounding soil,

three different *Neocosmospora* (previously *Fusarium*) spp. were isolated. These included *N. solani*, a pathogen that is known to infect citrus trees when they are under stress. From the isolations it was therefore clear that a complex of pathogens, that are known to cause disease in stressed hosts, are present in trees showing the decline symptoms. In order to evaluate some management options for this decline syndrome, two pot trials were established. In the first trial, Carrizo citrange rootstock seedlings were planted in soil from affected orchards. This soil was known to contain the abovementioned *Neocosmospora* spp. Four different chemical fungicide treatments were applied as soil drenches. The first three applications took place within three months after planting and the second set of 3 applications, 3 weeks apart, was done the first spring after planting. Seedlings were uprooted four months after the application and evaluated for root weight, volume and stem diameter. Results indicated that seedlings receiving the benomyl drench had significantly bigger root systems and also thicker stems compared to the other three chemical treatments. The rootstock pot trial, evaluating the growth performance of 12 different rootstocks in infected soil, did not yield conclusive results after one year. This could be due to the stress factors present in diseased orchards not being replicated in the pot trial. The rootstock trial will be repeated as an orchard planting in infected soil. The promising benomyl drench treatment will also be further evaluated in the orchard trial.

Opsomming

'n Agteruitgang en afsterwing van sitrusbome is al vir 'n aantal jare in Swaziland, Hoedspruit en die Gamtoos- en Sondagsriviervalleie aangeteken. Opnames van siek bome is in die Gamtoos- en Sondagsriviervalleie produksie-areas gedoen. Isolاسies vanuit simptomatiese stam- en wortelmateriaal het *Diaporthe* spp. isolate en isolate vanaf genera binne die Diatrypaceae familie opgelewer. Hierdie patogene is daarvoor bekend dat hulle houtagtige gashere wat onder strestoestande verkeer, infekteer. Drie verskillende *Neocosmospora* (voorheen *Fusarium*) spp. is vanuit die geïnfekteerde wortelweefsel en omliggende grond geïsoleer. Hierdie sluit *N. solani* in, 'n patogeen bekend daarvoor om sitrusbome wat onder strestoestande verkeer, te infekteer. Dit was dus duidelik uit die isolاسies, dat 'n kompleks van patogene, wat bekend is om siekte in gashere onder stres te veroorsaak, teenwoordig is in bome wat die agteruitgang simptome toon. Ten einde bestuurs-opsies vir hierdie agteruitgang sindroom te evalueer, is twee potproewe gevestig. In die eerste proef is Carrizo citrange onderstamsaailinge in grond vanaf geïnfekteerde boorde geplant. Dit was bekend dat hierdie grond die bogenoemde *Neocosmospora* spp. bevat het. Vier verskillende chemiese fungisiedbehandelings is as gronddrinkings toegedien. Die eerste drie toedienings het binne drie maande na plant plaasgevind en die tweede stel van 3 toedienings, 3 weke uitmekaar, is die eerste lente na plant gedoen. Saailinge is vier maande na die toediening uitgehaal en vir wortelgewig, volume en stamdeursnit geëvalueer. Resultate het getoon dat saailinge wat die benomyl drenking ontvang het, betekenisvol groter wortelsisteme en ook dikker stamme gehad het in vergelyking met die ander drie chemiese behandelings. Die onderstam potproef, wat die groeiprestasie van 12 verskillende onderstamme in geïnfekteerde grond geëvalueer het, het nie afdoende resultate na een jaar gelewer nie. Dit kan die gevolg wees van strestoestande teenwoordig in siek boorde, wat nie in die potproef herhaal kon word nie. Die onderstamproef gaan as 'n boord-aanplanting in geïnfekteerde grond herhaal word. Die belowende benomyl drenkbehandeling gaan ook verder in die boordproef geëvalueer word.

4.3.7 PROGRESS REPORT: Potential biocontrol agents and host/pathogen interaction of citrus replant pathogens

Project 1215 by Dr JM van Niekerk (CRI), Dr Elodie Stempien, Prof Lizel Mostert, Gray-Lee Carelse and Sonè Reens (USPP)

Summary

Previously the causal agents associated with replant disease in South African citrus orchards have been regarded as the citrus nematode, *Tylenchulus semipenetrans* and the soilborne pathogens, *Phytophthora nicotianae* and *P. citrophthora*. However, a recent study found that apart from the abovementioned pathogens and nematode, *Pythium* spp. and *Fusarium* spp. might also potentially be involved in citrus replant disease. These organisms were shown to build up in an orchard soil during the lifetime of the orchard. Once the orchard is removed, inoculum of the pathogens remains in the soil and reinfests the newly planted trees. As these pathogens were found to occur together in the same soils, it could be expected that they would interact to cause typical citrus replant symptoms. The interaction of this whole complex are at this stage unclear and needs to be studied, both in controlled co-inoculation trials, as well as in naturally infested orchard soils. Management of these pathogens in soil has previously relied on soil fumigation or the application of fungicides and nematicides. Concerns over the environmental impact of soil fumigation and fungicide resistance build-up in these pathogens, have necessitated investigation of biological control options. In this project DNA sequence data of abovementioned soilborne pathogens have been obtained and are currently being used to develop species specific q-PCR primers. These will be used to study the interaction of the different pathogens *in planta* in controlled glasshouse trials and also old orchards. The primers will also have the potential to be used in pathogen identification and quantification as part of disease diagnostic services. In the search for potential biological control agents, soil and root samples were collected from healthy and declining trees in mature orchards in the Citrusdal and Kirkwood production areas. Isolations from the roots and rhizosphere soil has yielded several *Bacillus* and *Pseudomonas* spp. isolates along with *Trichoderma* and other fungal genera. These potential biological control isolates are currently being used in *in vitro* screening against abovementioned pathogens. The screening aims to identify the most successful isolates that will be developed and evaluated further *in planta*.

Opsomming

Voorheen is die sitrus-aalwurm, *Tylenchulus semipenetrans*, en die grondgedraagde patogene, *Phytophthora nicotianae* en *P. citrophthora*, as die veroorsakende agente wat met herplantsiekte in Suid-Afrikaanse sitrusboorde geassosieer word, beskou. 'n Onlangse studie het egter bevind dat, behalwe vir bogenoemde patogene en aalwurm, *Pythium* spp. en *Fusarium* spp. ook moontlik in sitrusherplantsiekte betrokke kan wees. Daar is getoon dat hierdie organismes gedurende die leeftyd van die boord, in die boordgrond opbou. As die boord verwyder word, bly die inokulum van die patogene in die grond en herinfekteer die nuut-aangeplante bome. Aangesien hierdie patogene sáám in dieselfde grond voorkom, word daar verwag dat daar interaksie tussen hulle sou plaasvind ten einde tipiese sitrusherplantsimptome te veroorsaak. Die interaksie van hierdie hele kompleks is op hierdie stadium nog nie duidelik nie en moet bestudeer word, beide in beheerde ko-inokulasie proewe, asook in natuurlik geïnfesteerde boordgronde. Bestuur van hierdie patogene in grond het voorheen op grondberoking of die toedien van fungisiedes en aalwurmdoders staatgemaak. Kommer oor die omgewingsimpak van grondberoking en fungisied weerstandsopbou in hierdie patogene, het die ondersoek van biologiese beheer-opsies noodsaak. In hierdie projek is die DNS volgorde-data van bogenoemde grondgedraagde patogene verkry en word tans gebruik om spesie-spesifieke q-PCR inleiers te ontwikkel. Dit sal gebruik word om die interaksie van die verskillende patogene *in planta* in beheerde glashuisproewe en ook in ou boorde te bestudeer. Die inleiers sal ook die potensiaal hê om in patogeen-identifikasie en -kwantifisering as deel van siekte diagnostiese dienste gebruik te word. In die soeke na potensiële biologiese beheer-agente, is grond- en wortelmonsters van gesonde en agteruitgaande bome in volwasse boorde in die Citrusdal en Kirkwood produksie-areas versamel. Isolates vanuit die wortels en risosfeergrond het verskeie *Bacillus* en *Pseudomonas* spp. isolate opgelewer, tesame met *Trichoderma* en ander swamgenera. Hierdie potensiële biologiese beheer-isolate word tans in *in vitro* evaluering teen bogenoemde patogene gebruik. Die evaluering het ten doel om die suksesvolste isolate te identifiseer wat verder *in planta* ontwikkel en geëvalueer sal word.

4.4 PROGRAMME: POSTHARVEST DISEASES

Programme coordinator: W du Plooy (CRI)

4.4.1 Programme summary

The postharvest environment is a rapidly shifting one, and an integral part of the research projects at the CRI is to stay abreast of any changes. During 2018/19 the service project in this programme, Project 123, looked at new products and alternative actives, testing them for efficacy and compatibility with currently used actives. Most of these were still focussed on sanitation, and included bioflavonoids from Vibacsan. The products tested were not able to withstand the bio-load pressure in the aqueous environment. With expanded use, some application issues were uncovered on PAA products, and one of the application improvements investigated was the feasibility of online pH adjustment in the fungicide bath. This was done successfully, with no detrimental effect on the PAA, or the fungicides in the bath. Assessment of sanitisers will be continued, as the increasing pressure to limit the use of synthetic chemicals means sanitation is critical in postharvest disease management. In support of recommendations for resistance management in the packhouses, alternative actives, and combinations of current actives were evaluated on a small scale. The work by the CRI on azoxystrobin directly resulted in the active being registered for postharvest use on citrus. Furthermore, combinations with other fungicides seem to be successful, and registration of an azoxystrobin and fludioxonil combination in particular will be pursued. More such combinations will be tested in 2019.

The particular phytochemical relationship between *Phyllosticta citricarpa* and citrus has not been investigated in depth. With Project 1135 an initial investigation of three citrus types with varying susceptibility to the pathogen did result in good data. Using GC, HPLS and UHPLC phytochemical profiles were compiled for each citrus type. All chromatography data were exported to SIMCA P+ (13.0) software (Umetrics, Sweden) and used to construct chemometric models. Principal component analysis (PCA) score plots of chromatographic data demonstrated that the metabolomics profiles of the volatiles were clustered into three main classes, according to variety. This separation into clusters indicates chemical compositional differences between the clusters. Within variety, group separation was seen corresponding to developmental stages of the fruit. The project has been terminated, but with RCE funding, two MTech students (Mss Melida Mabogoane and Puseletso Tswaai) from Tshwane University of Technology are continuing the research in a new project from 2019.

Understanding continued development of pathogen resistance against current chemical interventions was clearly highlighted in Project 1141. An MSc student, Lindokuhle Mamba successfully completed his thesis, evaluating propiconazole (PPZ) as a possible alternative against sour rot (*Galactomyces citri-aurantii*). He also did a baseline study on *Penicillium digitatum* and *G. citri-aurantii* from the Western and Eastern Cape against this active, using isolates collected from packhouses in these two provinces. All isolates were previously exposed to either PPZ or imazalil, both FRAC 3 actives and thus demethylation inhibitors. *Galactomyces citri-aurantii* had a 1.6% resistance frequency at discriminatory concentrations (DC) of 0.5 and 0.9 $\mu\text{g}\cdot\text{ml}^{-1}$, and a high number of propiconazole resistant *P. digitatum* were recovered with 22.2% resistance frequency at a DC of 0.5 $\mu\text{g}\cdot\text{ml}^{-1}$ compared to *G. citri-aurantii*. Lastly, through his work, application of the active was optimised in terms of aqueous applications on the packline. The study pointed out that for 80% sour rot and green mould control, PPZ drench should be done as early as 11.3 and 12.3 h for Clementine, 13.45 and 16.1 h for lemon, and 19.4 and 16.2 h for navels. A second student, Mr Charles Stevens, is currently using the benchmark set by this work to develop a sensitivity testing model for the pathogens against PPZ.

The pressure to remove methylbromide as a permissible product in wood treatment, has resulted in the use of compounds not researched before. Due to this, several issues need to be resolved (Project 1165). The increased prevalence of fungi and probable resultant wood degradation of the pallet bases are a concern for two reasons: (1) potential structural failure of the wood, and (2) possible phytosanitary threats. Another issue is the deposition of SOPP on fruit that was never treated with any chemical actives, but were shipped in a container that may have been exposed to SOPP. This exposure may originate from either the pallet bases that are being dipped in SOPP due to the use of this chemical as a wood preservative, or the soaps being used to sanitise the container between shipments. With the support of PPECB, samples of the cleaning materials are being collected and will be analysed. An initial survey of the saprophytes from pallet wood indicated that there are only a select few species, with *Trichoderma* and *Fusarium* species dominating. All isolated are being identified by using PCR techniques. Simultaneously, several wood preserving compounds are being procured for trials to evaluate their volatiles and subsequent residue threats.

A serious postharvest pathogen is *Phytophthora nicotianae*, often causing serious losses when it manifests as brown rot on citrus. No postharvest fungicides are currently registered for the control of this disease. Project 1198 aims to evaluate the in vitro baseline and non-baseline sensitivity of *P. nicotianae* towards two fungicides, namely azoxystrobin (AZO) and fludioxonil (FLU). Results indicated that isolates from both previously unexposed and previously exposed pathogen populations could be divided into different AZO and FLU sensitivity groups that were statistically different based on their mean EC50 and EC90 values. For azoxystrobin the EC50 values of the groups in the previously unexposed population ranged between 0.01 and 0.19 ppm and the EC90 values between 4.28 and 83.96 ppm. In the unexposed population the EC50 values for azoxystrobin was between 0.04 and 0.46 ppm and that of the EC90 values between 11.45 and 84.85 ppm. For fludioxonil sensitivity the EC50 values of sensitivity groups in the previously unexposed population was between 5.56 and 1613.52 ppm with the EC90 values of these groups ranged from 1988.50 and 9929.30 ppm. The values for the groups in the previously exposed groups were similar. Here the highest EC50 value was 84.79 and the lowest 3.10 ppm. The highest EC90 was 6809.90 and the lowest 1090.50 ppm. Azoxystrobin and fludioxonil were furthermore found to both have very good curative action, significantly reducing brown rot incidence when the fungicide was applied up to 12 h after inoculation. Applications done 24 h after inoculation also provided some curative action but not as good as earlier applications. Azoxystrobin furthermore provided very good protection against infection if inoculations were done up to 48 h after application. However, the preventative ability of fludioxonil was poor and not better than the untreated control. Both fungicides have been shown to have potential to control the development of postharvest brown rot on lemons.

Programopsomming

Verwikkelinge in die naoesomgewing gebeur vinnig en 'n integrale deel van die CRI se navorsing is om op hoogte te bly met enige veranderinge. Die diensprojek in hierdie program, Projek 123, het in 2018/19 na nuwe produkte en alternatiewe aktiewes gekyk. Die formulasies was getoets vir effektiwiteit en verenigbaarheid met huidig-gebruikte aktiewes. Meeste van die produkte het op sanitasie gefokus en het bioflavonoïede vanaf Vibacsan ingesluit. Die produkte was egte nie instaat om die biolading in die wateromgewings binne die pakhuis te weerstaan nie. Met uitgebreide gebruik was 'n aantal aanwendingskwessies met PAA produkte teëgekóm. Een van die ondersoeke na verbetering van die aanwending van PAA was om die pH te verstel in die funksiede bad. Dit was suksesvol gedoen, met geen nadelige effek op die PAA of die funksiede gebruik in die bad nie. Evaluering van saniteermiddels sal voortgaan, aangesien die druk op sintetiese aktiewes bly toeneem en sanitasie dus krities word in na-oessiektebeheer. Ter ondersteuning van die aanbevelings vir weerstandsbestuur was alternatiewe aktiewes, asook kombinasies daarvan, geëvalueer op klein skaal. Die werk op azoxystrobien gedoen deur

die CRI het direk bygedra tot die registrasie van die aktief vir na-oestoeëpassing op sitrus. Verder was van die kombinasies suksesvol, en is aansoek gedoen om spesifiek die azoxystrobien en fludioksonil kombinasie te registreer. Verdere oënskynlike potensiaal van die kombinasies sal in 2019 ondersoek word.

Die besondere fitochemiese interafhanklikheid tussen *Phyllosticta citricarpa* en verskillende sitrustipes is nie vantevore in diepte ondersoek nie. Met projek 1135 was 'n voorlopige ondersoek gedoen met drie sitrustipes wat verskillende vlakke van vatbaarheid teenoor die patoëen vertoon, gedoen, met goeie data wat verkry is uit die studie. Fitochemiese profiele is saamgestel vir elke sitrustipe, deur middel van GC, HPLC en UHPLC. Alle chromatografiese data was oorgedra na SIMCA P+ (13.0) sagteware (Umetrics, Swede), wat gebruik was om die chemometriese modelle saam te stel. Hoofkomponent analiese (HKA) tellingsgrafieke van die chromatografiese data kon demonstreer dat metabolomiese profiele van die vlugtige verbindings in drie hoogroepe volgens sitrustipe saamgroepeer. Hierdie skeiding in groepe kon die chemiese samestellingsverskille tussen die groepe uitwys. Binne elke sitrustipe kon ooreenstemmende skeiding gebaseer op ontwikkelingstadiums van die vrugte ook herken word. Die projek was getermineer, maar met RCE befondsing was twee M.Tech. studente, Me Melida Mabogoane en Puseletso Tswaai, vanaf Tshwane Universiteit van Tegnologie, aangestel en word die navorsing vanaf 2019 voortgesit as 'n nuwe projek.

Die belangrikheid daarvan om die voortdurende ontwikkeling in patoëenweerstand teen chemiese beheermiddels te verstaan, was uitgewys deur Projek 1141. Lindokuhle Mamba, 'n MSc-student, het sy tesis oor propikonasool (PPZ) as 'n moontlike alternatief teen suurvrot (*Galactomyces citri-aurantii*), suksesvol voltooi. Hy het ook 'n basislynstudie van die aktief teen *Penicillium digitatum* en *G. citri-aurantii* uit pakhuis in die Wes- en Ooskaap, gedoen, met isolate wat almal vantevore aan of imazalil, of PPZ blootgestel was. Albei hierdie aktiewes val binne die FRAC 3 groep en is dus demetileringsinhibeerders. *Galactomyces citri-aurantii* het 'n weerstandsfrekwensie van 1.6% getoon teen 'n diskriminerendskonsentrasie (DK) van 0.5 en 0.9 $\mu\text{g}\cdot\text{ml}^{-1}$, terwyl 'n groot aantal propikonasoolweerstandige *P. digitatum* isolate met 'n 22.2% weerstandsfrekwensie teen 'n DK van 0.5 $\mu\text{g}\cdot\text{ml}^{-1}$ (in vergelyking met *G. citri-aurantii*), gevind was. Laastens, as gevolg van sy werk, was die aanwendig van die aktief in waterbehandelings op die paklyn, geoptimeer. Die studie het aangedui dat, om 80% beheer van groenskimmel en suurvrot te behaal, moet PPZ so gou as 11.3 hr vir Clementine en 12.3 hr vir navel na oes aangewend word in die stortbadbehandeling. 'n Tweede student, Mnr Charles Stevens, is tans besig om die maatstawwe ontwikkel in hierdie studie te gebruik om 'n sensitiwiteitsvoorspellingsmodel te bou vir patogene teen PPZ.

Die druk om metielbromied te verwyder as 'n toelaatbare produk vir houtbehandeling, het gelei tot die gebruik van aktiewes wat nie vantevore nagevors was nie. As gevolg hiervan het verskeie kwessies opgeduik wat moes ondersoek word (Projek 1165). Die toename in fungi en waarskynlik houtdegradering as gevolg daarvan is 'n bekommernis vir twee redes: (1) moontlike strukturele faling van die hout, en (2) moontlike fitochemiese bedreiging. 'n Verdere kwelling is die neerlegging van SOPP op vrugte wat organies geproduseer was en dus nooit enige behandelings ontvang het nie, maar wat uitgevoer word in 'n behouingseenheid wat wel blootgestel was aan die aktief. Hierdie blootstelling kan ontstaan weens die gebruik van SOPP as 'n houtpreserveermiddel vir die palletbassise, of weens die sepe wat gebruik word om die behouingseenhede te saniteer tussen besendings. Die ondersteuning van PPECB was nodig om voorbeelde van die skoonmaakmiddels te kon bekom, sodat dit geanaliseer kan word. Die aanvanklike studie van die saprofiete vanaf die pallethout het aangedui dat daar slegs 'n beperkte aantal spesies betrokke is, met oorheersend *Trichoderma* en *Fusarium* spesies. Al die isolate word tans deur PKR tegnieke bevestig. Terselfdertyd word verskeie produkte wat moonlik effektief sal wees vir houtpreservering, ge-evalueer vir vlugtige stowwe en gevolglike residue probleme.

Phytophthora nicotianae is 'n ernstige na-oespatogeen wat ernstige verliese kan veroorsaak wanneer dit as bruinvrot op sitrus manifesteer. Daar is tans geen naoes fungisiede geregistreer vir gebruik teen hierdie siekte nie. Projek 1198 is gerig daarop om die *in vitro* basislyn en nie-basislyn sensitiwiteit van *P. nicotianae* teen twee fungisiede, azoxystrobien (AZO) en fludioxonil (FLU), te evalueer. Resultate dui daarop dat isolate uit populasies wat tevore blootgestel, of populasies wat nie blootgestel was nie, in statisties verskillende AZO en FLU groepe ten opsigte van hulle sensitiwiteite gegroepeer kan word na aanleiding van hulle gemiddelde EC₅₀ en EC₉₀ waardes. Die EC₅₀ waarde vir AZO in groepe wat nie vantevore blootgestel was aan die aktief nie, was tussen 0.01 en 0.19 dpm, terwyl die EC₉₀ waardes tussen 4.28 en 83.96 dpm was. Vir azoxystrobin het die EC₅₀ waardes van die groepe in die voorheen nie-blootgestelde populasie tussen 0.01 en 0.19 dpm gevarieer, en die EC₉₀ waardes tussen 4.28 en 83.96 dpm. In die nie-blootgestelde populasie, was die EC₅₀ waardes vir azoxystrobin tussen 0.04 en 0.46 dpm en vir die EC₉₀ waardes tussen 11.45 en 84.85 dpm. Vir fludioxonil sensitiwiteit was die EC₅₀ waardes van sensitiwiteitsgroepe in die voorheen nie-blootgestelde populasie tussen 5.56 en 1613.52 dpm, terwyl die EC₉₀ waardes van hierdie groepe van 1988.50 tot 9929.30 dpm gevarieer het. Die waardes vir die groepe in die voorheen blootgestelde groepe was soortgelyk. Hier was die hoogste EC₅₀ waarde 84.79 en die laagste was 3.10 dpm. Die hoogste EC₉₀ was 6809.90 en die laagste 1090.50 dpm. Daar is verder gevind dat azoxystrobin en fludioxonil baie goeie uitwissende aksie het, en het bruinvrotvoorkoms betekenisvol verminder wanneer die fungisied tot 12 ure na inokulasie toegedien is. Toedienings wat 24 uur na inokulasie gedoen is, het ook 'n mate van uitwissende aksie verskaf, maar nie so goed soos vroeër toedienings nie. Azoxystrobin het verder baie goeie beskerming teen infeksie gebied indien inokulasies tot 48 uur na toediening gedoen is. Die voorkomende vermoë van fludioxonil was egter swak, en nie beter as die onbehandelde kontrole nie. Beide fungisiedes het op suurlemoen getoon dat hulle die potensiaal het om die ontwikkeling van naoes bruinvrot te beheer.

4.4.2 FINAL REPORT: Epicuticular wax composition of CBS resistant and susceptible citrus cultivars.

Project 1135 (2017/2018) by Wilma du Plooy (CRI) and Wilma Augustyn (TUT)

Summary

The profile of water polar and apolar metabolites from five different citrus types was investigated in this study. Through chemometric comparison of the five profiles, significant differences that may point to unique phytochemistry of the citrus type involved, are being sought. Such unique compounds play a role in the ability of a citrus type to withstand challenges such as pathogens, cold damage and environmental pressure. This study, however, is the first where five different citrus types are compared side-by-side, with the ultimate focus on citrus type differences in susceptibility to infection by the citrus blackspot (CBS) infection, *Phyllosticta citricarpa*. Preliminary chromatographic studies point towards promising differences, with one set of peaks in particular (the biomarker on 5.04 min in the UPLC chromatogram, tentatively identified as naringenin-7-O-neohesperidoside, and the biomarker on 5.85 min in the UPLC chromatogram as hesperitin-7-O-rhamnoside) that will be investigated further. These compounds do not occur in limes and kumquats, but do occur with significantly different concentrations in "Bitter Seville" (low susceptibility to CBS), "Valencia" (susceptible) and lemons (highly susceptible). The current format of the project will be changed in order to accommodate two MTech students. This work will result in two theses, with the accompanying articles

Opsomming

Die profiele van die polêre en apolêre metaboliete van vyf verskillende sitrustipes was in hierdie projek ondersoek. Deur hierdie profiele chemometries met mekaar te vergelyk, word gekyk waar daar

noemenswaardige verskille is wat kan dui op 'n unieke fitochemiese samestelling vir die betrokke sitrustipe. Sulke unieke verbindings speel 'n rol in die vermoë van sitrustipes om uitdagings soos patogene, koue skade en omgewingsdruk die hoof te bied. Hierdie studie is egter die eerste waar vyf tipes sy-aan-sy vergelyk word, met die uiteindelijke fokus op verskillende sitrustipes se vatbaarheid vir infeksie deur die sitruswartvlek (SSV) patogeen, *Phyllosticta citricarpa*. Voorlopige chromatografiese studies dui op belowende verskille, met veral een stel unieke verbindings (biomerker op 5.04 min in UPLC chromatogram, tentatief geïdentifiseer as naringenin-7-O-neohesperidosied en biomerker op 5.85 min in UPLC chromatogram as hesperitin-7-O-rhamnosied) wat verder ondersoek sal word. Hierdie verbindings kom nie in lemmetjies en kumkwarte voor nie, met beduidend-verskillende konsentrasies daarvan in “Bitter Seville” (lae SSV vatbaarheid), Valencia (vatbaar vir SSV) en suurlemoene (hoogs vatbaar vir SSV). Die projek se huidige formaat sal verander word sodat dit kan hervat in 'n nuwe formaat wat twee nuwe MTech student kan akkommodeer. Die werk sal dus uiteindelik lei tot twee tesisse, met gepaargaande publikasies.

Introduction

Plant surfaces and the chemistries thereof play a very important role in host recognition and infection by pathogenic fungi. The most prevalent of the phytochemicals involved are the epicuticular waxes, the oils and lipids, and flavonoids (Kozłowski and Pallardy, 2007). The effect of species-related surface chemistry, as well as age related chemistry on the germination of fungal spores were demonstrated by Inyang et al. (1999). They found that spore germination can be elicited by phytochemicals present at different periods in the maturation of leaves and fruit. Lui et al. (1999) demonstrated that citrus wax deposition and composition were related to host defence responses against fungal interactions. In a study where the epicuticular wax of two different grasses was investigated, the susceptible / host species significantly enhanced the growth of germ tubes of the fungus, *Curvularia eragrotidis*, but had no effect on appressorium formation. However, a resistant / non-host grass species inhibited the extension of germ tubes and the differentiation of appressoria (Wang et al., 2008). A preliminary trial by Dr T. Schutte in 2015 found that the wax composition of “Valencia” oranges, lemons and “Bitter Seville” are significantly different during the earlier stages of fruit development (unpublished data). In terms of susceptibility to CBS, Bitter Seville has low susceptibility and lesion expression, Valencia's susceptible, but less than lemons, which are highly susceptible. These results indicated that citrus epicuticular wax composition of resistant and susceptible cultivars may play a role in infection success by *P. citricarpa*. In addition, if it can be proven that citrus wax does in fact harden off as early as March, it might explain why field applications of fungicides for CBS control are not necessary from this stage of fruit development until harvest.

Epicuticular wax plays a pivotal role in the maturing of the fruit rind. All aerial plant organs are covered with this substance, which occurs on the surface of the cuticle as well as within the cuticle. This layer has several functions in plant development, adaption and survival and is composed of alcohols, aldehydes, esters, fatty acids, hydrocarbons, ketones and related compounds. *n*-Alkanes with 20-35 carbon atoms have been found in the epicuticular wax of citrus fruit. Linear-chain alkanes were shown to represent at least 98% of the epicuticular wax total alkanes of mature Duncan grapefruit (Nagy et al., 1975). The carbon chains C₃₁, C₂₉, and C₂₇ were most abundant, comprising 41, 22, and 11% of the total alkane fraction respectively. Fruit development affects the composition and relative abundance of each component of the *n*-alkane fraction of the surface wax of citrus fruit. Substantial amounts of C₂₀ – C₂₇ carbon chains (soft) were present in the wax of immature fruit while C₂₈ – C₃₃ chains (hard) predominated in the wax of mature fruit (Norby and Nagy, 1977; El-Otmani and Coggins, 1985; El-Otmani, Arpaia and Coggins, 1987).

In addition to the waxes, other phytochemicals that occur in fruit rind are the phenolics such as flavonoids (Harborne and Williams, 2000), and the oils and lipids (Kozłowski & Pallardy, 2008). These groups of compounds all form an integral part of the cuticular composition, as do the wax precursors. Their role in

fungal interactions has been documented before (Inyang et al, 1999; Harborne and Williams, 2000; Reina-Pinto & Yephremov, 2009), and they may therefore be suspected to play a similarly relevant role in the CBS resistance / sensitivity of the citrus types studied. Currently used cultivars include “Bitter Seville”, which is accepted to be resistant towards CBS infection, highly susceptible lemon and “Valencia” orange with medium susceptibility. Determining the composition of the wax layer of the different citrus cultivars over time (from fruit set until harvest) and comparing them over the same developmental periods will reveal the epicuticular wax composition of all citrus cultivars studied.

Metabolomics involves the measurement of a set of low molecular weight metabolites, such as phenolic compounds and their intermediates, in biological systems (Wishart, 2008). It allows for the observation of variations in total metabolite profiles, and is capable of detecting complex biological changes using chemometrics, a statistical multivariate pattern recognition method (Putri et al., 2013). In chemometric analysis, the chemical compounds are not identified. Instead, separation and detection are recorded and then statistically compared to reveal features that distinguish samples. This type of analysis is particularly useful in systems such as those in fruit rind and fruit pulp, where the complexity undergoes rapid developmental changes (Kozłowski & Pallardy, 2008).

Principal component analysis (PCA) allows for the visualization and clustering of multiple data sets based on linear combinations of their shared features (Eriksson et al., 2006: 36). This model type is normally used to determine differences between samples, to identify variables that contribute the most to these differences and to establish whether the variables contribute in the same way (correlated) or independently (uncorrelated). Orthogonal partial least squares projections to latent structures discriminant analysis (OPLS-DA) is a regression technique that rotates PCA-components to improve the separation between assigned groups of observations (Wishart, 2008) and can be used to explain differences between groups or classes. However, OPLS-DA can also separate predictive from non-predictive (orthogonal) variation and can be used to identify variables that are responsible for class discrimination (Mehl et al., 2014, Bylesjö et al., 2006).

The project made good progress and excellent results were obtained, however, it was still less progress than was projected at the onset, due to logistical and resource constraints. Regardless, due to the interest generated in this project at the Tshwane University of Technology, two masters’ students have been enlisted. This gives new impetus on the project, relieving issues with available research capacity. The project in its current format is therefore terminated, and a new proposal to continue the work with a new time line and objectives were submitted.

Stated objectives

Phase 1

Analyses of wax layers

1. Sample and extract epicuticular chemicals from fruit of different citrus cultivars, from fruit set until harvest to determine the phytochemical changes taking place as the fruit matures.
2. Electron microscope study to study the wax layers of “Bitter Seville”, lemon and “Valencia” orange fruit during different stages of development.
3. NIR study of the epicuticular wax layers of “Bitter Seville”, lemon and “Valencia” orange.
4. Exploratory analysis of non-wax phytochemicals using TLC and HPLC.
5. Advanced chromatographic analysis of phytochemicals.
6. Determination of bioactive compounds in resistant cultivars, if any is found.

Phase 2

Biological infection study

1. Extraction of phenolic compounds and lipids from the rind of different citrus cultivars during different stages of development.
2. Evaluation of the biochemical activity of the extracted components against *P. citricarpa* in each stage of extraction.
3. Infection studies using any active phytochemicals against *P. citricarpa*.
4. Infection studies on fruit from different phenological stages

Materials and methods

1. Extraction of essential oils

The peel rind of three types of citrus fruits, lemon (*Citrus limon*), “Valencia” (*Citrus sinensis*) and “Bitter Seville” (*Citrus aurantium*) were finely grated using a steel grater. Five grams of grated peel rind were weighed, in triplicate, into 50 mL centrifuge tubes to which, 5 mL of hexane was added. The samples were shaken overnight at 100 rpm (Labcon platform shaker) after which it was vortexed for 10 min. The hexane extracts were decanted and dried over anhydrous sodium sulphate.

2. Extraction for of secondary metabolites

Frozen citrus peels rinds were freeze dried (Telstar Cryodos, Labotec, Johannesburg, South Africa). The dried peels rinds were finely ground (IKA industrial grinder) and sieved (Test Sieve, Endecott's LTD, 500 microns, London, England). The powdered samples were stored in 50 mL centrifuge tubes. Powdered peels rinds (0.5 g) was extracted with 15.0 mL of methanol: acetone: water (7:7:1, v:v:v) with the aid of an industrial microwave oven (MarsX Express microwave extractor, Mad Technology, South Africa). The temperature of the mixture was ramped to 75 °C in 5 min, held at this temperature for 15 min and cooled for 15 min. Thereafter, the samples were concentrated to about 3.0 mL using a Genevac evaporator (EZ-2 Personal Evaporator, United Scientific, South Africa). The volume was adjusted to 5.0 mL with distilled water and then defatted using 5.0 mL hexane. Chlorophyll was removed from each extract by adding 5.0 mL chloroform to the tube followed by vortexing for 1 min and centrifuging (TD4K-Z) at 4000 rpm for 10 min. The aqueous phase was drawn off and The final volume of the aqueous phase was adjusted to 5.0 mL with 20 % aqueous methanol.

3. Determination of total phenolic content

Total phenolic contents were determined by a modified Folin Ciocalteu method (Bray and Thorpe, 1954). A further modification of the classic Folin Ciocalteu method, reported by (Du Plooy et al., 2009), incorporating a micro-assay carried out in flat-bottom 96 well Elisa plates (Merck, Germany) was used. Four replicates of each extract were subjected to the assay. Distilled water (275 µL), 25 µL Folin Ciocalteu reagent (Sigma, South Africa), 5 µL plant extract and 50 µL freshly prepared 20% (w/v) sodium carbonate solution were placed in each well, in this order. The reagents were mixed, and the plates incubated in an oven at 40°C for 20 min, where after they were cooled on ice. An Elisa plate reader (Multiscan Ascent VI, Finland) was used to determine the absorbance of each well-content at 740 nm. The concentrations of total phenolics in the samples were expressed as gallic acid equivalents [(mg.g⁻¹ dried mass, (DM))] using a standard curve ranging from 100 to 600 mg/L gallic acid ($y=0.0016x-0.0254$, $R^2 = 0.9983$).

4. Gas Chromatography

The hexane extracts were analysed by gas chromatography (Varian Chromapack CP- 3800 GC) equipped with an autosampler (Varian CP-8400) and a reverse phase capillary column (ZB-5, 30 m x 0.25 mm, I.D. x 25 µm). Helium was used as carrier gas at a flowrate of 1.5 mL/min. The column oven temperature was maintained at 30 °C for 5 min, increased to 200 °C in 5 min and finally increased to 250 °C in 30 min and held for 1 min, total analysis time was 41.67 min.

5. High Performance Liquid Chromatography

Phenolic compounds were analysed with a high performance liquid chromatograph (Agilent Technologies 1200 Infinity, Chemetrix, South Africa) equipped with an C18 reversed phase column (Inertsil 5, 250 mm x 4.6 mm i.d. x 5 µm particle size) at a flow rate of 1.0 mL/min: solvent A (aqueous solution, distilled water acidified with H₃PO₄ to a pH 3.6 and solvent B (acetonitrile). The gradient programme was as follows: solvent A:B (80:20) for 12 min, then ramped to (25:75), in 2 min and a post run time of 2 min. Compounds were detected with a fixed wavelength UV/Vis detector at 280 nm.

6. Ultra-high performance liquid chromatography

Extracted phenolic compounds (4 µL) were analysed with an ultra-high performance liquid chromatographic (UPLC) system (WatersTM, South Africa) comprising a binary solvent manager and a photodiode array detector (PDA) for verification of compound identities. Profiling was achieved through the use of a Micromass –LCT Premier Quadrupole Time of Flight mass spectrometer (QToF-MS). Chromatographic software Masslynx 4.1 was used to process and obtain all the chromatographic data. The following ESI conditions were used: capillary voltage 3500 V, cone voltage 30 V, MCP detector voltage, source temperature 100 °C, desolvation temperature 350 °C, cone gas flow 20 L/h, desolvation gas flow 500 L/h, Determination was performed in positive ion mode over an m/z range of 100–1500 Da and a scan time of 0.5 s. Mass calibration was automatically performed using leucine enkephalin as the lock-mass. To normalise the abundances of the phenolic compounds, 5 µL of each sample was mixed and analysed. Two sets of data were collected in parallel, one set at low collision energy, 6 eV, to determine the mass of the molecular ion, and another set at higher collision energy, 30 eV, to detect all fragment ions.

Separation was achieved with a UPLC Acquity BEH C18 (2.1 x 150 mm, 1.7 µm particle size) column (Waters, South Africa) maintained at 40 °C. Two solvents were combined for gradient elution at a flow rate of 0.3 mL/min: Solvent A (distilled water acidified with 0.1% formic acid to a pH of 3.5 ± 0.02) and Solvent B (acetonitrile). The gradient programme was as follows: initially Solvent A:B (95:5), ramped to 70:30 in 8.5 min, then ramped in 1 min to 40:60, held for 1 min and back to A:B (95:5) in 1 min with a total run time of 12 min. The recorded range for the UV spectra was 200-400 nm with a spectral resolution of 1.2 nm.

7. Chemometric analysis

Chromatography data were exported to SIMCA P+ (13.0) software (Umetrics, Sweden) and used to construct chemometric models. Pareto (Par) scaling was applied to the GC data and univariate scaling to HPLC data. Clustering patterns were investigated and outliers identified by constructing a PCA model. Principal component analysis (PCA) and orthogonal partial least squares projections to latent structures discriminate analysis (OPLS-DA) models were constructed to identify biomarkers that can be used to differentiate between citrus fruits susceptible to citrus black spot (CBS).

Results and discussion

Objective	Achievement
Phase 1	
Objective 1. Study cultivars with low, medium and high susceptibility against CBS.	'Eureka' lemons, "Bitter Seville" orange, 'Late' "Valencia" orange, 'Nagami' kumquat and 'Tahiti' lime were sampled from fruit set and throughout the season until harvest.
Objective 2. Determining the thickness and architectural structure of the	This objective has still not been met, due to logistical issues.

	epicuticular wax of the different citrus cultivars using electron microscope imagery.	
Objective 3.	Determining the composition of the epicuticular wax layer to distinguish between the soft C ₂₀ – C ₂₇ chains of immature fruit and hard C ₂₈ – C ₃₃ chains of the mature fruit.	These three objectives are linked. Peel from the different growth stages were collected and frozen at -80°C for processing. Samples were freeze dried and extracted. Polar and apolar fractions were analysed using chromatographic techniques. Chemometric analysis of the results are presented as principal component analysis (PCA) score plots. From these it is clear that the phytochemical profiles of Bitter Seville, Valencia and lemons undergo changes that is depicted as the spatial shifts on the score plots (figures 1 – 2).
Objective 4.	Using NIR spectroscopy to study of the epicuticular wax layer of different citrus cultivars.	
Objective 5.	Using biochemical techniques such as TLC and HPLC-MS, and GC-MS when relevant, to determine the composition and distribution of phenolics, and lipids and oils in the rinds of the different cultivars.	
Phase 2		
Objective 1.	Expansion of the cultivar range studied: including more cultivars with a range of susceptibility/resistance.	'Nagami' kumquat and 'Tahiti' lime have been added as a CBS free control. Variability of cultivar/type susceptibility needs more expansion, however, the resources available at the moment is the limiting factor for expansion of the range.

Volatile secondary metabolites

The PCA model obtained accounts for 96.3% of the variation in X ($R^2_{cum} = 0.963$ and $Q^2_{cum} = 0.872$). The scores scatter plot for the first two principal components, Figure 4.4.2.1, revealed only one outlier, but this value was retained in the model because removing the data point did not improve the model parameters. The plot demonstrates that the citrus cultivars are clustered into three main classes, separated by the first principal component along the X-axis and the second component along the Y-axis. "Bitter Seville" (blue) is separated from "Valencia" (red) and lemon (green) and is grouped to the top of the Y-axis, while "Valencia" and lemon are clustered at the bottom of the Y-axis. However, along the X-axis, two groups are found, one consisting of "Valencia" and lemons and then another group containing only lemons. This grouping indicated that there are chemical compositional differences between these groups. In Figure 4.4.2.1B, the same scores plot as in 4.4.2.1A was coloured according to developmental stages. It can clearly be seen that there is separation within the cultivar groups according to developmental stages. For "Bitter Seville" only green small, green big and colour break data were available. The "Valencias" were already fully developed when samples and only colour break and fully mature were analysed. In figure 4.4.2.1B, the separation according to developmental stages are apparent. However, for lemons, samples of small green, big green, colour break and fully mature fruit were analysed. Two sets of ripe and colour break fruit were samples, one set with visible CBS infection and the other with no visible infection. Lemons separated into two groups, one group close to "Valencia" contained CBS infected colour break fruit and uninfected colour break fruit as well as fully mature CBS infected lemons. The other group to the right of the Y-axis contained all the other stages of lemon; a clear separation between infected and uninfected mature lemons can be seen.

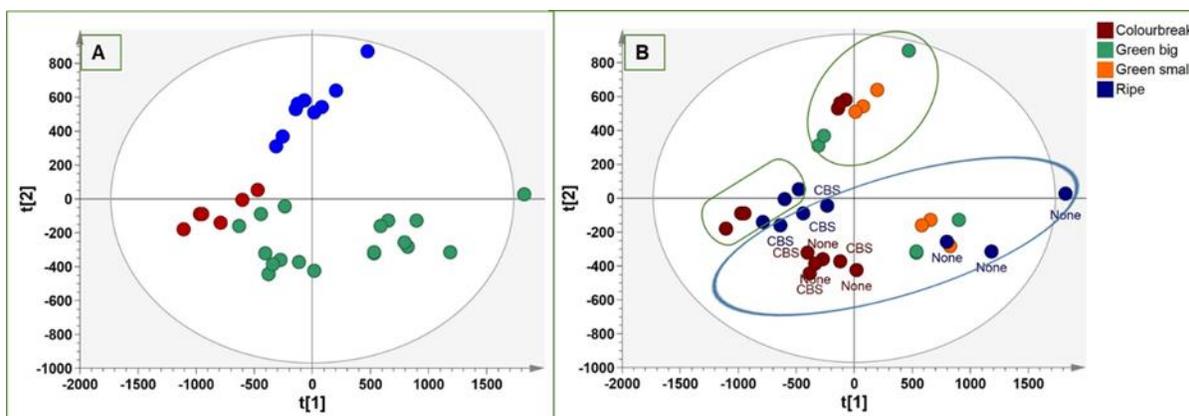


Figure 4.4.2.1. A: PCA scores plot of gas chromatographic data “Bitter Seville” (blue), “Valencia” (red) and lemons (green). B: PCA scores plot of selections coloured according to developmental stage with the three citrus fruits encircled as in Figure 1A.

The major volatile components identified by gas chromatography are limonene and ethanol. Minor components with relatively higher levels (higher than 1% relative to the major peak) were 2,4-bis(1,1-dimethylethyl)-phenol in “Bitter Seville”, myrcene and linalool in “Valencia”, β -pinene, γ -terpinene and citral in lemons.

Table 4.4.2.1: Percentage peak area, relative to the major peak, of gas chromatographic analysis of the three citrus varieties.

Compound	“Bitter Seville”	“Valencia”	Lemon
α -pinene	nd	0.56	nd
β -pinene	0.72	nd	4.51
sabinene	nd	nd	0.79
myrcene	0.53	2.08	nd
n-dodecane	0.51	0.41	nd
limonene	35.6	100	29.1
eucalyptol	0.6	nd	nd
3,3-dimethyl hexane	0.84	nd	nd
γ -terpinene	nd	nd	4.76
n-hexadecane	0.62	nd	nd
1,3-bis(1,1-dimethylethyl)-benzene	0.85	nd	nd
Octacosane	0.75	nd	nd
linalol	0.53	1.23	nd
n-eicosane	0.63	nd	nd
α -terpineol	nd	nd	0.56
citral	nd	0.5	2.1
8-heptyl-pentadecane...	0.39	0.3	nd
2,4-bis(1,1-dimethylethyl)-phenol	1.31	0.8	nd

Non-volatile secondary metabolites

The PCA model obtained accounts for 82.9% of the variation in X ($R^2 = 0.829$ and $Q^2 = 0.52$). The scores scatter plot (not shown) revealed no outliers and the three citrus fruits clustered into three separate groups.

The hierarchical cluster analysis of the PCA model, Figure 4.4.2A, indicated three main groups, with “Bitter Seville” (blue) in one group, lemons (green) in another and one group containing valecias (red) as well as lemon. Smaller differences (separations) can be seen on the dendrogram, indicating differences observed for the varieties at different sampling dates.

The samples were assigned to one of the three classes, based on the separation observed on the dendrogram. The groups revealed by the dendrogram allowed classes to be assigned in a less biased fashion than when using prior information regarding cultivars, since it is based purely on the chemical profiles of the fruit. An orthogonal projection to latent structures-discriminant analysis (OPLS-DA) model was subsequently constructed to identify the variables responsible for class discrimination (Bylesjö *et al.*, 2006, Mehl *et al.*, 2016). The model, Figure 4.4.2B, displays excellent model parameters, $R^2X_{cum} = 0.825$ and $Q^2 = 0.823$. The separation evident in Figure 4.4.2.2 can be attributed to differences in the phenolic composition of the various citrus samples. The loading scatter plot identified the variables responsible for the separation into the three groups, Figure 3. The “Bitter Seville” group separated as a result of very high levels of compounds eluting at 8.89 and 13.4 min as well as high levels of total phenolic content. “Valencia” on the other hand displayed high levels of the compound eluting at 11.2 min. Lemons had relatively low levels of all these compounds and therefore clustered into a separate group.

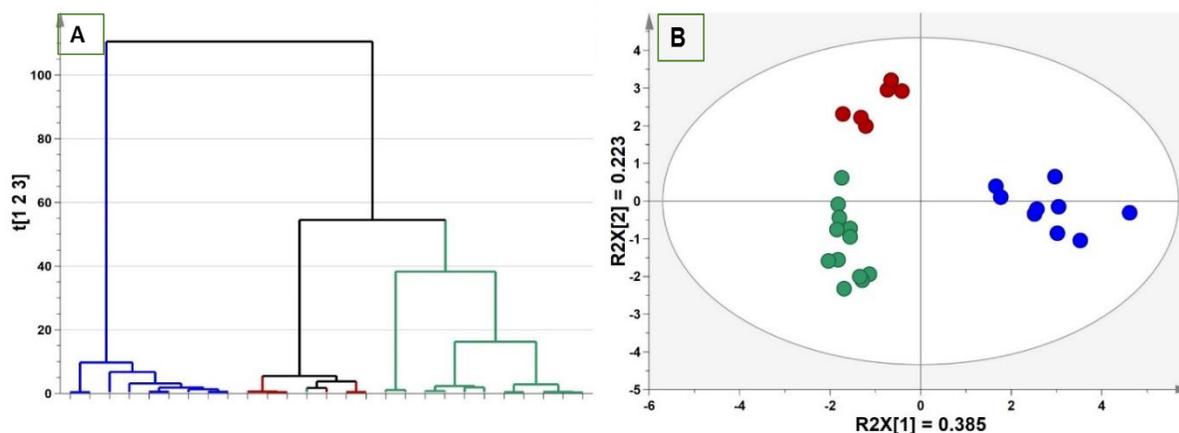


Figure 4.4.2.2. A: Hierarchical cluster analysis of HPLC data of all selections. B: OPLS-DA scores plot of HPLC data of all selections separated into three groups, with citrus varieties “Bitter Seville” (green), “Valencia” (red) and lemons (green) for both figures.

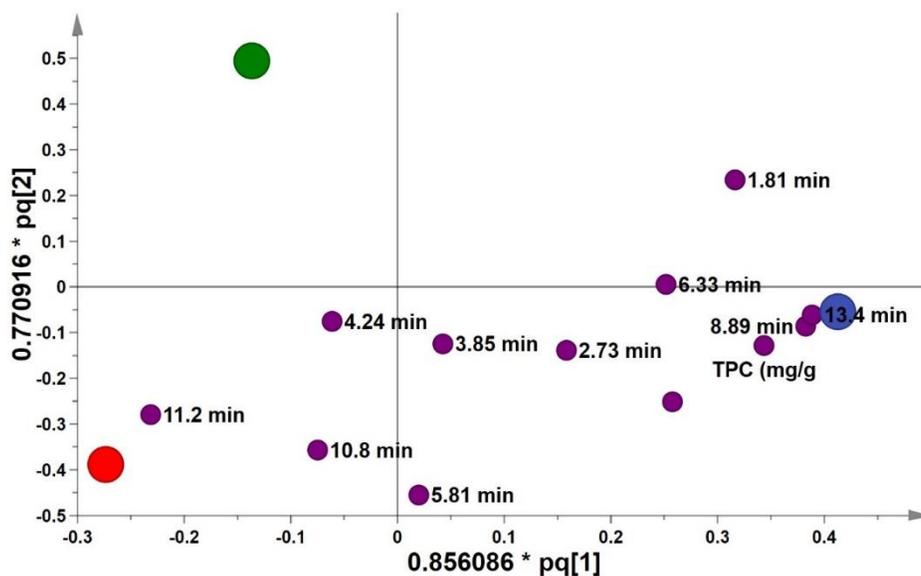


Figure 4.4.2.3. Loadings scatter plot of OPLS-DA, with Y-variables: Lemon (green), “Valencia” (red) and “Bitter Seville” (blue) as in Figure 4.4.2.2.

Tentative identification of the compounds responsible for the separation seen in Figures 4.4.2.2 and 3 were achieved by UPLC-QToF-MS analysis of the three citrus varieties. The retention times were much shorter as a result of UPLC analysis compared to HPLC, however, the same profiles were obtained. The red block in Figure 4.4.2.4, indicates the retention times of possible biomarkers for resistance of “Bitter Seville” compared to “Valencia” and lemons. The compound identified as biomarker at 8.89 min (5.04 min in UPLC chromatogram) was tentatively identified as naringin (naringenin-7-O-neohesperidoside) and the compound with retention time 13.4 min (5.85 min on UPLC chromatogram) as hesperitin-7-O-rhamnoside. However, the identities of these compounds must be confirmed by certified standards. Both these compounds are very prominent on the “Bitter Seville” chromatograms, Figure 4.4.2.4, and absent on the chromatograms of “Valencia” and lemon and may play a role in the observed tolerance of “Bitter Seville” to CBS infection. The compound eluting at 11.2 min on the loadings plot, Figure 4.4.2.3, and responsible for the separation of this variety from the other two cannot be identified as yet. It is a flavonoid, with a major mass fragment at 303 *m/z*, but the fragmentation pattern does not clearly indicate a specific compound.

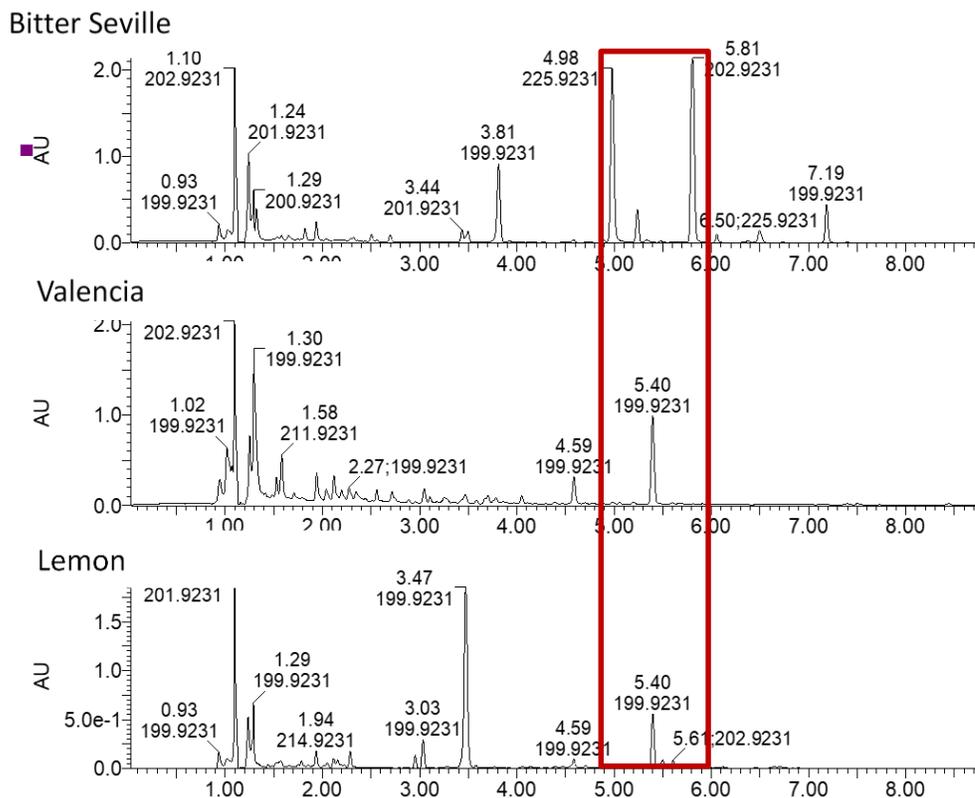


Figure 4.4.2.4. UPLS-DAD chromatograms of “Bitter Seville”, “Valencia” and lemon with the biomarker region marked in red.

In conclusion, metabolic profiling and chemometric modelling of volatile and phenolic compounds from citrus peel indicated a clear distinction between the profiles of the various citrus fruits. Significant differences between the groups could be demonstrated. These phytochemical differences will be correlated with differences such as CBS susceptibility, with particular reference to how, if identifiable, such compounds play a role in elicitation or inhibition of ascospore germination and infection establishment on the different citrus types used in this study.

Discussion of scope and techniques to end March 2019

- Dr Wilma Augustyn, a plant extract expert and analytical chemist at the Tshwane University of Technology is the main collaborator. Dr Augustyn has proven experience in the importance of cultivar-related phytochemical differences in bioactivities. The phytochemical analysis is being interpreted and evaluated by means of chemometric data analysis. We were unable to find a suitable postgraduate student for this project.
- The collected samples are analysed by GC, and GC-MS for the C₈-C₄₀ alkanes, as well as other phytochemical components. The analytical capabilities of the national asset base at TUT (Department of Analytical Chemistry) are being used.
- No gravimetric work was done, as the investigation has shifted from quantitative analysis to qualitative analysis.
- The water soluble fractions of the phytochemical content of the rind were studied using applicable extraction methods, followed by analysis using TLC and HPLC-MS.

- The proof-of-concept for using Near Infra-Red analysis of the epicuticular phytochemistry as a baseline reference study has been undertaken by Dr Obiro Wokadala (ARC).

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4.4.3 FINAL REPORT: Studies on the management of sour rot and green mould with propiconazole

Project 1141 (2018/19) by Lindokuhle C. Mamba, Cheryl Lennox, Julia Meitz-Hopkins (SU), Wilma du Plooy and Paul Fourie (CRI)

Summary

Following the withdrawal of guazatine from use in the European market, propiconazole (PPZ) is an important postharvest fungicide for use in the control of sour rot in South Africa. The active has some efficacy in controlling green mould, which is effectively controlled by imazalil, a demethylation inhibitor (DMI) like PPZ. *Galactomyces citri-aurantii* and *Penicillium digitatum* were evaluated for sensitivity to PPZ to assist in postharvest disease and resistance management. The sensitivity screening of DMI exposed isolates of both pathogens to PPZ was done using the discriminatory dose (DD) method. The sensitivity of 160 *G. citri-aurantii* isolates using DD of 0.5 and 0.9 $\mu\text{g}\cdot\text{ml}^{-1}$, and 141 *P. digitatum* isolates using DD of 0.2 and 0.5 $\mu\text{g}\cdot\text{ml}^{-1}$, were evaluated. Isolates were sampled from packhouses, juice factories, and orchards in the Eastern Cape and Western Cape citrus production regions. An isolate was classified as resistant when the relative mycelial growth was > 50% on PPZ amended growth media compared to non-amended growth media. *Galactomyces citri-aurantii* and *P. digitatum* isolates resistant to PPZ were recovered from packhouses where the fungicide was in use for three years. The *G. citri-aurantii* sensitivity range was wide (0 to approximately 100%) with a 15.5% resistance frequency. A high number of PPZ resistant *P. digitatum* isolates were recovered, with a 39.6% resistance frequency compared to *G. citri-aurantii*. The use of the DD was important in detecting shifts in fungicide sensitivity, and provided a threshold for differentiating between sensitive and resistant pathogen isolates.

Opsomming

As gevolg van die onttrekking van guasatien vir gebruik na Europa, het propikonasool (PPZ) 'n belangrike na-oes fungisied vir die beheer van suurvrot in Suid-Afrika geword. Die aktief het ook 'n mate van effektiwiteit teen groen skimmel, wat effektief beheer word met behulp van imazalil wat, soos PPZ, 'n demetileringsinhibeerder (DMI) is. *Galactomyces citri-aurantii* en *Penicillium digitatum* is geëvalueer vir sensitiwiteit teenoor PPZ om by te dra tot naoes siektebeheer en weerstandsbestuur. Die sensitiwiteitsifting van isolate van beide patogene wat reeds blootgestel was aan DMI's was gedoen met behulp van die metode vir die bepaling van die diskrimineringsdosis (DD). Die sensitiwiteit van 160 *G. citri-aurantii* isolate was ge-ëvalueer met DD van 0.5 en 0.9 $\mu\text{g}\cdot\text{ml}^{-1}$, terwyl 141 *P. digitatum* isolate met DD van 0.2 en 0.5 $\mu\text{g}\cdot\text{ml}^{-1}$ gedoen was. Isolate was versamel uit pakhuis, versappigsaanlegte en boorde uit die Oos- en Wes-Kaap produksie areas. Die isolate was as weerstandbiedend beskryf wanneer die relatiewe miseliumgroei > 50% was op media aangepas met PPZ groeimedium teenoor nie-aangepaste medium. *Galactomyces citri-aurantii* en *P. digitatum* isolate weerstandbiedend teenoor PPZ was gevind in pakhuis waar die fungisied al vir drie jaar in gebruik was. Die *G. citri-aurantii* sensitiwiteitsreeks was wyd (0 tot omtrent 100%) met 'n weerstandsfrekwensie van 15.5%. 'n Groot aantal PPZ weerstandbiedende *P. digitatum* isolate was gevind, met 'n 39.6% weerstandsfrekwensie in vergelyking met *G. citri-aurantii*. Die gebruik van DD was belangrik om sensitiwiteitsverskuiwings, asook die drempelwaarde vir onderskeiding tussen sensitiewe en weerstandige patoogeenisolate te verskaf.

Introduction

Sour rot, caused by the soil-borne pathogen *Galactomyces citri-aurantii* Butler, contributes significantly to postharvest losses incurred by the South African citrus industry. Outbreaks of the disease are sporadic, mainly being associated with fruit harvested during extended wet conditions. Stored, infected fruit disintegrates and can contaminate healthy fruit and pack-lines. Postharvest decay in citrus is routinely managed through the use of fungicides, which target *Penicillium* decays; however, none of these are effective against sour rot (Cohen, 1989; Smilanick *et al.*, 2008). In South Africa, the two fungicides specifically used to manage sour rot are sodium ortho-phenylphenate (SOPP) and guazatine (GZT). Both of these fungicides are at risk of being withdrawn for use as postharvest treatments due to pressure from the international markets. The use of SOPP by the citrus industry has declined recently because of disposal and human safety concerns (McKay *et al.*, 2012a).

Guazatine, although highly effective against sour rot, will never be registered in the USA as it contains multiple active ingredients and there is no method to analyse fungicide residues of the fungicide on fruit (McKay *et al.*, 2012a). Since May 2016 the EU has imposed a guazatine MRL of 0.05 mg/kg, which is comparable to a ban of the active ingredient. This is a major concern with Europe being one of South Africa's major export markets. It is vital that the citrus industry investigates alternatives to SOPP and GZT for the management of sour rot in South Africa. One such alternative is the use of the DMI-triazole propiconazole (PPZ) as a postharvest treatment (McKay *et al.*, 2007; 2012a). McKay *et al.* (2012a) found PPZ and cyproconazole to be highly effective in reducing postharvest sour rot of citrus. McKay *et al.* (2012a) chose to do additional studies with PPZ as a postharvest treatment as it has favourable toxicological characteristics for food crop registration in the US and the US registrant supports a world-wide registration. These are important considerations for the acceptance of alternatives to SOPP and GZT in South Africa. McKay *et al.* (2012a) found that tank mixtures of PPZ with the citrus postharvest fungicides fludioxonil and azoxystrobin were highly effective in reducing green mould caused by isolates of *Penicillium digitatum* sensitive or moderately resistant to imazalil and sour rot. The introduction of PPZ to the citrus postharvest industry poses a major threat to imazalil in terms of cross resistance, therefore the application of PPZ needs to be studied with the aim to optimise it for the best possible levels of control.

Stated objectives

- A. Determination of the *in vitro* sensitivity of South African sour rot and green mould isolates to the DMI-triazole fungicide PPZ.
- B. Determination of the most effective postharvest PPZ drench application strategy for the management of sour rot in South African pack-houses.
- C. Determination of PPZ residues associated with drench application strategies (Objective B) and determination of residue levels required to manage sour rot infection.
- D. Determination of the *in vitro* sensitivity of South African sour rot and green mould isolates to the DMI-triazole fungicide propiconazole.
 1. The baseline sensitivity of a previously unexposed population (N=50 each) of sour rot and green mould isolates will be determined on PPZ amended medium using FRAC protocols. From this the EC₅₀ will be determined.
 2. Sour rot and green mould isolates will be collected from pack-houses throughout the citrus industry and the sensitivity of the isolates tested on medium amended with the discriminatory dose (EC₉₅ X 10; calculated from the EC₅₀ using the Hill slope equation x 10).

- E. Determination of the most effective postharvest propiconazole drench application strategy for the management of sour rot in South African pack-houses on clementine and lemon.

Variables to be investigated

- i. Concentration: Registered dose (600 mg/L)
 - ii. Incubation time: 6, 14, 18 and 24 h
 - iii. Exposure time: 1, 2 and 3 min
 - iv. Pathogens
 1. *Galactomyces citri-aurantii*
 2. *Penicillium digitatum*
 - v. Evaluation
 1. Curative control
- F. Determination of PPZ residues associated with drench application strategies and determination of residue levels required to manage sour rot infection.

Materials and methods

1. Baseline sensitivity testing

Unexposed symptomatic fruits were sampled from orchards in the Western and Eastern Cape production regions. Fruit was brought to the lab for isolations. Fruit was surface sterilized and pathogens isolated. Single spore isolation was conducted for 50 isolates each (*G. citri-aurantii* and *P. digitatum*). Mycelial growth test of isolates grown on PDA media amended with propiconazole [range 0.009-4.5 mg/L] was conducted to determine the EC₅₀ from two unexposed populations of *G. citri-aurantii*, one from the Eastern Cape and one from the Western Cape and one *P. digitatum* population from the Western Cape.

Discriminatory dose testing

Technical grade of the active ingredient propiconazole (0.5 and 0.9 mg/L) was used in an amended agar test to measure the inhibition of mycelial growth of single spored isolates from packhouses and orchards of the regions Ashton, Citrusdal, Franschoek, Montagu, Simondium and Stellenbosch in the Western Cape and Addo, Kirkwood and Hermitage in the Eastern Cape.

2. Curative drench PPZ treatment

Commercial harvested fruit (clementines, lemons and oranges) were used for the trial. Fruit harvested on the same day were sanitized with 75 mg/L chlorine, and were dried and stored in a cold room (6°C). For *G. citri-aurantii*, fruit was also pre-treated with a protectant imazalil application (500 mg/L) and allowed to dry before used in the trial. Fruit (clementines and lemons) were moved from cold storage and left at ambient temperature a day before the trial. The fruit were inoculated with either *G. citri-aurantii* at 1x10⁷ spores per millilitre (isolate SR05) or *P. digitatum* at 1x10⁶ spores per millilitre (isolate GM03), making four inoculated wounds on each fruit. Inoculated fruits were put into cartons containing 12 fruit each, replicated 3 times per treatment incubated for 6 h, 14h, 18 h and 24 h before drench treatment. Cartons were covered with polyethylene bags. Incubation parameters for *G. citri-aurantii* further require the addition of 200 ml of distilled water soaked into sterile paper towel, and placed in the middle of the trays to create a suitably humid environment for disease development. A curative drench treatment with a formulated propiconazole

fungicide (PropiCure 250 EC, ICA Chemicals, Plankenburg, Stellenbosch) was applied on fruit at the recommended dose (600 mg/L) using three different exposure times (1 min, 2 min, 3 min). *Penicillium digitatum* inoculated fruit was stored at ambient temperature (21°C) in the lab and rated after 7 days. *Galactomyces citri-aurantii* inoculated fruit was stored in an incubation room at a temperature of 28°C and were rated after 6 days. Fruit was rated using a UV light and the infection incidence was recorded (*G. citri-aurantii* and *P. digitatum*). In the second season varying doses of PPZ (75, 150, 300, 450, 600 mg/L) were applied on clementine, orange or lemon fruit after inoculation with the same isolates of *G. citri-aurantii* (SR05) or *P. digitatum* (GM03). PPZ was either applied 8 h, 12 h, or 14 h after inoculation (as described above).

Results and discussion

Objective	Achievement
A. <i>In vitro</i> study	
A1. Baseline sensitivity	More than 50 isolates were collected from organic farms (one in the Eastern Cape, one Western Cape) for each of the two species, <i>G. citri-aurantii</i> and <i>P. digitatum</i> , respectively. In addition, more isolates were collected from conventional farms, giving a total of 158 isolates of <i>P. digitatum</i> and 191 isolates of <i>G. citri-aurantii</i> . Amongst the <i>G. citri-aurantii</i> isolates, two pathogenic isolates from a previous year's collection were identified as <i>G. geotrichum</i> . The isolates were single-spored and tested on propiconazole amended media. Mean EC ₅₀ values were established for both species. For <i>P. digitatum</i> (Western Cape isolates) the baseline sensitivity for PPZ was determined to be 0.15 µg/ml, and 0.21 µg/ml for Eastern Cape isolates. Similarly, for Eastern Cape isolates of <i>G. citri-aurantii</i> the baseline sensitivity was 0.68 µg/ml, and 0.31 µg/ml in the Western Cape. No attempt has been made to investigate the reason for the differences.
A2. Discriminatory dose resistance screening	Sour rot and green mould isolates were collected from packhouses and orchards in the Western Cape (N _{SR} = 128; N _{GM} = 70) and Eastern Cape (N _{SR} = 63; N _{GM} = 88) and tested at two discriminatory doses for propiconazole sensitivity in repeated trials (Fig.4.4.3.1).
B. Drench	
B1. <i>Galactomyces</i> spp. and <i>P. digitatum</i>	Two trials were conducted on clementine, lemon and orange fruit for the curative control of <i>G. citri-aurantii</i> and <i>P. digitatum</i> . After 6, 14, 18 and 25 hours' incubation, fruit was drench treated in a 600 mg/L PPZ solution for 1, 2, or 3 min. Curative control (incidence) was assessed.
B2. Residue analysis	Trials were done on clementine, navel and lemon to evaluate effective fungicide concentrations loaded in the drench at various fungicide concentrations and time intervals after inoculation (8 h, 12 h, 14 h).

A1. Baseline Sensitivity

Baseline sensitivity, measured as PPZ fungicide concentration that inhibits 50% of mycelial growth *in vitro* (EC₅₀) was determined for sour rot and green mould causing fungal isolates from each of two unexposed orchard populations (one from Eastern Cape, one from Western Cape). The results were similar to sensitivity reported from the USA, where the EC₅₀ for *G. citri-aurantii* was 0.34 µg/ml and *P. digitatum* was 0.008 µg/ml (McKay et al. 2012b). The sensitivity of populations of *G. citri-aurantii* populations to PPZ was EC₅₀ = 0.68 µg/ml (range 0.45-0.89 µg/ml) in the Eastern Cape (N=37 from Valencia oranges, Addo), and 0.31 µg/ml (range 0.004-1.09 µg/ml; N=52) in the Western Cape (clementines, Riviersonderend). For *P. digitatum*, the mean EC₅₀ was 0.15 µg/ml (range 0.03-0.41 µg/ml; N=70) in the Western Cape population and 0.21 µg/ml (range 0.031 – 0.606 µg/ml; N=88) in the Eastern Cape *P. digitatum* population.

A2. Discriminatory Dose Resistance Screening

Agglomerative Hierarchical Clustering (AHC) of the relative growth inhibition data for 191 *G. citri-aurantii* isolates grown on the discriminatory concentration of 0.5 µg.ml⁻¹ grouped the isolates into 3 classes, with relative growth inhibition percentages ranging from 7.0 – 29.5% (79 isolates), 30.3 – 62.3% (109 isolates) and 77.1 – 100% (3 isolates). Data for the 0.9 µg/ml discriminatory concentration were grouped into 4 classes, with percentages ranging from 2.7 – 11.7% (141 isolates), 12.3 – 19.8% (37 isolates), 21.7 – 38.2% (9 isolates) and 65.6 – 100% (3 isolates). For each discriminatory concentration assessment, the latter group of three isolates (GAL373, GAL085 and GAL087) was classified as PPZ resistant. The other classes identified in the AHC analyses of the 0.5 and 0.9 µg/ml discriminatory concentration data grouped in a normal and heavy-tailed distribution, respectively, and therefore not deemed separate from the sensitive sub-population. The relative growth thresholds were calculated as the means of the centroids for the resistant class and nearest neighbouring sensitive class and were 70.9% for 0.5 µg/ml and 55.3% for 0.9 µg/ml.

Using a discriminatory concentration of 0.5 µg/ml, it was found that 98.4% (188 out of 191) of the *G. citri-aurantii* isolates tested were sensitive to PPZ. All three resistant (2.3%) isolates were from the Western Cape and no resistant isolates were recovered from the Eastern Cape. A total of two out of 50 resistant isolates were previously exposed to PPZ (4%). None of the 21 isolates exposed to both IMZ and PPZ postharvest were classified as resistant. All three PPZ isolates resistant at 0.5 µg/ml were also resistant at the PPZ discriminatory concentration of 0.9 µg/ml.

A subset of *P. digitatum* isolates was screened at the discriminatory concentration of 0.2 µg/ml, and from these results it was clear that the discriminatory concentration was too low, since all exhibited relative growth of >50% (data not shown). Therefore, the tests were run at a discriminatory concentration of 0.5 µg/ml. Agglomerative Hierarchical Clustering (AHC) analyses were conducted for the 158 *P. digitatum* isolates from different citrus production regions of the Eastern and Western Cape screened at the discriminatory concentration of 0.5 µg/ml propiconazole. This grouped the *P. digitatum* isolates into 5 classes, with relative mycelial growth percentages ranging from 0 – 9.5% (53 isolates), 10.2 – 29.2% (70 isolates), 36.8 – 58.6% (16 isolates), 63.4 – 80.9% (14 isolates), 90.2 – 98.2% (5 isolates). The last three groups were classified as resistant to PPZ and therefore deemed separate from the sensitive, normally distributed sub-population. The relative growth threshold for the discriminatory concentration of 0.5 µg.ml⁻¹ propiconazole was calculated as the mean of the centroids for the nearest neighbouring sensitive and resistant classes, and was 33.3%.

A total of 123 out of 158 (77.8%) of the *P. digitatum* isolates tested at this concentration were sensitive to PPZ, with mycelial growth varying from 0 to 29% in comparison to the growth on the non-amended PDA control. Of all the isolates from the Western Cape (70 in total), 13 isolates (18.6%) were resistant to PPZ, whereas 22 of the 88 (25.0%) isolates from the Eastern Cape were resistant to PPZ. A total of 10 out of 38

(26.3%) and 22 out of 92 (23.9%) *P. digitatum* isolates previously exposed to PPZ and IMZ, respectively, exhibited resistance to PPZ. The IMZ resistant reference isolate STE-U6590 had a mean RG of 64.2% (ranging from 57- 72%) (Kellerman et al., 2017). Isolates from soft citrus showed a wider range of sensitivity (0 to approximately >90%) compared to isolates from other citrus types.

B1. Drench trial

Data from two season's drench trials using PPZ at the recommended dose (600 mg/L) were combined in an analysis to verify that results were consistent and combinable (Fig.4.4.3.1). Sour rot was most severe on soft citrus (clementine), followed by Eureka lemon and least problematic on Navel oranges. On lemon and clementine PPZ was found to be effective against sour rot when application was done within 8 h after inoculation, while it could be delayed on oranges for up to 18 hours. For green mould control on the other hand, PPZ had to be applied within 12 hours on all fruit types. Green mould incidence of more than 46% was observed when treatment was delayed for 18 h, when PPZ was applied on clementine and more than 39% for lemons and more than 32% incidence was observed on oranges when treatment was delayed for 24 h. In a second study (second season's trials) different PPZ dose rates were tested to determine how much fungicide was required to control sour rot or green mould. PPZ was most effective for sour rot control on lemons and oranges at a dose rate of 150 mg/L when applied within 8 h after inoculation, with less than 20% mean incidence, whereas 450 mg/L was required on clementine, resulting in less than 11.5 % mean infection. A dose rate of 150 mg/L was also found to be effective for sour rot control on oranges when applied within 12 h (mean disease incidence of 16.5%). Sour rot control was lost on clementine and lemon at the 12-hour treatment application with over 30 % mean disease incidence even at 600 mg/L PPZ. Conversely, on clementines, PPZ applied at 600 mg/L 14 h after inoculation was effective with 21% mean sour rot incidence. For oranges 450 mg/L PPZ was still effective at 14 h after inoculation with a mean sour rot incidence of 18%.

PPZ effectively controlled green mould on clementines and lemons at a dose of 300 mg/L up to 14 h after inoculation (mean incidence 23% and 7%, respectively). Green mould control on oranges was lost 14 hours after inoculation at all tested doses (> 32% mean incidence), but was still effective 12 hours after inoculation at a dose of 600 mg/L (12% mean incidence; Fig 4.4.3.2).

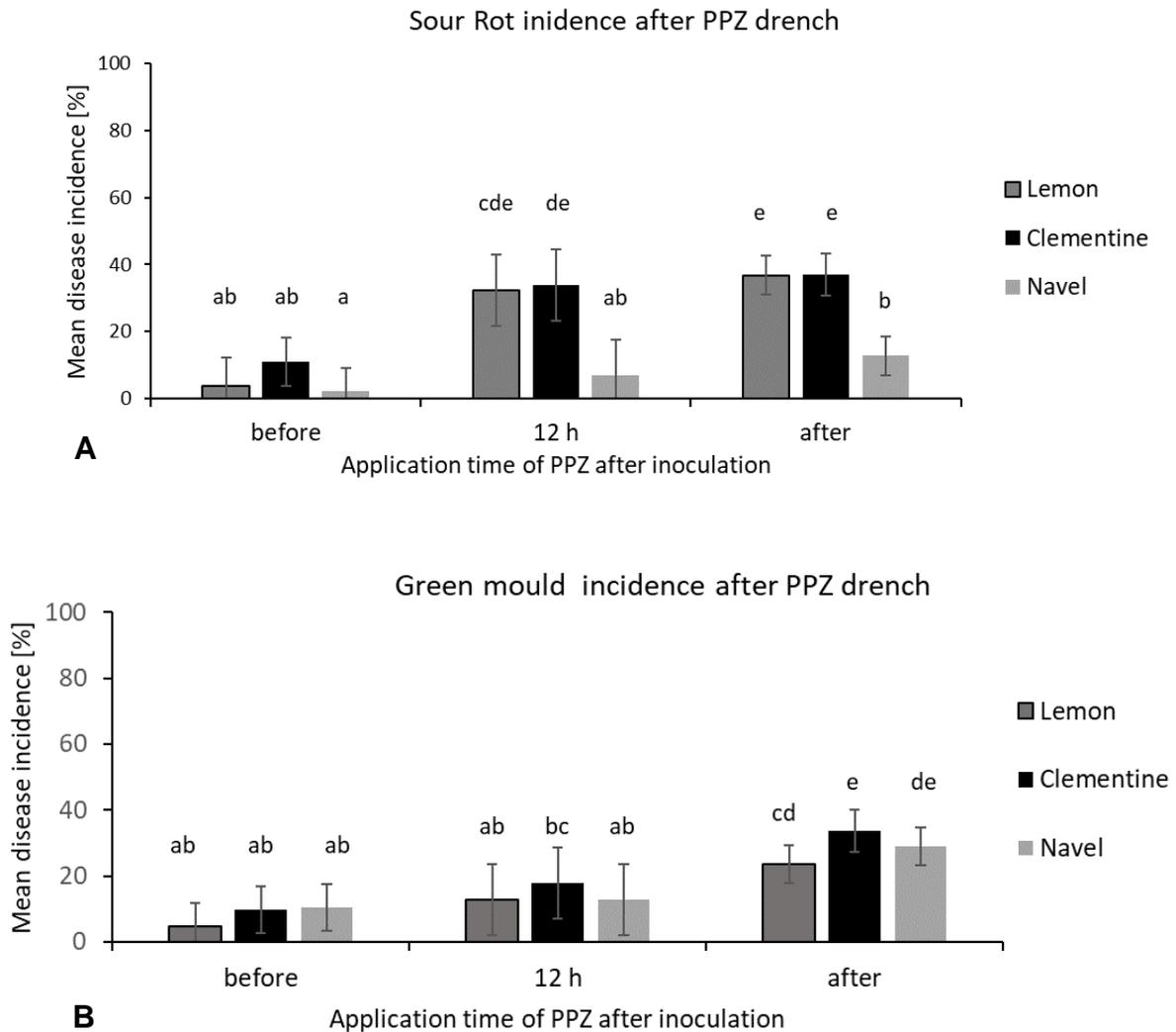


Fig. 4.4.3.1. A. Sour rot disease incidence on lemon, Clementine and Navel orange fruit after inoculation with *G. citri-aurantii* and drench application of 600 mg/L PPZ at different treatment times; either before 12 hours (“before”; i.e. 6 h or 8 h) or 12 hours (“12 h”) or more than 12 hours (“after”; i.e. 14 h, 18 h, 24 h). **B.** Green mould incidence on lemon, Clementine and navel orange after inoculation with *P. digitatum*.

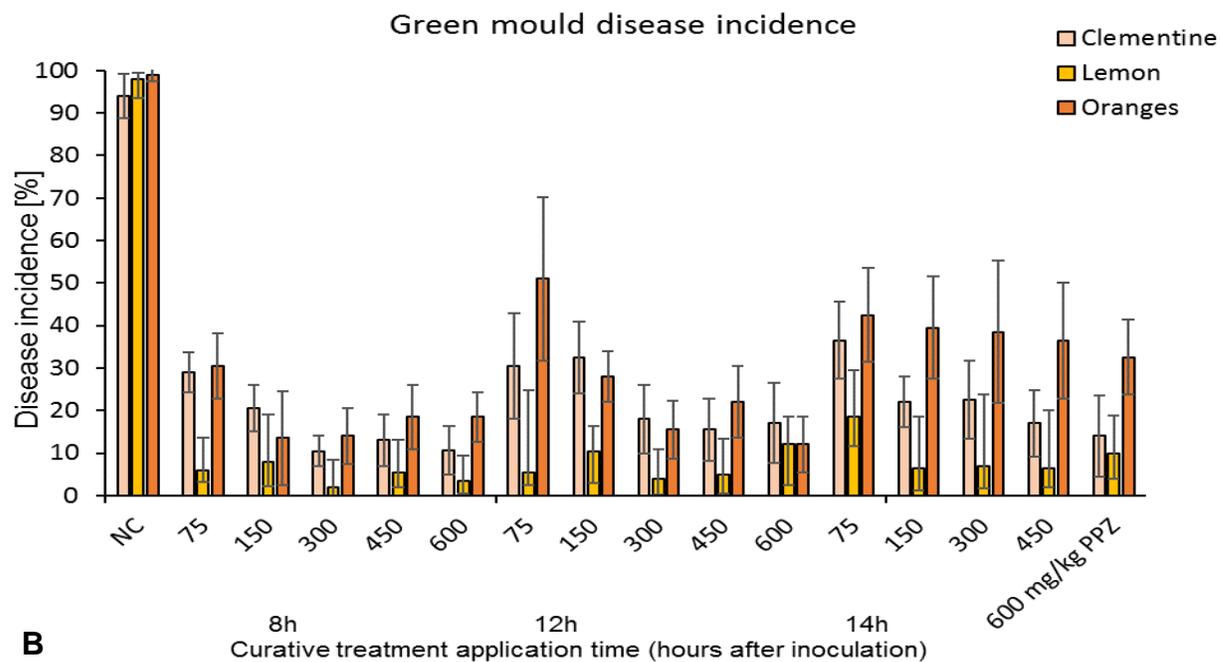
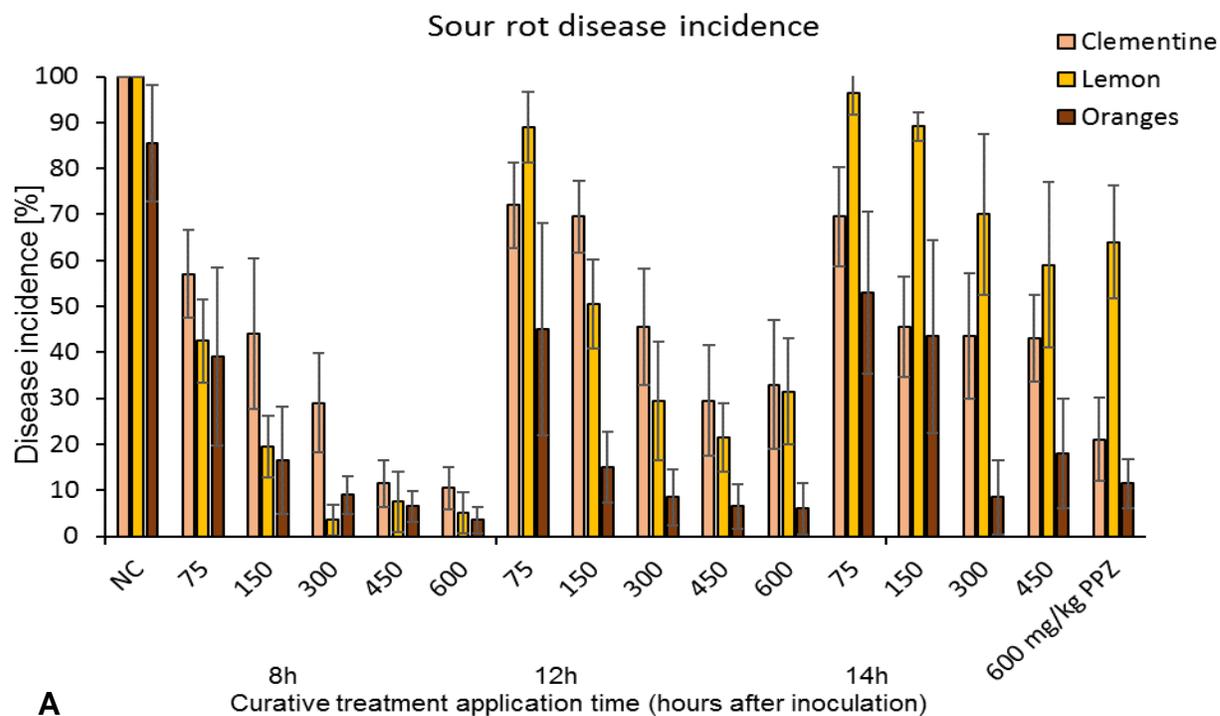


Fig. 4.4.3.2. A, Sour rot and **B**. Green Mould incidence when curative PPZ treatment was applied either 8 hours, 12 hours or 14 hours after inoculation with *Galactomyces citri-aurantii* (**A**) or *Penicillium digitatum* (**B**) at doses ranging from 150 mg/L to 600 mg/L. Error bars indicate 95% confidence intervals.

Conclusion

Propiconazole baseline sensitivity testing of *G. citri-aurantii* and *P. digitatum* isolates from unexposed orchards showed large variation within the sampled populations from the Eastern- and Western Cape, confirming ranges reported from American baseline sensitivity studies. Based on the data available from the literature and South African orchard populations discriminatory doses of 0.5 and 0.9 mg/L were chosen for resistance screening in *G. citri-aurantii* samples and 0.2 as well as 0.5 mg/L for *P. digitatum* isolates. Only 1.6 % of the tested *G. citri-aurantii* isolates from packhouses and orchards were categorised as PPZ resistant from populations in the Eastern Cape and Western Cape.

A large percentage of *P. digitatum* isolates were classified as resistant on PPZ amended media. These samples were mainly collected from lemons or oranges from packhouses in the Eastern Cape and satsuma samples from an orchard in the Western Cape. The drench trial showed significant differences in efficacy of propiconazole to control sour rot as well as green mould depending on application time on the different fruit types tested (Lemon, Clementine, Navel oranges).

Technology transfer

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- Mamba, L. C., du Plooy, W., Lennox, C.L. (2019) Citrus sour rot and green mould management by propiconazole drench application in South Africa. Postharvest Workshops, South Africa.

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APPENDIX A

Thesis emanating from this study is available from the University of Stellenbosch:

PROPICONAZOLE PRE-PACKHOUSE DRENCH APPLICATION AGAINST CITRUS SOUR ROT AND GREEN MOULD IN SOUTH AFRICA

By: LINDOKUHLE CYRIL MAMBA
 Supervisor: Dr. Cheryl. C. Lennox
 Co-supervisors: Dr. Julia Meitz-Hopkins
 Prof. Paul Fourie
 Dr. Wilma du Plooy

4.4.4 **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 (CRI), Thabani Mgwenya (CRI), Lindokuhle Mamba (CRI), Catherine Savage (CRI).

Summary

A single ring test was done and several products were evaluated. Once again, emphasis was placed on sanitation products. The powder formulation of PAA was tested for stability at different pH values – it did not affect the effectivity of fungicides at the range of pH values tested. Additionally, this formulation has a very low corrosive threat towards different metals. The formulation was also used in a preharvest trial to evaluate compatibility with preharvest phosphonates. At 0.2% application, no phytotoxicity was observed. The phosphonate is sensitive towards acidic solutions, and this has to be tested in the 2019 season. Fruit bioflavonoids were tested, but were ineffective in aqueous solutions. Further testing with azoxystrobin indicated good curative action. Several fungicide combinations were evaluated on an *ad hoc* basis, with initial results indicating the value of synergistic action.

Opsomming

'n Enkele ringtoetse was onderneem en verskeie produkte was getoets. Daar was weereens klem gelê op sanitasie produkte. Die poeierformulasie van PAA se stabiliteit was by verskillende pH waardes ondersoek - dit het nie die effektiwiteit van fungisied aktiewes by enige pH geïnhibeer nie. Hierdie formulasie van PAA het ook baie lae korrosiwiteit teenoor verskeie metale getoon. Verder was die poeier PAA in 'n boord (vooroes) proef gespuit om te bepaal of dit verenigbaar is met fosfonate wat vooroes gespuit word. Daar was nie fitoksisiteit in die boord waargeneem teen 0.2% van die poeierformulasie nie. Die fosfonaat is

egter gevoelig vir oplossings met 'n lae pH. Dit is dus iets wat in die 2019 seisoen moet herhaal word. Vrugte bioflavonoïde was getoets, maar is huidiglik nie effektief in 'n waterige omgewing nie. Verdere toets was met azoxystrobien gedoen, en dui op goeie kuratiewe effektiwiteit. Verskeie kombinasies van fungisiede was op 'n *ad hoc* basis getoets, met redelike goeie resultate, wat dui op die waarde van synergistiese aktiewes.

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 by 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 2018/19 report year.

Objective / Milestone	Achievement
1. New potential products will be tested as sanitation agents and/or fungicides.	No new actives were offered for trials, but some combined actives were investigated. These were all small, <i>ad hoc</i> trials with variable outcomes. A combination of FRAC groups as considered as part of support for the extension team when recommending resistance management strategies. Azoxystrobin and fludioxonil combinations seemed the most promising and was pursued further on behalf of ICA. A number of products were tested as sanitisers, with good results from the PAA products. A powder formulation was tested as an orchard spray one week before harvest, but failed to have the desired effect as a sanitiser against sour rot in the orchard. It was felt that the level of inoculation used on the fruit did put the product at a disadvantage, as even the control failed to have a positive outcome.
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 by Packhouse clients. Several of the samples indicated a shift in sensitivity, and the implicated packhouses were consulted on remedial action. The test methods are currently being revised, so that the results can be interpreted with more accuracy in terms of the actives implicated.
4. Analytical lab focus – ring test with the aim to reduce variability	A single test was conducted, but the German laboratory was not included. Once again, three South African laboratories had acceptable results, with the PPECB laboratory failing to detect correct levels of the spiked fungicide they were presented with. Unfortunately, due to the unacceptable time it takes to retrieve results from H&K, it is felt that this laboratory can no longer be used in routine

	work. We will, however, still send control samples there, only to verify that the results presented to us are accurate.
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Alternative products

A number of GRAS chemicals and alternative postharvest products were evaluated, in particular focussing on sour rot and green mould. Further testing with Azoxystrobin was conducted. The results of the different products were variable (Appendices 1-4). A preharvest trial with PAA was attempted in an attempt to evaluate the value of applying sanitisers in the orchard as a control measure against sour rot. This was not successful (Appendix 5).

Packhouse sanitation

Products offered as water sanitation options in citrus packhouses were evaluated. A powder formulation of PAA was evaluated to see if the pH of its solution can be adjusted readily, without any detrimental effects on the fungicide action. A bioflavonoid was tested, but that was not successful in an aqueous environment.

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 again. As in 2017, the test was presented without the laboratories knowing that it is part of a ring test. This was 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 2019. A few incidences of sensitivity shift in pathogen resistance were detected and the packhouses concerned consulted about the issue. A Standard Operating Procedure was compiled to enable uncontested repeatability of the tests (Appendix 6)

Technology transfer

Presentation of the extension-based work was done at the postharvest workshops, with complete revision of the fact sheets, which were presented as a booklet in January 2019. They are also available on the CRI website.

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

APPENDIX 1

Report of Pilot Trial: Wondercide at different pH levels

On behalf of: Ivan Davidson, Chemical Convertors
 Researchers: Wilma du Plooy, Catherine Savage
 Fruit type: Navels
 Origin: Nelspruit area
 Trial date: 2 July 2018
 Target fungi: *Penicillium digitatum* (green mould)
 Report date: 31 July 2018

Objective(s):

1. Determine the effect of pH adjustments on the efficacy of Wondercide as a sanitiser for use in aqueous applications in citrus packhouses, against *Penicillium digitatum* (PD).
2. Evaluate any possible signs of phytotoxicity after treatments.

Materials and methods:

Fruit	Healthy, untreated navels collected from a packhouse near Nelspruit, and stored at 22°C.
Trial design	3 replicates with 10 fruit each per pathogen and treatment combination
Spore suspension	A suspension of <i>P. digitatum</i> (1 x 10 ⁶ spores/mL) was prepared, and diluted to 1 x 10 ³ spores/mL in a 5 L water bucket.
Treatment	Wondercide were used at 0,2% (10 g/ 5 L) Imazalil (IMZ) was used at 500 ppm. The drench mix consisted of Thiabendazole (TBZ) and pyrimethanil (PYR), applied at 1000 ppm each, and 2,4-D at 250 ppm. The controls were: IMZ without added PAA, and drench mix without PAA. The treatments were applied as indicated in Table 1 below. Treatments were added to spore suspensions in 5 L buckets, and gently stirred for 180 seconds. Injured fruit were dipped into the solution for 120s.
Fruit injury	For inoculation, a custom plate with 10 x 8 mm prongs were used to injure the fruit on two equidistant sides of the equatorial area.
Storage	Each individual replicate was packed into a cavity liner and placed inside an open top display grape box, which was then placed in a polyethylene bag that was punctured 4 x with a 2 mm ² prong to allow gaseous exchange.
Incubation	Treated fruit were incubated at room temperature (≈ 22°C) for 4 – 10 days (when controls show >50% decay).
Assessment	Efficacy: Severity of infection as infected fruit per replicated carton was determined; any number of rotting lesions on a fruit resulted in a +1 count per fruit per box. Phytotoxicity: Untreated control fruit were compared to treated fruit and rind damage or defects rated after 72 hours.

Treatments:

No.	Trial	Active ingredient(s)	pH*	Dosage rate(s)
1	Untreated control	water	7.86	na

2	IMZ control	IMZ	3.64	500 ppm
3	Wondercide Normal use	PAA + IMZ	8.69	0.2% + 500 ppm
4	Wondercide	PAA + IMZ	5.51	0.2% + 500 ppm
5	Wondercide	PAA + IMZ	4.06	0.2% + 500 ppm
6	Drench mix control	PYR, TBZ, 2,4-D	8.15	1000, 1000 and 250 ppm resp.
7	Wondercide Normal use	PAA + (PYR, TBZ, 2,4-D)	9.75	0.2% + (1000, 1000, 250 ppm)
8	Drench mix + Wondercide	PAA + (PYR, TBZ, 2,4-D)	5.48	0.2% + (1000, 1000, 250 ppm)
9	Drench mix + Wondercide	PAA + (PYR, TBZ, 2,4-D)	3.02	0.2% + (1000, 1000, 250 ppm)

Results

1. The outcome of treatments in controlling induced infections is visually explained in Figure 1 below.

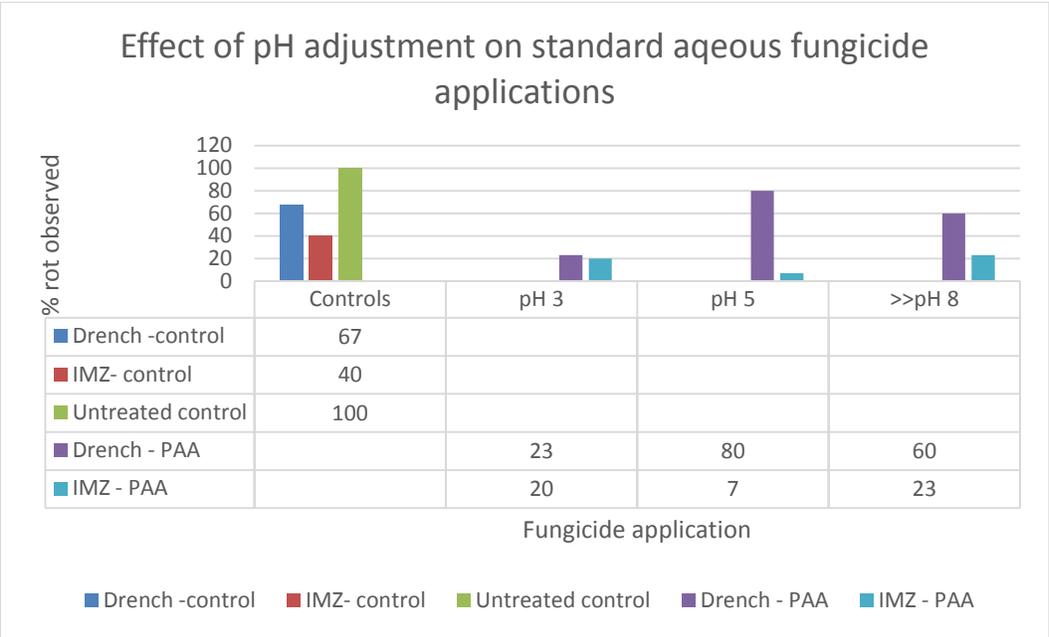


Figure 1 Graph representing the % rot seen in treated navel fruit, comparing Wondercide at different pH values, as well as unsanitised controls.

2. MRL data – not available from Hearshaw and Kinnes at the time of the report. Will be made available to Chemical Convertors as soon as the results are released to the CRI.
3. Phytotoxicity observed within 72 hours of the trials: NONE at any pH.

Discussion and Conclusion:

1. Prior to treatment the fruit receive more injuries than what normally happens on a packline, as this creates a pressure situation which enables the observation of the product potential.
2. The untreated water control resulted in a 100% rot, proving the viability of the culture used for inoculation, as well as the wounding technique applied.
3. The water temperature ranged between 16.4 and 16.6°C, which would have affected the pH marginally, but would not have skewed any of the results.
4. The adjustment of the pH had an effect on the fungicides' efficacy, with IMZ benefitting from its optimum pKa range at pH 5.
5. Wondercide added to the different aqueous solutions resulted in improved control for all IMZ applications, but not significantly so for the drench mixture.
6. The drench mix with added Wondercide did not perform well at pH 5, but performed slightly better at pH 8 than the drench mix control without Wondercide. It is suspected that the particularly poor performance of the drench mix at pH 5 is due to this range being unfavourable for fungicidal action by TBZ and PYR. Please note that 2,4-D is not a fungicide, but is included to ensure button retention to prevent interference from latent pathogens.
7. Navels have moderate sensitivity towards phytotoxicity, and no effect from the any of the Wondercide treatments was observed on the fruit tested.

Disclaimer:

No results from any pilot trial cannot be construed as an endorsement by the CRI of the trial product. Any product offered to the industry requires Act 36 registration and as such, successful trial products would still require further commercialisation with an accredited body.

Report by Dr Wilma du Plooy
31 July 2018

APPENDIX 2

Report of Pilot Trial: Determining the curative control of imazalil and fludioxonil in combination against resistant a *Penicillium digitatum* strain

On behalf of: CRI
 Researchers: Dr Wilma du Plooy, Catherine Savage and Thabang Mgwenya
 Fruit type: Navels
 Origin: Nelspruit area
 Trial date: 11 June 2018
 Target fungi: *Penicillium digitatum* (green mould)

MATERIALS AND METHODS

1. Trial layout summarised in table 1

Table 1 Layout of the trial

Curative	Fludioxonil	Imazalil	Temp of solution	pH of solution
Control	0	0	35.3	6.23

Control	0	1 X	36.2	5.78
	1 X (13 ml/5L)	0	35.2	9.03
	½ X (6.5 ml/5L)	½ X (1.68 g/5L)	35.8	6.67
	1 X (13 ml/5L)	1 X (3.35 g/5L)	35.6	5.93
	½ X (6.5 ml/5L)	1 X (3.35 g/5L)	35.2	5.89
	1 X (13 ml/5L)	½ X (1.68 g/5L)	35.9	6.35
	1 X (13 ml/5L)	0	22.3	9.06

2. Fruit was wounded 12 hours

before treatment – 4 wounds / fruit, administered equidistant from each other and 3 cm from the button.

3. Wounds were made with a 2 mm² prong.
4. Three reps x 12 fruit per treatment was used.
5. Fruit was laid in nectarine trays, placed in grape boxes and covered by lipping each box into a plastic bag. The bag was then punctured four times to allow some air exchange and moisture movement out of the box.
6. Fruit was incubated at 23°C for 5 days.
7. Residue samples were taken:
 - a. Fludioxonil in warm water
 - b. Fludioxonil in cold water
 - c. FLU + IMZ (1:1) in warm water

RESULTS

Table 2 The number of infected fruit and % control in each treatment combination with FLU and IMZ

Curative	Fludioxonil	Imazalil	# Infection	% Control
Control	0	0	100	0
Control	0	1 X	19/36	47,22
	1 X (13 ml/5L)	0	6/36	83,33
	½ X (6.5 ml/5L)	½ X (1.68 g/5L)	5/36	86,11
	1 X (13 ml/5L)	1 X (3.35 g/5L)	3/36	91.67
	½ X (6.5 ml/5L)	1 X (3.35 g/5L)	5/36	86,11
	1 X (13 ml/5L)	½ X (1.68 g/5L)	2/36	94,44
	1 X (13 ml/5L)	0	8/36	77,78

Table 3 Residues of FLU in cold water and at the temperature of the fungicide, as well as the equal ratio blend of LFU and IMZ

TEMP	RESIDUE (ppm)	
FLU cold	0,565561	
FLU warm	0,80589	
1:1 warm	0,802187	FLU residue
	2,051493	IMZ residue

CONCLUSION

1. The strain used was reasonably resistant against IMZ in typical conditions encountered in a fungicide bath.
2. Adding Fludioxonil to IMZ does improve the control of a resistant PD strain.

3. Fludioxonil in cold water had diminished control of the same PD strain. However, in warm water (typical temperature of a fungicide bath), the control is improved. From the residue data above, it is clear that the active does not load sufficiently in cold water.
4. A solution of ½ :1 (FLU : IMZ) seemed as effective as a ½ : ½ solution, giving some indication of a synergistic action. At a 1:1 ratio, however, some improvement in control was seen. The best result was at seen at a 1 : ½ ratio, indicating that FLU was able to improve the action of the combination.
5. This work needs to be repeated with more repeats and larger volumes of fruit, but from the results above adding fludioxonil to imazalil could be considered as a resistance management strategy. The number of residues on the fruit may, however, pose an issue.

Report by Wilma du Plooy

25 September 2018

APPENDIX 3

Report of Pilot Trial: The efficacy of ViBacSan as an alternative to sodium hypochloride as water sanitizer

On behalf of: VibacSan – represented by Marili Mouton

Researchers: Wilma du Plooy, Catherine Savage

Fruit type: Eureka lemons, Clementines

Origin: Nelspruit area

Trial date: 14 July 2018

Trial site: CRI, 2 Baker Street, Nelspruit, 1200

Report date: 17 August 2018

Target fungi: *Penicillium digitatum* (green mould); *Galactomyces citri-aurantii* (sour rot)

Objective(s)

1. Determine the efficacy of ViBacSan products (APSS and BC Stock) as alternatives to sodium hypochloride to sanitize water from *Penicillium digitatum* and *Galactomyces citri-aurantii* spores and prevent infection of injured fruit by these pathogens.
2. Evaluate any possible signs of phytotoxicity after treatments.

Materials and methods

Fruit	Collect healthy, untreated fruit and store at 10°C. Remove fruit from the cold room 24 h before commencing the trial.
Trial design	Use 5 replicates of 12 fruit each per pathogen and treatment
Spore suspension	Cover ¾ of sporulating <i>P. digitatum</i> cultures up with Tween water (1 drop of Tween/1 L water) and loosen spores with a glass hockey stick. With the use of a spectrophotometer at a 425 wavelength, prepare a concentration of 1 x 10 ⁶ spores/mL in the dip bath (120 L). Follow the same procedure for the preparation of a 1 x 10 ⁸ spores/mL <i>G. citri-aurantii</i> spore suspension.

Fruit injury	For <i>P. digitatum</i> inoculation, make four equidistant wounds into the albedo on the stem end, 2-3 cm from the button (navels) or 4 wounds across the same equatorial side (lemons). Follow the same method for <i>G. citri-aurantii</i> inoculation, but use picture hook with four 0.5 X 6 mm nails, resulting in 4 x 4 wounds
Treatment	Add treatments to the spore suspension and gently stir for 180 seconds. Dip the injured fruit into the solution for 120 seconds. Additionally, remove 8 mL of solution and plate out 100 µL of the mixture onto PDA, amended with antibiotics (PDA+) - replicate x 2.
Incubation	Allow fruit to dry before packing it on a pulp tray that is placed in a stone-fruit/grape carton. Cover the carton with a large transparent polyethylene bag. Fold the bag under the carton, but do not seal tightly. Make at least 4 holes (2-5 mm in diameter, use the inoculation tool, a pen etc.) in the plastic to reduce moisture and humidity. Incubate at room temperature (≈ 22°C) for 4 – 10 days (when controls show 50% decay). Follow the same method for <i>G. citri-aurantii</i> -exposed fruit, but add 100 ml water to the pulp tray before packing the fruit and incubate the cartons in the dark at 28°C for 4 days – 10 days. Additionally, incubate the agar plates in the laboratory at room temperature for 4 days.
Assessment	Efficacy: Determine severity of infection as infected fruit per replicated carton; Phytotoxicity: Compare untreated control fruit to treated fruit and rate rind damage or defects.

Treatments

Treatment no.	Product	Active ingredient	pH*	Dosage rate	
1	Untreated positive control	-	-	-	-
2	APSS	Bioflavonoid complex + fruit acids	4	1 X	0.5%
3	APSS	As above	4	2 X	1.0%
4	BC Stock	As above	4	1 X	1.0%
5	BC Stock	As above	4	2 X	2.0%
6	HTH Sodium hypochloride	Chlorine	7	1 X	150 ppm

Results

1. No Phytotoxicity was observed within 72 hours of the trials.
2. None of the treatments were successful in controlling the infections. All the controls and treatments displayed similar levels of rot.
3. This trial did not look at the possibility of the product being used as a surface sanitiser.
4. No residue was tested, as the product is based on bioflavonoids with no known residue issues.

Conclusion

None of the tested VibacSan products were effective as water sanitising products.

DISCLAIMER

No results from any pilot trial cannot be construed as an endorsement by the CRI of the trial product. Any product offered to the industry requires Act 36 registration and as such, successful trial products would still require further commercialisation with an accredited body.

Report by Wilma du Plooy
17 August 2018

APPENDIX 4

TRIAL: Azoxystrobin residue loading under different pH and temperature management regimes

On behalf of: ICA International Chemicals

By: Wilma du Plooy, Catherine Savage and Thabang Mgwenya

Objective

Testing the interactive effect of pH and temperature on azoxystrobin residue loading

Crop: Citrus - type mature Valencia

Origin: Naranja Packhouse, Burgersfort District

Fungicide: Azoxystrobin (Obstructo 250 SC at a rate of 450 ml/100L)

Trial date: 14 September 2018

Trial site: CRI, 2 Baker Street, Nelspruit, 1200

Initial report date: 17 November 2018

Updated report date: 18 January 2019

Materials and Methods

1. Freshly picked, mature Valencia fruit were collected from the commercial packhouse on the 8th of September and rinsed in chlorine (150 ppm total chlorine, pH 7 for 90 seconds), and allowed to air dry.
2. The fruit was stored at $\approx 8^{\circ}\text{C}$ for four days before the trial commenced. The day before the trial commenced the fruit was moved into ambient temperature ($\approx 22^{\circ}\text{C}$) in order for fruit temperature to also reach ambient, and to allow any possible condensation to evaporate.
3. pH adjustments were done with HCl or NaOH, whichever was appropriate to get to a pH range of 3, 5, 6.5, and 8.

4. Solutions were prepared with tap water at the correct temperature immediately before the fruit was dipped, with a temperature range at each pH of 25, 35, 42°C. These temperatures were selected as they most closely resemble typical temperatures found in a postharvest packhouse.
5. Each treatment had six replicates with 10 fruit in each repeat.
6. The fruit was dipped in a standard Azoxystrobin solution for 120 seconds, removed, dried in a forced air drying tunnel and stored at ambient temperature.
7. From each rep two fruit was randomly collected.
8. One fruit from each set was then resorted to constitute two replicates for residue analysis.
9. For each of the two residue reps, all six fruit were pooled together and prepared for residue determinations.

Table 1: Schedule of treatments used to evaluate pH + temperature interaction

Trmt no	pH	Temperature (°C)
1	3	25
2		35
3		42
4	5	25
5		35
6		42
7	6.5	25
8		35
9		42
10	8	25
11		35
12		42

Sample Preparation

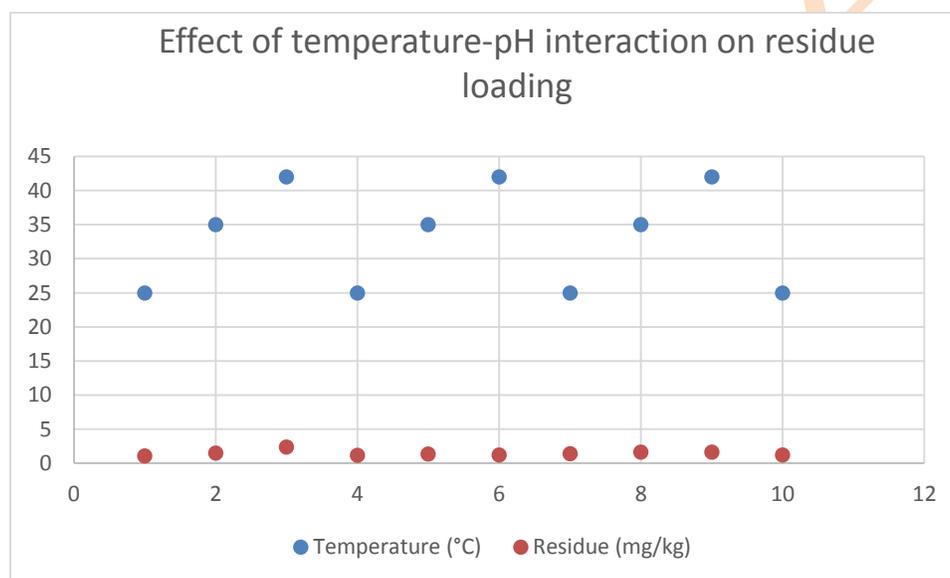
1. Fruit samples for residue analysis consisted of two replicates of six uninoculated fruit. Each sample of six fruit per treatment was macerated to a fine pulp using a blender and frozen at -20 °C. After treatment, they were chopped and blended (Salton Elite Blenders, Amalgamated Appliance Holdings Limited, Reuven, South Africa) into pulp using distilled water to dilute the fruit into a soft, paste-like consistency. The dilution factor for each sample was recorded. The pulp was frozen into sub-samples that were couriered to an accredited analytical laboratory (Hearshaw and Kinnes Analytical Laboratory, Cape Town, South Africa) for AZO residue analyses. Acetonitrile was used for extraction, which was followed by a matrix solid phase dispersion extraction. The extracts were analysed using liquid chromatography tandem mass spectrometry (LCMS/MS; Agilent 6410, Agilent Technologies Inc., Santa Clara, CA, USA) according to an accredited procedure. Residue data received from the analytical laboratory were recalculated using the dilution factor recorded when the pulp was blended. Residue results are expressed as mg·kg⁻¹ fresh fruit weight.

Results and discussion

Table 2: Residues loaded on Valencia fruit subjected to Obstructo 250 SC at different pH values and different temperatures.

Trmt no	pH	Temperature (°C)	Residue (mg/kg)
1	3	25	1,0903
2		35	1,5305
3		42	2,3853
4	5	25	1,1923
5		35	1,3625
6		42	1,2446
7	6.5	25	1,4226
8		35	1,6301
9		42	1,6665
10	8	25	1,2231
11		35	1,2279
12		42	1,7487

1. Throughout all temperatures and pH combinations, AZO residue loading was consistent, with the only anomolous result from pH 3 at 42°C.
2. This specific combination is highly unlikely to occur, as such low pH's are not found in the controlled working packhouse environment.



Graph 1: Temperature-ph interaction had very little effect on the residues loaded on mature Valencia fruit

Conclusion

Obstructo 250 SC has a very stable chemical behaviour in water applications, with the pH-temperature interaction very low. Being from FRAC group 11, this is an important consideration in resistance management of the pathogen. The owner of the product should pursue registration thereof.

DISCLAIMER

No results from any pilot trial cannot be construed as an endorsement by the CRI of the trial product. Any product offered to the industry requires Act 36 registration and as such, successful trial products would still require further commercialisation with an accredited body.

Report by Wilma du Plooy
17 November 2018

APPENDIX 5

TRIAL: Evaluation of PAA as a pre-harvest solution to control sour rot occurrence

BY: Wilma du Plooy, Charl Kotze
Crop: Citrus - Clementine two weeks before harvest
Origin: Larten Estates
Sanitiser: Powder PAA
Trial date: 19 July 2018

Objectives

1. Sour rot originates in the orchard, and the possibility to control the disease from rampant postharvest expression by pre-harvest sprays, were investigated.
2. The use of a phosphonate and PAA together may pose a risk of rind burn or breakdown. By applying them together, this risk was evaluated.

Materials and methods

1. Two litres of 1×10^4 spore suspension of *Galactomyces citri-aurantii* was prepared.
2. Small volumes of the spore suspensions were dispensed in the orchard during inoculation.
3. Per treatment, ten trees and 30 fruit per tree was inoculated by hand, using inoculation plates with 7 to 9 prongs.
4. Every inoculated fruit was clearly marked for future harvesting.
5. All spent and unspent inoculum was destroyed by adding 3% Jik to the solutions once inoculation was completed.
6. Treatments were freshly made in the orchard, just before application.
7. Treatments were applied two weeks before harvest, to comply with the Fighter label.
8. The treatments were prepared in 200 L batches, and applied with the custom build sprayer owned by the CRI
 - a. PAA at 0,2%
 - b. Fighter – applied as per label, as a light cover spray of 2000 l spray water per hectare.
 - c. PAA and Fighter in compatible ratio (the powder PAA ups the pH to about 8,5 and is therefore well-suited for the tank mix)
 - d. Untreated control (water)

Results

1. The disease control component of the trial was unsuccessful, due to the intense inoculation. The controls did rot with an 87% infection rate. Unfortunately, all of the treatments managed only about 45% control.
2. There was no phytotoxicity observed at the rates applied.
3. The trial should be repeated, but three more parameters need to be considered:

- a. the inoculation method should be less stringent
- b. the sanitiser should only be applied the day before harvest
- c. a more acidic PAA must be evaluated for phytotoxicity when applied shortly after (or with) Fighter.

Conclusion

No phytotoxicity between Fighter and the more alkaline PAA product was observed.

Report by Wilma du Plooy
21 August 2018

APPENDIX 6

TITLE: Discriminatory dose trial for loss of sensitivity testing

PURPOSE: Setting up and determining discriminatory doses for indication of loss of sensitivity by *Penicillium digitatum* against imazalil, pyrimethanil, and thiabendazole. This Standard Operating Procedure is intended to allow technicians to deliver reliable, repeatable results from swabs entered into the CRIDC by packhouses and extension officers.

DEFINITIONS: Imazalil (IMZ), pyrimethanil (PYR), thiabendazole (TBZ)

PROCEDURE:

Part A:

1. Prepare 6 x 250 mL PDA media in suitable Schott bottles as follows (label them bottle 1 – 6):
 - a. In 250 mL deionised water dissolve 9.75 g of PDA
 - b. Autoclave for 15 min.
 - c. Once cool (50°C), add 0.01 g of chloramphenicol (bacterial antibiotic).
2. Prepare 3 x 250 mL GAA media in suitable Schott bottles as follows (label them bottle 7 – 9):
 - a. In 250 mL deionised water dissolve
 - 1 g peptone
 - 0.25 g yeast extract
 - 3.75 g gelatine
 - 3.75 g agar
 - b. Autoclave for 15 min.
 - c. Once cool (50°C) add 0.01 g of chloramphenicol (bacterial antibiotic).
3. Autoclave 2 L deionised water, 3 x 500 mL empty Schott bottles, and a 500 mL measuring cylinder (with opening covered with tin foil).
4. Prepare 3 x stock solutions using the autoclaved water and glassware:
 - a. Add 0.67 g IMZ to 500 mL sterile water for a 1000 ppm stock solution.
 - b. Add 1 mL TBZ to 500 mL sterile water for a 1000 ppm stock solution.
 - c. Add 1 mL PYR to 400 mL sterile water for a 1000 ppm stock solution.
5. Continuously stir all stock solutions to prevent actives from settling out.
6. PDA medium with IMAZALIL
 - a. To bottle 1, add 250 µl of 1000 ppm IMZ stock solution to create a 1 ppm medium.

- b. To bottle 2, add 750 µl of 1000 ppm IMZ stock solution to create a 3 ppm medium.
 - c. To bottle 3, add 1250 µl of 1000 ppm IMZ stock solution to create a 5 ppm medium.
7. PDA medium with THIABENDAZOLE
 - a. To bottle 4, add 250 µl of 1000 ppm TBZ stock solution to create a 1 ppm medium.
 - b. To bottle 5, add 750 µl of 1000 ppm TBZ stock solution to create a 3 ppm medium.
 - c. To bottle 6, add 1250 µl of 1000 ppm TBZ stock solution to create a 5 ppm medium.
8. GAA medium with PRIMETHANIL
 - a. To bottle 7, add 250 µl of 1000 ppm PYR stock solution to create a 1 ppm medium.
 - b. To bottle 8, add 750 µl of 1000 ppm PYR stock solution to create a 3 ppm medium.
 - c. To bottle 9, add 1250 µl of 1000 ppm PYR stock solution to create a 5 ppm medium.
9. Pour media, and clearly mark the plates using different coloured stickers on the lids to differentiate between media.
10. Once cool, store in a refrigerator.

Part B:

1. Follow the SOP: PH – Preparation of swabs for testing for loss of sensitivity
 - a. Using swabs taken from active cultures, prepare 10⁻³ dilutions.
 - b. Using all the different concentrations of media for the determination of loss of sensitivity, streak 0.5 ml of each dilution onto plates prepared as in PART A above.
 - c. Streak out three repetitions per swab, per dilution of active, and per active being tested.
2. Use both the sensitive and the resistant strain of *Penicillium digitatum* as control organisms.
3. Incubate at 27°C for a minimum of 4 and maximum of 7 days.

4.4.5 PROGRESS REPORT: Fungal degradation of wood pallets used in export of citrus fruit Project 1165 (2017/18) by Wilma du Plooy, Elaine Basson and Paul Fourie (CRI)

Summary

Samples of filamentous fungi were collected from pieces of wooden pallets received from various packhouses. These fungi were cultured on PDA and two sets prepared for further studies. The first set was used to re-infect pallet wood. The wood used in these trials was collected from a pallet manufacturer, cut into 25 cm sections and sterilised before inoculation. Establishing infection is a slow process, but without it the alternative treatment products cannot be evaluated.

The second set was used to determine fungal diversity. The colony morphology and spore structures were documented using standard microscopy. From these, representative fungal cultures (n=267) were obtained for identification and characterisation. Identification was done using DNA sequence comparison on Genbank using sequence data from the β-tubulin and ITS gene regions of the different isolates. The following genera of fungi were identified by means of sequencing and Genbank Blast searches, *Acervuloseptoria* spp., *Alternaria* spp., *Cladosporium* spp., *Clonostachys* spp., *Drechslera* spp., *Fusarium* spp., *Gibberella* sp., *Lecanicillium* spp., *Leptosphaerulina* spp., *Mucor* spp., *Neocosmospora* spp., *Penicillium* spp., *Pithomyces* sp., *Trichoderma* spp.

The rest of the samples need to be sequenced with the ITS primers, as well as the following primer sets: beta-tubulin, elongation factor and large subunit (LSU). This will ensure an accurate identification of genera and species to confirm the absence of any phytosanitary threat amongst the pallet wood contaminants. Selected fungi will be also sent for further sequencing to determine species representativeness. It is planned that a profile of the mycological diversity on the wood types used for palletising, as well as the

different areas of production, will be drawn up. At the same time alternative products for the treatment of the pallet wood will be trialled in a commercial environment.

Opsomming

Monstersneming vir filamentagtige fungi is gedoen vanaf stukke houtpallet wat deur pakhuis voorsien is. Hierdie fungi is op ADA gekweek, met twee stalle wat voorberei is vir verdere werk. Die eerste stel was gebruik om skoon hout te herinfekteer. Hierdie hout was verkry vanaf 'n palletvervaardiger, opgesny in 25 cm stukke en gesteriliseer voor inokulasie. Houtinfeksie is 'n stadige proses, maar daarsonder kan alternatiewe produkte vir houtbehandeling nie getoets word nie.

Die tweede stel was gebruik om fungusdiversiteit te bepaal. Koloniemorfologie en spoorstruktuur was gedokumenteer met behulp van standard ligmikroskopie. Uit hierdie groep was verteenwoordigende kulture verkry (n=267) vir identifikasie en karakterisering deur die DNA volgordes van die β -tubulin en ITS geenareas van geselekteerde isolate te vergelyk met bestaande volgordes op GenBank. Die volgende genera is met behulp van DNA volgorde bepaling geïdentifiseer: *Acervuloseptoria* spp., *Alternaria* spp., *Cladosporium* spp., *Clonostachys* spp., *Drechslera* spp., *Fusarium* spp., *Gibberella* sp., *Lecanicillium* spp., *Leptosphaerulina* spp., *Mucor* spp., *Neocosmospora* spp., *Penicillium* spp., *Pithomyces* sp., *Trichoderma* spp.

Die ITS primêre paar volgorde bepaling vir res van die monsters moet nog gedoen word, sowel as vir die primêre stalle vir β -tubulin, verlengingsfaktor en groot subeenhede (LSU). Dit sal verseker dat akkurate indentifikasie van die genera en spesies gedoen word, asook bevestig dat geen fitosanitêre risiko teenwoordig is in die pallethoutkontaminante nie. "n Aantal fungi sal uitgekies en weggestuur word vir verdere volgorde bepaling om spesie verteenwoordiging te bepaal. 'Die optrek van 'n profiel van die mikoliese verskeidenheid op die hout wat vir palletisering gebruik word, asook vir verskillende areas, word beplan. Terselfdertyd sal alternatiewe produkte vir houtbehandeling getoets word binne 'n kommersiële omgewing.

4.4.6 PROGRESS REPORT: Evaluation of new postharvest fungicides for the control of *Phytophthora brown rot*

Project 1198 (2018/9 – 2019/20) by Jan van Niekerk (CRI); Lize van der Merwe and Cheryl Lennox (USPP)

Summary

Phytophthora brown rot caused by *Phytophthora nicotianae* can often cause serious losses postharvest. Currently there are no postharvest fungicides registered for the control of this disease on citrus. The aim of this project was to evaluate the baseline and non-baseline sensitivity of *P. nicotianae* towards these two fungicides *in vitro*. Results indicated that isolates from both previously unexposed and previously exposed pathogen populations could be divided into different azoxystrobin and fludioxonil sensitivity groups. These groups were statistically different based on their mean EC₅₀ and EC₉₀ values. For azoxystrobin the EC₅₀ values of the groups in the previously unexposed population ranged between 0.01 and 0.19 ppm and the EC₉₀ values between 4.28 and 83.96 ppm. In the unexposed population the EC₅₀ values for azoxystrobin was between 0.04 and 0.46 ppm and that of the EC₉₀ values between 11.45 and 84.85 ppm. For fludioxonil sensitivity the EC₅₀ values of sensitivity groups in the previously unexposed population was between 5.56 and 1613.52 ppm with the EC₉₀ values of these groups ranged from 1988.50 and 9929.30 ppm. The values for the groups in the previously exposed groups were similar. Here the highest EC₅₀ value was 84.79 and the lowest 3.10 ppm. The highest EC₉₀ was 6809.90 and the lowest 1090.50 ppm. Azoxystrobin and

fludioxonil were furthermore found to both have very good curative action, significantly reducing brown rot incidence when the fungicide was applied up to 12 hrs after inoculation. Applications done 24 hrs after inoculation also provided some curative action but not as good as earlier applications. Azoxystrobin furthermore provided very good protection against infection if inoculations were done up to 48 hrs after application. However, the preventative ability of fludioxonil was poor and not better than the untreated control. Both fungicides have shown to have potential to control the development of postharvest brown rot on lemons.

Opsomming

Phytophthora bruinvrot, veroorsaak deur *Phytophthora nicotianae*, kan ernstige naoes verliese veroorsaak. Daar is tans geen naoes fungisiedes vir die beheer van hierdie siekte op sitrus geregistreer nie. Die doel van hierdie projek was dus om die basisvlak en nie-basisvlak sensitiviteit van *P. nicotianae* teenoor hierdie twee fungisiedes *in vitro* te evalueer. Resultate het aangedui dat isolate van beide voorheen nie-blootgestelde en voorheen blootgestelde patoogeenpopulasies, in verskillende azoxystrobin en fludioxonil sensitiviteitsgroepe verdeel kan word. Hierdie groepe het verder statisties verskil, gebaseer op hul gemiddelde EC₅₀ en EC₉₀ waardes. Vir azoxystrobin het die EC₅₀ waardes van die groepe in die voorheen nie-blootgestelde populasie tussen 0.01 en 0.19 dpm gevarieer, en die EC₉₀ waardes tussen 4.28 en 83.96 dpm. In die nie-blootgestelde populasie, was die EC₅₀ waardes vir azoxystrobin tussen 0.04 en 0.46 dpm en vir die EC₉₀ waardes tussen 11.45 en 84.85 dpm. Vir fludioxonil sensitiviteit was die EC₅₀ waardes van sensitiviteitsgroepe in die voorheen nie-blootgestelde populasie tussen 5.56 en 1613.52 dpm, terwyl die EC₉₀ waardes van hierdie groepe van 1988.50 tot 9929.30 dpm gevarieer het. Die waardes vir die groepe in die voorheen blootgestelde groepe was soortgelyk. Hier was die hoogste EC₅₀ waarde 84.79 en die laagste was 3.10 dpm. Die hoogste EC₉₀ was 6809.90 en die laagste 1090.50 dpm. Daar is verder gevind dat azoxystrobin en fludioxonil baie goeie uitwissende aksie het, en het bruinvrotvoorkoms betekenisvol verminder wanneer die fungisied tot 12 ure na inokulasie toegedien is. Toedienings wat 24 uur na inokulasie gedoen is, het ook 'n mate van uitwissende aksie verskaf, maar nie so goed soos vroeër toedienings nie. Azoxystrobin het verder baie goeie beskerming teen infeksie gebied indien inokulasies tot 48 uur na toediening gedoen is. Die voorkomende vermoë van fludioxonil was egter swak, en nie beter as die onbehandelde kontrole nie. Beide fungisiedes het op suurlemoen getoon dat hulle die potensiaal het om die ontwikkeling van naoes bruinvrot te beheer.

4.4.7 PROGRESS REPORT: Comparison of the rind phytochemistry and wax composition of CBS resistant and susceptible citrus cultivars.

Project 1242 / RCE 2-13 (2019/20) by Wilma du Plooy (CRI) and Wilma Augustyn (TUT)

Summary

Volatile and non-volatile secondary metabolites from the rinds of lemon (*Citrus limon*), Valencia (*Citrus sinensis*) and Bitter Seville (*Citrus aurantium*) were investigated. From the 2017/2018 season fruit ranging from unripe to fully ripe provided the first insights into the phytochemical differences in the three types. More comprehensive sampling through all developmental stages from 2018 and 2019 seasons are currently used. Two M.Tech. students have joined the project in March 2019 and all pre-2019 samples were reanalysed to ensure consistency and continuance of the analytical methods used. Volatile fractions were analysed by GC-MS to identify metabolites as well as variation in metabolomics profiles of the three varieties at different developmental stages of the fruit. Non-volatile fractions were analysed by HPLC and the individual fractions thereafter analysed by UPLC-MS to obtain the metabolomic profiles of the three citrus varieties.

The major volatile components identified by gas chromatography were limonene and ethanol. Minor components with relative higher levels (higher than 1% relative to the major peak) are 2,4-bis(1,1-dimethylethyl)-phenol in Bitter Seville, myrcene and linalool in Valencias, β -pinene, γ -terpinene and citral in lemons.

All chromatography data were exported to SIMCA P+ (13.0) software (Umetrics, Sweden) and used to construct chemometric models. Principal component analysis (PCA) scores plot of gas chromatography data demonstrated that the metabolomics profiles of the volatiles were clustered into three main classes, according to variety. Separation into clusters indicate chemical compositional differences between the clusters. Within variety group separation was seen corresponding to developmental stages of the fruit. Two sets of ripe and colour break fruit were sampled, one set with visible CBS infection and the other with no visible infection. Lemons separated into two groups: one group closely clustering to Valencias contained CBS-infected fruit at both colour break and full ripeness. The second group clustered separately and contained all the other stages of lemon, enabling a clear chemical distinction between infected and uninfected lemons. An orthogonal projection to latent structures-discriminant analysis (OPLS-DA) model was subsequently constructed from HPLC data to identify the variables responsible for class discrimination. The separation can evidently be attributed to differences in the phenolic composition of the various citrus samples.

Tentative identification using UPLC-QToF-MS analysis were made of these compounds responsible for the phytochemical separations of the three citrus varieties. Two compounds are very prominent on the Bitter Seville chromatograms, and absent on the chromatograms of Valencias and lemon and may play a role in the observed tolerance of Bitter Seville against CBS infection. The compound identified as a biomarker at 8.89 min (5.04 min in UPLC chromatogram) has tentatively been identified as naringin (naringenin-7-O-neohesperidoside) and the compound with retention time 13.4 min (5.85 min on UPLC chromatogram) as hesperitin-7-O-rhamnoside. However, the identities of these compounds must be confirmed by certified standards.

Opsomming

Vlugtige en nie-vlugtige sekondêre metaboliete uit die skil van suurlemoen (*Citrus limon*), Valencia (*Citrus sinensis*) en Bitter Seville (*Citrus aurantium*) was ondersoek. Uit die 2017/2018 seisoen was vrugte vanaf onryp tot volledig ryp gebruik om die aanvanklike fitochemiese verskille tussen die drie groepe te bepaal. Tans word 'n baie meer volledige reeks deur alle ontwikkelingstadiums uit die 2018 en 2019 seisoene gebruik. Twee M.Tech. studente het in Maart 2019 aangesluit by die projek en all pre-2019 monsters word oorgewerk om eenvormigheid en kontinuïteit van die analitiese metodes te verseker. Die vlugtige fraksies was deur middel van GC-MS ontleed om sodoende die metaboliete te identifiseer, asook om die variasie in die metabolomiese profiele van die drie tipes teen verskillende ontwikkelingstadiums te betaal. Die nie-vlugtige fraksies is deur middel van HPLC geanaliseer, waarna die individuele fraksies met UPLC-MS ontleed was om die metabolomiese profiele van die drie tipes te verkry.

Die hoof vlugtige komponente soos deur gaschromatografie bepaal, was limoneen en etanol. Mindere komponente met relatiewe hoë vlakke (meer as 1% relatief tot die hoofpiek) was 2,4-bis(1,1-dimethyleetil)-fenol in Bitter Seville, mirseen en linalool in Valencia, β -pineen, γ -terpineen en citral in suurlemoene.

Die chromatografie data was volledig oorgeplaas na SIMCA P+ (13.0) sagteware (Umetrics, Sweden) en gebruik om die chemometriese modelle mee te bou. Hoofkomponent analiese (PCA) punte grafieke van die gaschromatografiese data het gedemonstreer dat die metabolomika profiele van die vlugtige komponente in drie hoofklasse gegroepeer word, volgens sitrus variëteit. Hierdie skeiding het verder die

chemiese samestellingsverskille tussen die groepe bevestig. Binne elke variëteit kon daar verder ook onderskei word tussen ontwikkelingstadiums. Twee stelle vrugte by kleurbreek en volryp was ondersoek: een stel met swartvlekletsels en een stel sonder enige sigbare letsels. Suurlemoene het in twee groepe verdeel: die een groep met swartvlekletsels in beide kleurbreek en volrypheid het naby die Valencias gegroepeer. Die tweede groep het al die ander stadiums van suurlemoene bevat, wat daarop dui dat duidelike chemiese onderskeiding tussen geïnfekteerde en nie-geïnfekteerde suurlemoene getref kan word. Gevolglik was 'n ortogonale projeksie op latente struktuur-diskriminant analiese (OPLS-DA) model gebou vanaf die HPLC data ten einde die veranderlikes wat verantwoordelik is vir die klasonderskeiding, te bepaal. Hieruit was afgelei dat skeiding toegeskyf kan word aan verskille in die fenoliese samestelling van die verskillende sitrusmonsters.

Voorlopige identifikasie van die komponente wat lei tot die fitochemiese onderskeiding van die drie sitrustipes, is met behulp van UPLC-QToF-MS analiese gedoen. Twee komponente was prominent op die Bitter Seville chromatogramme, maar afwesig in Valencia en suurlemoene. Hierdie komponente mag 'n rol speel in die weerstandigheid van Bitter Seville teen swartvlek infeksie. Die komponent op die biomerker by 8.89 min (5.04 min in UPLC chromatogram) is voorlopig geïdentifiseer as naringien (naringenien-7-O-neohesperidosied), terwyl die biomerker met 'n retensietyd van 13.4 min (5.85 min op die UPLC chromatogram) geïdentifiseer is as hesperitien-7-O-rhamnosied. Die identiteit van die twee verbindings sal met gesertifiseerde standaarde bevestig word.

4.5 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	38	1259	33	661
Nematode:Soil	4	27	58	611
<i>Phytophthora</i>	6432 ¹	1732	156	710
Water spore trap	195	0	4	0
Black spot identification (PCR)	0	251	0	32
Black spot benzimidazole resistance	0	69	0	0
Citrus greening (PCR)	0	35	0	0
Post Harvest Sensitivity	0	147	0	12
Fruit & Foliar identification	0	82	93	23
Soil dilution plating	0	275	57	6
VIRUS/VIROID PCR	0	8	0	0
SUB-TOTALS	6669	3885	401	2055

¹ Total samples received for citrus nurseries – includes quarterly samples, re-tests and non-certified nurseries

Footnote:

¹ Sample number and the percentage positive are only for certified nurseries and only for the quarterly samples received.

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, 5398² nursery samples were received by the diagnostic centre for *Phytophthora* analyses. Of these samples, 3.91% 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, Kwazulu-Natal, Limpopo, Mpumalanga, Northern Cape, North West, Swaziland, and Western Cape. Most of the samples received from citrus growers were analysed for *Phytophthora nicotianae* and the citrus nematode, *T. semipenetrans*. Fifty-one percent of the 1259 samples analysed for citrus nematode had counts above the threshold value of 1000 females per 10g of roots, and nematicide treatments were recommended. Forty-four percent of the 1732 samples analysed for *Phytophthora* tested positive.

Other crops

Nematode counts were done on soil or root samples of Avo, Banana, Blueberries, Macadamia and Peppers. Nematodes found present on these crops included: *Criconema*, *Helicotylenchulus*, *Hemicycliophora*, *Longidorus*, *Meloidogyne*, *Paratylenchus*, *Pratylenchus*, *Rotylenchus*, *Rotylenchulus*, *Scutellonema*, and *Xiphinema*. *Phytophthora* and *Pythium* analyses were done on Avocado, Blueberries, Grape, Macadamia and Peppers. The diagnostic centre analysed 60 soil samples from macadamia nurseries for the presence of *Phytophthora cinnamomi*.

Research samples

Nematode and *Phytophthora* analysis were done on 1982 samples from experimental trials. The Diagnostic Centre assisted in trials to identify possible citrus black spot lesions using PCR protocols.

CRI Diagnostiese Sentrum (Elaine Basson, Charmaine Olivier, Aubrey Metane, Samuel Ndlovu, en Jan van Niekerk)

Ontleding	Sitrus kwekerie	Kommersiële monsters	Ander gewasse	Navorsings-monsters
Aalwurms: Wortels	38	1259	33	661
Aalwurms: Grond	4	27	58	611
<i>Phytophthora</i>	6432 ¹	1732	156	710
Water spoorlokval	195	0	4	0
Swartvlek (PKR)	0	251	0	32
Swartvlek benzimidazole bestandheid	0	69	0	0
Sitrusvergroeningsiekte (PKR)	0	35	0	0
Na-oes bestandheid (Imazalil)	0	147	0	12
Vrug- en blaar identifikasie	0	82	93	23
Grondverdunningsplate	0	275	57	6
Virus / Viroïedes PCR	0	8	0	0
TOTAAL	6669	3885	401	2055

¹ Totale hoeveelheid monsters ontvang van gesertifiseerde kwekerie – sluit in kwartaal monsters, hertoets monsters en nie-gesertifiseerde kwekerie

Voetnota:

¹ Monster hoeveelheid en die persentasie positief is net vir gesertifiseerde kwekerie en slegs vir die kwartaal monsters ontvang.

Sitrus Gesertifiseerde Kwekerye

Dit is verpligtend vir al die sitruskwekerye wat aan die Sitrus Verbeteringskema deelneem om kwartaalike monsters vir *Phytophthora* te laat ontleed. Die besproeiingswater moet ook deur middel van die spoorlokval metode vir *Phytophthora* getoets word. In totaal 5398² monsters is deur die diagnostiese sentrum vir *Phytophthora* ontleding ontvang, waarvan 3.91% positief getoets het. Benewens die water en grondmonsters, moet kwekerye een keer per jaar 'n wortelmonster instuur om vir die teenwoordigheid van *Tylenchulus semipenetrans* te toets. Van die wortel- en grondmonsters wat ontvang is, het 0.0% positief getoets vir die teenwoordigheid van *T. penetrans*.

Kommersiële monsters

Monsters is uit die volgende sitrusverbouingsareas ontvang: Oos-Kaap, Kwazulu-Natal, Limpopo, Mpumalanga, Noord-Kaap, Noord-Wes, Swaziland, en Wes-Kaap. Die meeste van die monsters wat van sitrusprodusente ontvang is, is vir *Phytophthora nicotianae* en die sitrusaalwurm, *Tylenchulus semipenetrans*, ontleed. Een-en-vyftig persent van die 1259 aalwurmmonsters wat ontleed is, het tellings hoër as die drempelwaarde van 1000 wyfies per 10g wortels gehad. Aalwurmdoderbehandelings is in daardie gevalle aanbeveel. Vier-en-veertig persent van die 1732 monsters wat vir *Phytophthora* ontleed is het positief getoets.

Ander Gewasse

Aalwurmtellings is op grond- of wortelmonsters van Avokado, Bloubessies, Macadamia, Pepers, en Piesang gedoen. Aalwurms teenwoordig gevind op hierdie gewasse sluit in: *Criconema*, *Helicotylenchulus*, *Hemicycliophora*, *Longidorus*, *Meloidogyne*, *Paratylenchus*, *Pratylenchus*, *Rotylenchus*, *Rotylenchulus*, *Scutellonema*, and *Xiphinema*. Avokados, Bloubessies, Druive, Makadamias, en Pepers monsters is vir *Phytophthora* en *Pythium* ontleed. Die diagnostiese sentrum het 60 monsters vanaf makadamia kwekerye ontvang om vir *Phytophthora cinnamomi* te ontleed.

Navorsingsmonsters

Aalwurm en *Phytophthora* ontledings is op 1982 monsters afkomstig uit navorsingsprojekte gedoen. Die Diagnostiese Sentrum het ook hulp verleen aan navorsingsprojekte in die identifikasie van moontlike sitrus swartvlek letsels deur middel van PKR.

5 PORTFOLIO: CITRICULTURE

5.1 PORTFOLIO SUMMARY

By Paul Cronje (Portfolio Manager: CRI)

The aim of the Citriculture portfolio is firstly to improve yield and quality in the orchard and secondly to reduce postharvest quality loss in the cold chain, in order to increase the value and suitability of the citrus industry. To realise this aim, relevant research projects are undertaken on a wide array of producer identified research priorities. These include improvement of aspects surrounding nutrition requirements of citrus trees to address aspects such as fruit set, fruit size and rind condition. Momentum has been gained in this vital programme which will be expanded in scope in future seasons. The previous season saw the successful completion of a significant project for the industry on shade netting. This new technology,

increasingly employed in all production areas and for a wide range of cultivars, is changing how citrus production is viewed. The fundamental information supplied on the eco-physiological changes of the tree and fruit stemming from this project will enable decision makers at farm level to successfully produce high volumes of export quality fruit. The negative impact of rind disorders and in particular chilling injury mandate a more complete understanding of cultivar specific responses to the relevant pre- and postharvest conditions affecting incidence. For lemon fruit a clear impact of temperature during the last stages of fruit development has been identified. Furthermore, successful interventions in the postharvest process i.e. wax application, has been expanded on. The SA citrus cold chain is ever increasing in complexity and in the 2018 season projects to improve the conditions, in terms of the FMS, have been completed. Cultivar and rootstock evaluation remain a key component of continuous improvement efforts of the industry. The reliable flow of information from this programme underpin long-term decisions in the industry.

PORTEFEULJE OPSOMMING

Die doel van die Citriculture-portefeulje is eerstens om die opbrengs en kwaliteit in die boord te verbeter en tweedens om kwaliteitverlies ná oes in die koueketting te verminder, ten einde die waarde en volhoubaarheid van die sitrusbedryf te verhoog. Hierdie doel word verweselik, deur toepaslike navorsingsprojekte te onderneem op 'n wye verskeidenheid produsente geïdentifiseerde navorsingsprioriteite. Dit sluit in aspekte rondom die voedingsbehoefte van sitrusbome om aspekte soos vrugset, vruggrootte en rooikondisie aan te spreek. Hierdie belangrike program is besing om momentum gekry en sal in die komende seisoene voortgeste word. Die afgelope seisoen het die suksesvolle voltooiing van 'n beduidende projek vir die industrie op skadunet gesien. Hierdie nuwe tegnologie, wat toenemend in alle produksiegebiede en vir 'n wye verskeidenheid kultivars gebruik word, verander hoe ons dink oor sitrusproduksie. Die fundamentele inligting wat verskaf was uit die projek oor die ekofisiologiese veranderinge van die boom en vrugte, sal besluitnemers op plaasvlak in staat stel om verhoogde produksie van uitvoergehalte te produseer. Die negatiewe impak van na-oes defekte, veral koueskade, vereis 'n meer volledige begrip van kultivar-spesifieke respons op die relevante voor- en na-oes toestande wat die defek se voorkoms kan beïnvloed. Vir suurlemoenvrugte was 'n duidelike invloed op temperatuur gedurende die laaste stadium van vrugontwikkeling geïdentifiseer. Daarbenewens is suksesvolle praktyke in die na-oes omgewing soos waksaanwending op uitgebrei. Die SA sitruskoueketting raak konstant meer kompleks en in die 2018-seisoen was projekte om die hierdie tempertuur beheer te verbeter, soos gesien vanuit die FMS perspektief, voltooi. Evaluering van kultivars en onderstamme bly 'n belangrike onderdeel van die voortdurende pogings van verbeterde vrugkwaliteit van die bedryf. Die betroubare vloei van relevante inligting uit hierdie program ondersteun die langtermynbesluite in die bedryf.

5.2 PROGRAMME: RIND CONDITION AND COLD CHAIN

Programme coordinator: Paul Cronje (CRI)

5.2.1 Programme summary

Postharvest handling and the effect on firstly the incidence of rind disorders and secondly maintaining target temperatures to markets under a cold sterilisation protocol or the system approach, will remain a key focus of CRI research effort. The increase in export volume of mandarin and lemons already experienced in the last two to three seasons are adding strain to the citrus cold chain and new strategies and technologies are being tested for implementation. One such example was the successful inclusion into the FMS of ambient loading citrus at Tzaneen in containers on trains to Durban which were on target temperature on arrival. Various aspects of the FMS were also investigated in order to improve the accuracy of temperature control in containers. The temperature range used to the EU has an unfortunate negative impact on fruit quality in

the form of increased chilling injury incidence. A large scale project on aspects impacting on CI of lemon fruit indicate significant differences between areas and seasons. However, some mitigating actions such as use of wax have been found to reduce CI. The use of shade netting is increasing and the effect on all the various cultivars is to a large extent unknown. The data in the first season from the Eastern Cape indicate no noticeable difference in susceptibility to rind disorders. This programme will continue to identify and improve areas in the postharvest handling and cold chain which could influence fruit quality, as the increase in export volume will make this a key aspect in suitability.

Programopsomming

Na-oes hantering en die uitwerking op eerstens die voorkoms van skildefekte en tweedens die handhawing van verlangde temperatuur na markte wat 'n koue sterilisasieprotokol of die stelselbenadering verlang, sal 'n fokus bly vir die CRI-navorsingsprogram. Die toename in die uitvoervolume van mandaryne en suurlemoene wat reeds in die afgelope 2-3 seisoene ervaar was, het die druk op die sitrus-koueketting verhoog en moet daar dus na nuwe strategieë en tegnologieë getoets word wat geïmplementeer kan word. Een so 'n voorbeeld was die suksesvolle insluiting in die FMS van warm-gelaaide vrugte in houe op 'n trein van Tzaneen na Durban, wat by aankoms op die teiken temperatuur was. Verskeie aspekte van die FMS is verder ondersoek om die akkuraatheid van temperatuurbeheer te verbeter in die stelsel. Die temperatuurkeuses wat na die EU gebruik mag word, het ongelukkige 'n negatiewe invloed op die vrugkwaliteit in die vorm van verhoogde koueskade. 'n Grootseprojek oor aspekte wat 'n invloed op koueskade van suurlemoenvrugte kan het, dui op 'n beduidende verskil tussen produksie gebiede en seisoene. Daar is egter gevind dat sommige maatreëls soos die gebruik van waks koueskade effektief kan verminder. Die gebruik van skadunet in produksie neem toe en die uitwerking op al die verskillende kultivars is tot 'n groot mate onbekend. Informasie na die eerste seisoen uit die Oos-Kaap dui op geen noemenswaardige verskil in die vatbaarheid vir skildefekte nie. Hierdie program sal voortgaan om die aspekte in die hantering van die na-oes en koueketting wat die vrugkwaliteit kan beïnvloed, te identifiseer en te verbeter, aangesien die toename in uitvoervolume kwaliteit sentraal gaan maak in volhoubaarheid.

5.2.2 FINAL REPORT: Chilling injury of lemon fruit

Project number: 1169 (2017/8 – 2019/20) by Paul Cronje, Jade North (CRI), Lorenzo Zacarias (IATA), Nicola Kirsten and Lynn Hoffmann (SU)

Summary

The export-driven South Africa citrus industry relies on the sustainable production of high-quality blemish-free fruit. Various countries require citrus fruit to be cold stored during export at low temperatures (<2°C), for >22 days, reducing fruit quality. Low postharvest temperature leads to a physiological rind disorder (chilling injury) developing as sunken, necrotic spots that tend to coalesce and cover the entire fruit rind in sunken brown spots, leading to market rejection. This study aimed to identify some pre-harvest factors that contribute to a more tolerant or susceptible lemon fruit rind and to evaluate postharvest technologies to counter chilling injury development in sensitive fruit. In addition, factors influencing the rind physiological condition such as pigments and rind sugars were quantified. The pre-harvest factors evaluated for the impact on CI included production areas, i.e. climates, canopy position, harvest date (maturity) and also differences between cultivars. The results are indicating that CI is mostly dependent on climate and harvesting period, as shown by the different levels of CI developed by fruit harvested from various climatic areas and harvesting periods within one area. It was found that a higher temperature at harvest resulted in higher CI and a rapid drop in temperature during 60 days prior to harvest led to the production of a more CI tolerant fruit. Storage temperature and duration directly affect CI incidence, with higher temperatures, and shorter duration, resulting in less injury. Fruit stored at -1°C for 12 days would be thus more or less

similar in terms of CI development as if stored at 7°C for >30 days. A postharvest method that is commercially thought to prevent the development of CI is to apply a wax coating consisting of higher solids wax that would provide more protection to fruit under these cold conditions, and it reduces moisture loss in fruit which contributes to symptom development. Furthermore, an increased volume of high solids wax leads to slower rind colour development and leads to paler fruit compared to fruit wax with lower solids wax, thus care should be taken not to over-wax fruit. In general, this study provides the first insight into the effect of pre-harvest factors on lemon fruit susceptibility to CI, which will help the industry to formulate better preventive actions. The study also provides the industry with practical and effective postharvest strategies to counter the development of CI in cold-sensitive fruit.

Opsomming

Die uitvoergedrewe Suid-Afrika sitrusbedryf maak staat op volhoubare produksie van hoë gehalte vrugte. Verskeie lande vereis egter dat sitrusvrugte vir meer as 22 dae gedurende uitvoer teen lae temperature (<2°C) verskeep word, wat die vrugkwaliteit verlaag. Lae na-oes temperatuur lei tot 'n fisiologiese skildefekte (koueskade) wat ontwikkel as gesonke, nekrotiese vlekke wat geneig is om aan een te loop en ewekansig en die hele vrugteskil in bruin, gesonke kolle kan bedek, wat tot onbemarkbare vrugte lei. Hierdie studie het ten doel gehad om enkele vooroes faktore te identifiseer wat bydra tot 'n meer verdraagsame of vatbare suurlemoenvrug en om na-oes tegnologieë te evalueer wat die ontwikkeling van koueskade teë kan werk. Daarbenewens is faktore wat die skil se fisiologiese toestand beïnvloed soos pigmente en skil suikers, gekwantifiseer. Die voor-oes faktore wat geëvalueer was vir die impak op CI, sluit in produksiearea, (d.w.s. klimaat), posisie in die boom, oesdatum (volwassenheid) en ook verskille tussen kultivars in. Die resultate dui aan dat CI meestal afhanklik is van die klimaat en oestyd, soos gesien was in die verskillende vlakke van CI wat ontwikkel het in vrugte wat uit verskillende klimaatstreke asook tussen oestydperke binne een gebied. Daar is gevind dat 'n hoër temperatuur tydens die oes gelei het tot 'n hoër CI en 'n vinnige daling in temperatuur gedurende 60 dae voor die oes het gelei tot die produksie van 'n meer CI-verdraagsame vrug. Koelopbergings temperatuur en tydsduur beïnvloed direk die CI-voorkoms, met hoër temperature en die korter duur, wat minder letsels tot gevolg het. Vrugte wat vir 12 dae by -1°C gestoor word, sou dus min of meer dieselfde CI-ontwikkel as vrugte opgeberg vir >30 dae teen 7°C gestoor word. 'n Na-oes tegnologie wat kommersieel gebruik word om die ontwikkeling van CI te voorkom, is om 'n waslaag aan te wed wat bestaan uit 'n hoër vastestowwe, wat meer beskerming aan vrugte sal bied onder koue toestande, en ook die vogverlies verminder in vrugte, wat bydra tot die ontwikkeling van simptome. Verder lei 'n verhoogde volume waks van met 'n hoër vastestof inhoud tot 'n stadiger ontwikkeling van die skilkleur en lei tot ligter vrugte in vergelyking met waks met 'n laer vaste. Daar moet dus gewaak word dat vrugte nie ge-oorwaks word nie. Samevattend beskou bied hierdie studie die eerste insigte in die effek van voor-oesfaktore op die CI sensitiwiteit van suurlemoene, wat die bedryf sal help om beter voorkomende aksies te formuleer. Die studie bied ook praktiese en effektiewe na-oesstrategieë aan die bedryf om die ontwikkeling van CI in kouesensitiewe sitrus vrugte teen te werk.

Introduction

South Africa is currently the third-largest exporter of citrus fruit, following Spain with a record volume of 1.9 million tons exported during the 2017/18 season. Of this total export volume, 15% constituted lemon fruit at approximately 300 000 tons (CGA, 2019). The aim of South African citrus producers is to secure and expand existing market access to various countries, including the USA and China. Market access is currently limited by strictly enforced phytosanitary regulations such as mandatory cold storage requirements to ensure the elimination of pests such as fruit fly and false codling moth, as these long-term storage regimes are mostly detrimental to fruit quality. To ensure that export quality of fruit is maintained under

these low-temperature shipping conditions, postharvest special treatments and protocols are required to limit the development of chilling injury on cold-sensitive lemons.

Chilling injury is a physiological disorder of the flavedo rind section of citrus fruit, resulting from storage at suboptimal temperatures for an extended duration. Lemon fruit, like many other citrus varieties, being of subtropical origin, is sensitive to low-temperature storage and generally sustain postharvest blemishes when stored at temperatures of 10-12°C or below (Klein *et al.*, 2016; Ladaniya, 2004). The primary mechanism of this disorder is not well understood, but it is hypothesized that reactive oxygen species (ROS) accumulate in the fruit rind when exposed to low storage temperatures, causing cell disruption and subsequently cell membrane damage that then result in loss of membrane integrity (Siboza *et al.*, 2017). Membrane degradation is considered the primary mechanism of chilling injury, as the degree of unsaturated fatty acids in the cell membrane has consistently been found to positively correlate with chilling injury incidence (Lee *et al.*, 2005; Sibozza *et al.*, 2014; Biswas *et al.*, 2017). Furthermore, cell walls of damaged cells have been found to exhibit large empty gaps between the cell membrane and a much thinner cell wall (Lado, 2015). The symptoms of chilling injury in lemon fruit is evident as intense brown, sunken lesions on the flavedo rind of the fruit. These lesions most likely result from internal oxidation of oil gland content that, on release, tends to coalesce and spread over the entire fruit rind, rendering the fruit unmarketable. However, it is only during shelf-life conditions at ambient temperature that the symptoms of chilling injury manifest and become visible, even though the rind injuries were sustained during cold storage, where after a cascade of secondary physiological events finally result in the development of characteristic symptoms associated with chilling injury (Biswas *et al.*, 2017; Chalutz *et al.*, 1985).

For South African citrus producers to maintain or expand high, existing export targets, estimated currently at 70% of total citrus production, it is essential to continue to produce blemish-free fruit of high external and internal quality (CGA, 2017). Physiological disorders, including chilling injury, oleocellosis, and peteca, greatly reduce fruit quality. In addition, fruit predisposed to rind disorders may also be more susceptible to pathogen infections due to a compromised rind condition. Rind factors that are thought to induce more stress tolerance, especially within the flavedo, includes a higher sugar content and elevated carotenoid concentration. Carotenoids, by nature, are free radical scavenging molecules, acting as antioxidant species to reduce reactive oxygen species. A higher ratio of these pigments present in the rind of the fruit may thus offer higher tolerance to chilling injury (Krinsky, 1989; Ladaniya, 2004). This could offer some explanation on why citrus types such as lemon, grapefruit, and pummelos, which has about 3-5 times lower carotenoids present in the rind than mandarin and orange, are generally classified as chilling sensitive citrus species (Fishman and Chikovani, 1988). For instance, 'Marsh' grapefruit are more susceptible to chilling damage than red coloured 'Star Ruby' grapefruit, possibly due to the lack of the powerful antioxidant, lycopene, known to be present in red coloured grapefruit cultivars (Lado *et al.*, 2015).

Some environmental factors, in particular, light and temperature that are known to affect the rind physiological condition are also considered to be directly involved in the colour development of the rind, thereby influencing the accumulation of antioxidant pigment species (Rodrigo *et al.*, 2013). Therefore, prevailing climatic factors of the area of production may significantly influence the fruit tolerance to physiological disorders (Barry and Rabe, 2006). In addition, fruit susceptibility to chilling injury and the development of various rind disorders may equally be affected within a season by the time of harvesting (Dou, 2005).

When considering postharvest fruit handling, there are several stages during the supply chain, which could have an impact on the final fruit appearance. During fruit handling, dirt is removed from the fruit by washing, which might alter the structure of the cuticular wax of the fruit rind, rendering the fruit possibly more prone to postharvest disorders. Alternatively, treatment with fungicides to prevent *Penicillium* decay, such as the

application of Thiabendazole, as a dip treatment or added to the wax, has shown to prevent chilling injury in citrus fruit. In addition, wax treatments significantly reduce moisture loss that occurs during chilling injury and as such prevents the development of severe chilling injury symptoms on the fruit rind (Kellerman *et al.*, 2014; Hordijk *et al.*, 2013).

The aim of this study was to provide a better understanding of the factors that may contribute to an enhanced fruit rind physiological condition that may provide tolerance to chilling injury. The first objective was to study a range of pre-harvest factors that may have an impact on fruit rind quality including cultivar variation, specific production areas, the position of the fruit in the canopy and different periods of harvest, and be able to find a conclusion as to when a certain fruit will be more susceptible to postharvest stress conditions. In the second objective, the interaction between the temperature range and its associated storage duration on the incidence of chilling injury in lemon fruit was studied and quantified. The third and final objective was to evaluate current technology, namely wax and TBZ applications for its efficacy to reduce chilling injury in cold-sensitive lemon cultivars, in order to optimize postharvest protocols to control or prevent the development of chilling injury symptoms. Results from this intend to provide the South African citrus industry with greater insights into the physiological disorder chilling injury, whilst providing protocols and technology to control or prevent the development of this disorder in lemon fruit, in order to ensure high-quality citrus fruit exports and market access to countries requiring cold storage.

(Note: This report consists of part of a complete MSc Thesis available from the researchers and only the two relevant chapters are included in this termination report.)

Pre-harvest factors are influencing the susceptibility of lemon fruit (*Citrus limon* (L) Osbeck) to chilling injury.

ABSTRACT

Production of lemon fruit in Southern Africa has increased with 60% from 2015 to 2018. To maintain the current export target of 75% of citrus produced, only blemish-free fruit of the highest quality will be able to continuously satisfy market demand. Lemon fruit, known to be a chilling sensitive citrus variety, is found susceptible to a physiological disorder that manifests as necrotic areas on the rind, following extended periods of storage at low temperatures of <4°C, resulting in suboptimal fruit quality and consequently, market rejection. It is hypothesized that fruit with a rind of improved physiological properties may exhibit enhanced tolerance to cold storage conditions, thus reducing and preventing the development of chilling injury. The aim of this paper was to evaluate various pre-harvest factors that may have an influence on the physiological condition of the fruit rind and its components, particularly as pertaining to rind pigment and sugar content. The factors considered that might have an impact on fruit and rind quality included possible climatic differences between different production areas; microclimate differences between fruit within an orchard; as well as the position of the fruit within a tree canopy. The impact of harvest time, particularly with reference to the different periods during stage III of fruit development, as well as cultivar differences, based genetic differences, were also investigated. Results indicate that pre-harvest factors such as climatic conditions especially referring to maximum and average temperature before harvest and harvesting at certain stages of fruit development contribute significantly to fruit quality, thus influencing the tolerance or susceptibility of the fruit to postharvest stresses, including those leading to chilling injury development. It is suggested that a more rapid fall in temperature and higher temperature at harvest will lead to fruit more tolerant to developing CI and a fruit harvested prior to or after commercial harvest would develop less injury after storage than fruit harvested during the commercial harvest period. This might again refer to the temperature during this specific time of harvest, leading to a more tolerant or susceptible fruit. A better understanding of how these studied factors may have an impact on rind quality is crucial when aiming to

produce citrus fruit within an optimum environment, where pre-harvest stresses, that may result in fruit of lower quality, rendering fruit more susceptible to postharvest stress, is minimized.

1. Introduction

The ability of lemon fruit to tolerate cold storage is influenced by both genetic and climatic factors where, with regards to the latter, even microclimate differences within an orchard may have a significant impact. South Africa has a wide range of distinct climatic areas that are considered suitable for citrus production. These areas vary from the coastal production areas of the Eastern Cape experiencing its highest rainfall in both spring and autumn, to areas in the North-Eastern part of the country, where summer rainfall and dry winters dominate, but also include the Western Cape winter rainfall region with its predominately Mediterranean climate (Barry and Rabe, 2006). Given these distinct climatic differences between areas, fruit from a variety of cultivars can be produced throughout the year. However, this may also result in a great variation in fruit quality, particularly with respect to the susceptibility of fruit to physiological disorders, when the same cultivars are produced, but in areas with distinctly different environmental and climatic conditions (Alferez, 2003; Barry and Rabe, 2006).

With the South African citrus industry aiming for consistent growth in production volumes to deliver high-quality fresh fruit to an expanding export market, lemon fruit has made a significant contribution to the total increase in citrus plantings over the last few seasons. Of the 6 million citrus trees planted in 2017, 1.7 million were represented by lemon trees. Furthermore, it is expected that the export volumes of lemons will increase from the reported 15 million cartons in 2015 to an expected 35-40 million in 2025 (CGA, 2018). To include countries such as China and USA as potential export markets for lemon fruit, will present a serious challenge to growers and exporters alike as these markets demand cold treatment protocols of below 7°C for extended periods. Long-term storage at these low temperatures are almost certain to induce chilling injury (CI) on lemon fruit and will most likely compromise fruit quality.

Extended exposure of chilling sensitive citrus varieties to cold (<7°C) temperatures result in cellular damage of the rind, manifesting as brown, necrotic areas that increase in size as the severity of the injury progresses (Yuen and Tridjaja, 1995). With CI, in citrus as well as in other fruit types, an interaction is found to exist between the actual temperature and time of storage, where, with short term storage at a low temperature will result in a similar chilling incidence as would occur under long-term storage at higher temperatures. Such induced CI can have a drastic impact on the market value of the fruit as external damage associated with CI typically becomes visible only after cold storage, during the shelf life period (Biswas *et al.*, 2017; Biswas and Brummell, 2019). Furthermore, in addition to a genetic predisposition to CI, pre-harvest factors might influence the quality of fruit as physiological disorders are not always only the result of postharvest handling practices, but can also originate from suboptimal conditions prevalent during fruit development stages such as maturity and ripening (Ferguson *et al.*, 1999). Climatic conditions prevailing during all stages of fruit development are considered major pre-harvest factors are determining the internal and external quality of exported fruit (Soule and Grierson, 1986b). The effect of climate on the fruit quality is demonstrated by some citrus cultivars produced in subtropical areas which may develop inadequate rind colour despite a high sugar content in the pulp, whereas citrus fruit cultivated in a Mediterranean climate with its characteristic hot, dry summers and cool, wet winters will generally produce fruit with a desirable external quality, but exhibiting a thicker rind, together with low sugar and juice percentages. In contrast, the fruit produced in warm, humid climates have higher internal sugar and juice percentage, but often fail to develop optimum rind colour (Reitz and Embleton, 1986; Soule and Grierson, 1986b). The impact of climate on the fruit also differs during the various developmental stages. A lower than normal temperature before bloom and higher than normal temperatures after bloom, and during fruit growth, resulted in re-greening of

'Valencia' orange. This may be indicative of a possible relationship between rind pigments and temperature prevailing during a certain stage of development (Reitz and Embleton, 1986).

Furthermore, several other factors besides climate, such as cultural practices including that of water management, nutrition, pruning, and rootstock selection can also influence fruit quality (Reitz and Embleton, 1986; Agustí, 2003). Water stress during phase III of fruit development can reduce the juice percentage of the fruit and increase the rind thickness, thus influencing fruit quality (Pérez-Pérez *et al.*, 2009). Alternatively, high rainfall two months prior to harvest has a similar negative influence on internal parameters of 'Clementine' mandarin, whereas high irrigation volumes in orange and grapefruit can result in increased juice percentage (Reitz and Embleton., 1986). Amount and formulation of fertilizer application as well as the timing of application are equally crucial in producing high-quality citrus fruit. Nitrogen and potassium application are known to have a positive influence on fruit quality, yet over-fertilizing should be avoided as this can negatively impact overall fruit quality, especially rind colour (Reitz, and Embleton, 1986). Citrus fruit produced in the same area, but in orchards with different microclimates and soil types may also influence fruit quality (Reitz and Embeton, 1986). There is evidence that climatic and cultural factors are integrated by the tree throughout all various growth stages of fruit, resulting in fruit at harvest with a certain physical rind condition along with a specific internal quality. In terms of physiological disorders such as chilling injury (CI), the susceptibility and tolerance to stressful postharvest conditions can therefore already be pre-determined on the tree. Thus, in order to control or reduce the impact of CI in lemon fruit, it is important to determine the impact of various pre-harvest factors that might contribute to the sensitivity of the fruit rind to low temperatures. In addition to the contributing factor of different production areas to variability in rind quality, the position of the fruit within the tree canopy may also be a factor determining the rind condition as well as tolerance to non-chilling related physiological disorders, in addition to low temperature related disorders (Cronje *et al.*, 2011; Lado *et al.*, 2015). 'Star-Ruby' grapefruit, for instance, developed a deeper red colour when grown in shaded conditions due to the accumulation of the red pigment, lycopene, resulting in a significant reduction in CI incidence, most likely due to the protective action of the lycopene pigments (Lado *et al.*, 2015). The fruit maturity stage is considered an important factor that may contribute to CI sensitivity, as seen in 'Marsh' grapefruit that is known to be less susceptible to CI during mid-season compared to early-or late-season fruit. In general, citrus fruit harvested very early (immature) or very late (over mature) in the season, are generally known to have a lower fruit quality and thus are likely to have a higher susceptibility to postharvest physiological disorders (Dou, 2005; Soule *et al.*, 1986 a). Even variations between seasons have been reported to affect rind condition in lemons, indicating the relevance of evaluating the impact of different harvest dates within a season on the lemon rind (Houck *et al.*, 1990). Genetic differences between citrus types such as lemon or mandarin result in significant differences in rind colour, total soluble sugar (TSS), citric acid content, and juice percentage. Therefore, it is not surprising that various citrus species and cultivars might react differently to cold storage, based on known variations in rind physiology. Chalutz *et al.* (1985) reported the development of severe decay and pitting of 'Marsh' grapefruit and 'Shamouti' at 6°C, however a lower amount experienced by these varieties at 2°C. In contrast, lemon fruit stored at 2°C experienced the highest injury, and 'Valencia' orange had the lowest overall pitting. This study shows that immense variation exists in the number of factors influencing the rind condition and susceptibility to chilling injury or rind pitting in citrus fruit as the lower temperature does not always result in the development of the most severe case but depends on an array of factors including the genetic background. However, to a large extent, differences in chilling injury sensitivity between cultivars within citrus types as eluded above have mostly been unexplored. The main aim of this study was thus to obtain a better understanding of the influence of the various pre-harvest factors that may affect lemon fruit sensitivity during low-temperature storage. Objectives were to evaluate production area, canopy position, different harvest periods, along with within orchard variation and cultivar differences as possible factors that may affect the susceptibility of lemon fruit to CI. A final objective was to elucidate the possible role of biochemical composition in contributing to physiological differences of the rind

by considering rind sugar, pigment, and pectin content. This analysis was aimed to understand the functioning of the rind and how these factors contribute to the sensitivity of the fruit to chilling injury.

2. Materials and methods

1. 2.1. Areas and plant material

The study was carried out over two seasons (2017 and 2018) in three different climatic areas that were representative of the three main distinct lemon production areas of South Africa. These areas included a cold winter-rainfall area (Citrusdal and Somerset West) in the Western Cape; an intermediate temperature inland summer rainfall area (Groblersdal) in Mpumalanga and a cold coastal, summer rainfall area, (Sundays River Valley) in the Eastern Cape (Table 1). The focus was placed on 'Eureka' lemon (*Citrus limon* L. Burm. F.) being the most produced lemon cultivar in South Africa, whilst other such as 'Genoa', 'Lisbon', 'Limoneira' and two seedless selections, 'Eureka' seedless and '2PH' seedless were also evaluated. Except where stated otherwise, all lemon fruit from the main set in September/October were harvested at commercial maturity as determined by producers in each area, thus resulting in different harvesting times within each of these three respective areas. To reduce sample variation fruit of similar diameter (50-60mm) and comparable colour plate scores (CRI color plate, 4-6) were selected for harvest. In all the experimental orchards, standard commercial management practices as recommended for the production of export quality fruit were applied (Table 1). After harvesting, the fruit were transported, at ambient temperature, to the Department of Horticultural Science, Stellenbosch University, where it was placed in cold storage at a delivery air temperature of -1 °C for 32 days to induce CI. In all experiments reported in this chapter, a low temperature of -1°C was used to induce CI. The selected temperature regime is similar to the delivery air temperature required for export of citrus fruit when shipping to cold sterilization markets to ensure pulp temperature of -0.6°C was attained. Furthermore, a 32-day cold storage period was selected as being a representative storage duration as fruit within a commercial cold chain may often exceed the minimum protocol stipulation of 22 days to the USA, due to various logistical challenges that may be experienced. Shelf-life conditions was simulated during which possible CI symptoms could manifest i.e. at room temperature (20-25°C) for a seven-day period prior to CI incidence evaluation. As climate forms the main difference between the three different areas, the climate (temperature and rainfall) of the areas from which fruit were sampled, in the last 60 days leading up to harvest in the various areas, were analyzed, to supply insight in possible climate contrast. Minimum, maximum and average temperature, relative humidity (RH) and rainfall (mm) was obtained using automated weather stations (data supplied by Schoemans farm, Moutons farm, SRCC and Ileaf for the different areas), positioned on each farm where fruit was harvested, and VPD was calculated from these various parameters by means of the following calculation (i).

$$i. \quad VPD = 0.6108 \left(\frac{17.3 \times \text{average temperature}}{\text{average temperature} + 237.3} \right) - 0.6108 \left(\frac{17.3 \times \text{average temperature}}{\text{average temperature} + 237.3} \right) \times \frac{RH \text{ average}}{100}$$

Heat Units was calculated for each day in the respective areas using the following formula (Hardy and Khurshid, 2007).

$$ii. \quad ((\text{Minimum temperature} + \text{Maximum temperature}) / 2) - 13$$

The accumulated heat values for each area was then also calculated using the heat values per day.

2. 2.2. Treatments and experimental layout

3. 2.2.1. Production area

To evaluate the effect of contrasting climatic conditions of production areas on CI susceptibility, 'Eureka' lemon fruit were harvested from two commercial orchards per farm, from three different farms per area respectively, located in a 10km radius from each other. In every orchard, ten adjacent trees, uniform in

size and vigor, were selected, from which 15 fruit per tree were picked from the outside up to 30 cm into the canopy, to obtain a total of 150 fruit per orchard. On arrival at the laboratory, 10 fruit from each tree attained from each orchard, were placed in cold storage as described to induce CI, with the remaining five fruit used for maturity indexing, representing day 0 (at harvest). The experimental layout was done according to a randomized design, where the production area was considered as the treatment (n=3), whilst orchards (n=6) within a production area provided statistical replication.

2.2.2. *Canopy position*

To evaluate the influence fruit position within the canopy on the predisposition of susceptibility to CI, 25 'Eureka' lemon fruit of similar size and colour from the inside (no direct sunlight) and the outside of the canopy (first 30 cm of the canopy), respectively were harvested from ten collective trees (n=10) per one orchard from one farm per production area. Five fruit per tree replicate was used for day 0 (at harvest) maturity indexing where after the remaining 20 fruit were placed into cold storage as described earlier to induce CI. The impact of different areas was not considered as a treatment. Therefore data from each area were analyzed separately.

2.2.3. *Various sampling periods*

To determine the impact of different sampling periods on CI susceptibility of 'Eureka' lemon fruit, the fruit of similar size and colour, were picked at different harvest dates for a 12-week period at two-weekly intervals, to obtain six sampling periods. The fruit were sampled from the same set of ten pre-selected trees, in a commercial orchard situated in the Stellenbosch/Somerset West area (Table 1). The first sampling date was set four weeks prior to the historical commercial harvest time for the experimental orchard (week 16) and continued until six weeks after the completion of commercial harvest for this orchard (week 26). On each sampling date, 15 fruit were harvested from the outside of each tree (first 30cm of the canopy). The experimental layout was a complete randomized design with ten tree replicates, where the sampling period was considered the treatment. Five of the 15 fruit sampled per tree were subjected to maturity indexing on day 0 (at harvest), whilst and the remaining fruit were cold-stored as described above to induce CI.

2.2.4. *Cultivars*

Six commercial lemon cultivars namely Eureka, Genoa, Lisbon, Limoneira, Eureka seedless, 2PH seedless harvested, as available, from the three production areas and evaluated to determine possible differences in CI susceptibility (Table 1). Ten fruit per cultivar were harvested from ten tree replicates according to a complete randomized design. Thereafter five fruit per replicate were subjected to day 0 (at harvest) maturity indexing, whilst the remaining five were cold-stored to induce CI as described above. In this trial, the predisposition of the different cultivars to CI was considered the treatment. Therefore data from each production area was analyzed separately.

2.2.5. *Orchard*

A twelve ha, 20-year-old, 'Eureka' lemon orchard based in Citrusdal, in the Western Cape production area (Table 1), was used to establish if variation in CI susceptibility may occur between fruit within the same orchard, being subjected to the exact same climatic conditions and cultural practices during the second season of this study. The orchard was divided into 5 different blocks with 4 blocks located on each corner of the orchard and one central block, position to the middle of the orchard. The first and second blocks were spaced 340m apart, with the second and third blocks at 236m apart, whilst the third and fourth block were 456m apart and fourth and first block spaced 334m (Fig. 1). The fifth block was positioned exactly in

the central region of the orchard to include any possible variation in vegetative development between trees within the orchard (Fig. 1). From each block, 40 fruit were harvested from six adjacent trees, at the commercial harvesting time. At harvest, 10 fruit per block was used for fresh maturity indexing, whilst the rest of the remaining 30 fruit was cold-stored as described earlier and evaluated for CI. A randomized design was followed where each individual fruit served as a replicate and the sampling positions represented as blocks within the orchard considered as the treatment.

2.3. Data collection

2.3.1. Chilling injury evaluation

CI incidence was assessed using a four-stage rating scale (Fig. 2), following a seven-day shelf life period.

0 = no damage, without any chilling injury symptoms (no pitting)

1= slight pitting of the rind

2= moderate pitting of the rind

3= severe chilling injury symptoms, covering more than 30% of the flavedo rind, rendering the fruit completely unmarketable (Siboza *et al.*, 2017).

In order to quantify the injury severity, rating scores were used to calculate the chilling injury severity (CIS) index value for each replicate according to the following formula (iii):

$$\text{iii. CI index} = \frac{\text{Chilling injury score (0–3)} \times \text{number of fruit in each score class}}{\text{Total number of fruit evaluated in replicate}}$$

Chilling injury index was presented as either chilling injury percentage (CI%) or chilling injury incidence, or severity index (CIS). CI % was calculated using the following formula (iv):

$$\text{iv. CI percentage (\%)} = \frac{\text{Total number of fruit with a CI score of } \geq 1}{\text{Total number of fruit evaluated}} \times 100$$

4.

5. 2.3.2. External and Internal fruit quality evaluation

Both fruit weight (g) and diameter (mm) was determined using an electronic scale (ADW, UWE Scales, and Calibrations, Cape Town, South Africa) and caliper (CD-6" C, Mitutoyo Corp, Tokyo, Japan) at harvest and following the seven-day shelf-life period respectively. The rind colour, expressed as Hunter *a/b* ratio, which depending on the colour, were either more negative (greener rind colour) or more positive (more yellow), of each fruit replicate was determined by means of a colorimeter (Konica Minolta CR-400, Tokyo, Japan) by taking readings on the two opposing sides of the fruit (180° plane), to include colour readings of both the sun and the shade sides of each fruit. Sun and shade values of each fruit obtained were averaged to produce a representative ratio for each fruit as flavedo-sampling could not be restricted to specific areas on the fruit. Colour was also recorded using the number 37 standard CRI lemon colour plate (CRI, 2004) where a visual colour score is assigned to each fruit. As the Hunter *a/b* ratio and the colour plate produced similar results, only the colorimeter values were included for discussion. The internal quality of the fruit was determined by cutting along the longitudinal plane of the fruit for juice extraction using a citrus juicer (8-SA10, Sunkist®, Chicago, USA). Pulp particles were removed from the juice by straining through a muslin cloth to produce a pure lemon juice sample of each replicate. A 50mL aliquot of juice from all ten fruit per replicate was used to determine the citric acid content of the juice using a potentiometric titrator (888 Titrand, Metrohm, Switzerland) and Tiamo™ software. The total sugar content of the fruit pulp (measured as °Brix and expressed as % TSS in the pulp) was determined using a digital refractometer (PR-32 Palette, ATAGO CO, Tokyo, Japan). The sugar content of the pulp, together with the citric acid percentage, was used to calculate the TSS/TA ratio representative of the fruit taste.

2.3.3. Flavedo rind analysis

Sample preparation. Following fruit quality assessments at harvest and subsequent shelf-life simulation following cold storage, an extraction from the flavedo rind to determine the rind sugars, pigments and extractible pectin's was performed, to establish possible relationships with CI. A lemon zester was used to remove the fruit rind, whereafter it was immediately flash-frozen in liquid nitrogen (N₂) and transferred for storage at -80°C in a brown paper bag until further processing by freeze-drying where moisture was extracted for a 48-hour cycle, using a Christ BETA 1-8 LO plus Freeze dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). The dry flavedo samples were then milled in a semi-dark environment with an analytical grinder (Yellow line, A10, IKA-Werke, Staufen, Germany) to produce a fine powder as would be required for chemical analysis. Extraction. Milled flavedo powder samples of 0.1g were weighed, where after 4mL of 80% ethanol, prepared from 835mL ethanol (96.2%v/v) [Illuvo (Pty) Ltd, Merebank, Durban, South Africa] diluted with 165mL deionized water to yield 1L were added to the milled powder, before being vortexed (GS60E Vortex-Genie® 2, Scientific Industries Inc, Bohemia, New York, US) and placed on a heating block (QBB3 Dry block heating system, Grant Instruments Ltd, Shepreth, Cambridgeshire, UK) at a temperature of 80°C for 30 minutes to facilitate extraction of the ethanol-soluble sugars. Thereafter samples were again vortexed, before being centrifuged (5810R centrifuge, Eppendorf, Hamburg, Germany) for 5 minutes at 10000g_n to separate the alcoholic supernatant from the residue. Once the supernatant was decanted and retained, the remaining residue pellet was subjected to two more extraction cycles to ensure complete extraction. The three collected supernatant fractions were pooled and retained in a sealed vial until further analysis, to prevent evaporation (E: alcoholic/ethanol fraction). Further analysis performed on the collected supernatants was done within a maximum of 24 hours of collection to ensure reliable results. Following the ethanol extraction process, 4mL of 100% deionized water was added to the residue. After vortexing, the mixture was placed on a heating block for 24 hours at 80°C. Thereafter the samples were vortexed, centrifuged and the supernatants collected in a separate vial than that of the combined E extraction fraction. The water extraction process was repeated two more times to ensure complete removal of the polysaccharide fraction of the rind sugars and the water-soluble pectin fraction in the flavedo. The three supernatants were also pooled and retained in a sealed vial until further analysis performed within 24 hours of extraction (W: water fraction). In the final extraction step, to obtain the non-water-soluble pectins, the residue pellet was submerged in 4mL 50mM trans-1,2-Diaminocyclohexane-N,N,N',N'-tetraacetic acid (CDTA) solution, prepared by dissolving 18.2g trans-1,2-Diaminocyclohexane-N,N,N',N'-tetraacetic acid Monohydrate [Sigma-Aldrich Chemicals, Riedsrt, Steinheim, Switzerland] and 4.1g sodium acetate [Merck Chemicals (Pty) Ltd, Darmstadt, Germany] in 1000mL deionized water solution, with the final solution adjusted to a pH of 6 whereafter it was placed on a mechanical shaker (IKA KS 500, IKA-Werke, Staufen, Germany) for 12-15 hours at a speed of 265 revolutions per minute (rpm). The samples were then centrifuged (5810R centrifuge, Eppendorf, Hamburg, Germany) for 5 minutes at 10000g_n to obtain a clean CDTA-solution fraction that was retained in a sealed vial until further analysis taking place within 24 hours of extraction (C: CDTA fraction). Ethanol- and water-soluble carbohydrate content determination, using fractions E and W. The analysis of the rind sugars was done on a 20 times dilution of fraction E and a 10 times dilution of fraction W, using the phenol-sulphuric assay (Brummer and Cui, 2005). A colorimetric reaction was achieved when 200µl of the diluted carbohydrate-containing fraction E or W was pipetted into a test tube, to which 200µl of 5% phenol solution (prepared from 50mg phenol [Merck Chemicals (Pty) Ltd, Modderfontein, Gauteng, South Africa] dissolved in 1000mL deionized water), followed by 1mL of concentrated sulphuric acid (Sulphuric acid (98.08g·mol⁻¹) [Merck Chemicals (Pty) Ltd, Darmstadt, Germany]), whereafter it was vortexed and left to stand for 30 minutes at room temperature before absorbance was measured at a wavelength of 490nm on a Cary 50 Conc spectrophotometer (Varian Technologies, Palo Alto, CA, United States). A glucose solution was used as a standard solution during extraction for the conversion of the sample absorbance values to mg glucose equivalents, permitting the results to be expressed as mg glucose equivalents per gram dry weight (DW) flavedo. Total uronic assay (using fractions W and C). The analysis of extractible pectins was performed on a 10 times dilution of W and C fractions, following the same steps for both fractions using a modification

of the total uronic acid assay as described by Filisetti-Cozzi and Carpita (1991). A 200µl aliquot of the 10 times diluted fractions was pipetted to a test tube, containing 20µl of a 4M sulfamate reagent (prepared from 38.84g Sulfamic acid [Sigma Aldrich Co., Spruce Street, St. Louis, Missouri, USA] added to 20mL of saturated Potassium hydroxide Pellets (KOH solution) [Scienceworld, Stellenberg Road, Parrow, Cape Town, South Africa]) followed by the addition of 1.2mL H₂SO₄-Tetraborate reagent (prepared by dissolving 28.6g di-sodium tetraborate-10hydrate [Merck Chemicals (Pty) Ltd, Wadeville, Gauteng, South Africa] in 1000mL sulphuric acid [Merck (Pty) Ltd, Darmstadt, Germany]). After vortexing, the tubes were inserted into a heating block (QBB3 Dry block heating system, Grant Instruments Ltd, Shepreth, Cambridgeshire, UK) set to 100°C for 20 minutes. Thereafter samples were left to cool down to room temperature before adding 40µl of m-hydroxy diphenyl (MPP) reagent (prepared by dissolving 0.15g m-hydroxyphenyl [Sigma-Aldrich Chemie, Riedstr, Steinheim, Germany] in 200 mL of 5% saturated sodium hydroxide solution (1g NaOH in 200ml deionized water) [Scienceworld, Stellenberg Road, Parrow, Cape Town, South Africa]). The final mixture was vortexed until the solution developed a pink coloration, where after, following the last waiting period of 10 minutes, the solution was transferred to a 1.5mL cuvette for absorbance readings at 520nm, using a spectrophotometer (Cary 50 Conc Spectrophotometer Varian Technologies, Palo Alto, CA, United States). A galacturonic solution of known concentration was used as the standard during extraction and permitted the conversion of sample absorbance values to be expressed as mg galacturonic equivalents per gram flavedo dry weight (DW). Pigment extraction. The rind pigments in the flavedo, namely chlorophyll a, b and carotenoids, were determined both at harvest and following shelf-life simulation after cold storage, using spectrophotometric methods. In a shaded semi-dark environment, flavedo powder of 0.1g was added to 2mL of ethanol (99.9% v/v) [Merck (Pty) Ltd, Modderfontein, Gauteng, South Africa] and vortexed before being transferred to a mechanical shaker (IKA KS 500, IKA-Werke, Staufen, Germany) where samples were mixed for 30 minutes at a speed of 265 rpm. The samples were then centrifuged at a temperature of 4°C for 5 minutes at 10000g_n (5810R centrifuge, Eppendorf AG, Hamburg, Germany). The ethanol supernatant was decanted to a 20mL vial, provided with a lid. This ethanolic extraction step was repeated once more, with the second supernatant pooled with the first ethanol extract, where after 2mL hexane (>97.0% v/v) [SIGMA-ALDRICH, Co., St. Louis, Missouri, United States] containing 0.1% of butylated hydroxytoluene (BHT: 2, 6-Di-t-butyl-p-cresol) (No. B-1378; Lot 62F-0573, Sigma chemical company, St Louis, USA) solution was added to the residue. After vortexing and being shaken with the mechanical shaker for 15 min at 265 rpm, the samples were again centrifuged for 5 minutes at 10 000 g_n. The hexane-BHT supernatant was then combined with the ethanol fractions in a 20mL vial. This hexane-BHT extraction was repeated another two times, where after the supernatants were pooled with the previous pigment extracts, raising the final volume of the fraction to 10mL. The pooled pigment fraction was then dried under vacuum in a Savant SC210A SpeedVac concentrator (Thermo Scientific Inc., Waltham, MA, USA) for 3 hours under a low-medium temperature (30-45°C). The dried residue was then reconstituted with 4mL of acetone (100% v/v) [Merck (Pty) Ltd, Modderfontein, Gauteng, South Africa] and vortexed. Spectrophotometer readings were performed on 1 mL of the pigment extract in UV- cuvettes using a Cary-60 UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) at wavelengths of 470nm, 644.8nm and 661.6nm respectively, with acetone as the blank solution. The concentrations of each of the pigments (chlorophyll a, chlorophyll b and carotenoids) per sample was converted to µg pigment per mL sample (µg · mL⁻¹) by using the equations of Lichtenthaler (1987) for 100% acetone (v-vii).

v. Chlorophyll a = $11.24A_{662} - 2.04A_{645}$

vi. Chlorophyll b = $20.13A_{645} - 4.19A_{662}$

vii. Carotenoid = $(1000A_{470} - 1.90C_a - 63.14C_b)/214$

These concentrations of each of the pigments were converted to µg·g⁻¹ dry weight (DW) by using the following equation (viii):

viii. $\mu\text{g}\cdot\text{g}^{-1} = (\text{pigment concentration} \times 4 \times 1000)/\text{flavedo mass}$

2.4. Data analysis

Data analysis of the respective trials was carried out by Statistica 13's VEPAC module (TIBCO Software Inc., 2018) in order to test for interactions between the main effects and to determine significant differences between treatments (production area, canopy position, harvesting date and cultivars) for parameters such as chilling injury, internal quality parameters and rind components. *P*-values smaller than 0.05 indicate significant differences between treatments and mean separation was achieved through the employment of Fishers LSD test. Partial Least Squares regression (PLS-R) were conducted with CIS and CI% as the dependent variables (Y matrix), and internal quality parameters, external rind parameters, and weather aspects as explanatory variables (X matrix). Variable Importance in the Projection (VIP) values >1 were considered to identify which explanatory variables contribute the most in predicting the dependent variables. The parameters are projected on two different axes relative to the amount of variation explained by these parameters and their influence on the dependent variables. The position of the parameters relative to the dependent variable is an indication of their relationship to this variable. This analysis was done to determine which factors have the highest contribution to CI development and was done using XLStat (Version 17.04, Addinsoft, New York, USA).

2.4.1. Production area

Data concerning the possible differences in CI susceptibility in fruit from various production areas were analyzed using a one-way ANOVA to determine the treatment (area) effect on the CI incidence (CI % and CIS) of the fruit. A factorial ANOVA was used to test for any interaction between the main effects of the production area and cold storage duration (at harvest, referred to as day 0 and after shelf-life, seven days following cold storage duration of the fruit) on maturity indexing and quality parameters such as titratable acidity (TA) expressed as citric acid percentage, total soluble solids (TSS), TSS/TA ratio, juice percentage in the pulp and rind colour, expressed as Hunter a/b ratio and also considering rind components, including that of rind sugars, pigments and extractible pectins. If no interaction persisted between the cold storage and the area as the main effects, data on the effect of area and cold storage duration were presented separate.

2.4.2. Canopy position

To determine the effect of canopy position on CI, data were analyzed using a one-way ANOVA. A factorial ANOVA was used to test for an interaction between cold storage duration and canopy position as main effects with regards to the maturity parameters of citric acid %, TSS/TA ratio, juice %, %TSS in the pulp and fruit rind colour. Where no interaction was detected, data on the canopy position and cold storage duration effects were analyzed separately.

2.4.3. Sampling period

A one-way ANOVA was used to analyze the effect of different sampling periods on the CI susceptibility of the fruit. A possible interaction between the sampling period and duration of cold storage with regards to maturity indexing and quality parameters was tested by means of a factorial ANOVA. When no interaction emerged, the effect of the harvest date and cold storage duration on the maturity parameters citric acid %, TSS/TA ratio, juice %, %TSS, and rind colour as well as on the rind components of rind sugars, pigments and extractible pectins were evaluated separately.

2.4.4. Cultivars

A one-way ANOVA was used to analyze the effect of cultivars as a treatment on the development of CI. A factorial ANOVA was used to detect a possible interaction between cultivars and cold storage duration as the main effects. In the absence of such an interaction, data on the maturity parameters such as citric acid

%, TSS/TA ratio, juice %, TSS% and rind colour between different lemon cultivars were analyzed separate from the effect of cold storage duration on these parameters.

2.4.5. Orchard

The data from this trial were analyzed using a one-way ANOVA to determine if there was any effect of the different picking position on the CI sustained by the lemon fruit. A factorial ANOVA was performed to evaluate the interaction between the cold storage duration and picking position as the main effects. If no interaction was evident, data for the two main effects were analyzed and presented separately with regards to rind colour and maturity indexing parameters.

3. Results

6. 3.1. Production area

Chilling injury percentage (CI %) and severity (CIS) differed significantly between the three production areas, during both seasons (Fig. 3). However, the CI differed from one season to the next between the respective production areas, with the fruit from the Eastern Cape having sustained a higher CI % and CIS during the first season compared to the other areas, whereas Mpumalanga sustained a significantly higher CI score than fruit from the Eastern Cape during the following season. The Western Cape production area had the lowest CI % and CIS, regardless of season (Fig. 3). The CI sustained by fruit from the Western Cape did not differ significantly from that sustained by the fruit from Mpumalanga during the first season. Comparing climatic data between the production areas, the difference between the maximum and minimum temperatures leading up to harvest for both seasons in the Western Cape ($\pm 18^{\circ}\text{C}$ over the last 60 days prior to harvest), were larger than in Mpumalanga ($\pm 13^{\circ}\text{C}$) (Fig. 4).

However, in 2017, when the Eastern Cape scored the highest CI of all the production areas (Fig. 3), the difference between the minimum and maximum temperature in the 60 days prior to harvest was more pronounced ($\pm 19^{\circ}\text{C}$) compared to 2018 ($\pm 14^{\circ}\text{C}$). Furthermore, the Western Cape and Mpumalanga had more constant differences between seasons than for the Eastern Cape (Fig. 4). In addition, 2017 was a colder season for the Eastern Cape than 2018, as temperatures close to 0° was experienced on three occasions, which contributed to fewer heat units accumulated 2017 at 129, compared to the total of 219 heat units accumulated in 2018. In 2018, when Mpumalanga experienced the highest CI of the three respective areas, the difference between the minimum and maximum temperatures immediately prior to harvest was also more distinct compared to that recorded for 2017. In 2017, the Western Cape and Mpumalanga had lower minimum temperatures compared to 2018, and the CI in both areas was lower in this season compared to the first.

By evaluating the partial squares regression (PLS) analysis, the first two components, influenced mostly by average- and maximum temperature slopes, generated by the regression explained 85% of the variation in CI (Y) (Fig. 5). The temperature recorded, thus in general, had a significant effect on CI development as showcased by the PLS plot (Fig. 5) and the highest VIP value was reached by the maximum and average temperature slopes, showing that these two variables were of the highest importance in the PLS projection, supporting the fact that these variables have a relationship with CI development (Table 5.1). Furthermore, the maximum temperature slope and the average temperature slope had the highest correlation with CI% and CIS in this analysis. However the correlation was not significant with CIS, but with CI%. supporting the fact that these parameters had a significant effect on CI% sustained by the fruit (Table 5.2). Maximum temperature- and average temperature slopes are negatively correlated to CI % and CIS (Table 5.2), identifying that a more drastic reduction in temperature in the 60 leading up to harvest (steep-slope) resulted in lower CI. This result explains the data from the Eastern Cape (2017), as a large slope was only evident

in 2018 during lower CI% and CIS recorded (Fig. 4). The average rainfall, over the 60-day period before harvest, was higher in the first season in the Eastern Cape compared to Mpumalanga, but lower during the second (Fig. 4). In the 2018 season, both the Eastern Cape and Mpumalanga experienced less rainfall than the Western Cape, in that 60 days prior to harvest, is a winter rainfall region (Fig. 4). PLS analysis indicates that an increase in days prior to harvest with less than 5mm rainfall, the higher the CI% would be, thus wetter days before harvest leads to a more tolerant fruit at harvest (Fig. 5), this correlation is however not significant and thus merely an observation (Table 5.2).

3.1.1. *Rind colour and pigment concentration as external quality parameters*

No significant interaction was found regarding the rind colour and pigment concentrations between the respective production areas and cold storage duration during the 2018 season (Table 2.1), suggesting that the rind colour and pigment concentration of fruit harvested in different areas, reacted similar during storage. When comparing fruit between different production areas, the Western Cape fruit showed a more pronounced yellow colour compared to the other areas during the 2018 season, despite the Eastern Cape having a higher carotenoid content (Table 2.1). However, regardless of area, lemon fruit displayed a more intense yellow colour after the cold storage- and subsequent shelf-life period, compared to the colour that was recorded at harvest as evident in the significant decrease in the Hunter *a/b* ratio (Table 2.1). During both seasons, carotenoid content decreased significantly during cold storage, whilst a similar, significant decline in chlorophyll content was recorded in fruit following storage and shelf-life in the 2018 season (Table 2.1). During the 2017 season, the Western Cape had the highest carotenoid content (Table 2.1). The interaction found between the cold storage duration and production area as main effects during the 2017 season regarding the rind colour and chlorophyll content and showed that chlorophyll breakdown in the Western Cape during storage was at the same rate compared to the other two areas during this season (Table 2.2), but still had the most intense yellow colour at harvest and after cold storage and shelf-life period (Table 2.1; Table 2.2).

7. 3.1.2. *The total free rind sugar and polysaccharide content*

No interaction was evident when considering the effect of production area and storage duration as main effects on rind sugar and polysaccharides of fruit during the 2018 season and on rind sugar in the 2017 season (Table 3.1). During the 2017 season, the Eastern Cape had the highest rind sugar content followed by Western Cape- and Mpumalanga fruit. However, in the 2018 season, fruit harvested from the Western Cape production area had significantly higher rind sugars than the other two production areas, and no significant difference in polysaccharide content was recorded, between the respective production areas (Table 3.1). The rind sugar and polysaccharide content of the rind was not significantly affected by cold storage and subsequent shelf-life (Table 3.1). During the 2017 season, where interaction was documented between the production area and the cold storage duration regarding polysaccharide rind content, both the Mpumalanga and Eastern Cape fruit had a higher content after storage than at harvest compared to the Western Cape (Table 3.2). During the PLS analysis, it was evident that the polysaccharides after storage and subsequent shelf-life, contributed to the PLS plot projection with a relatively high VIP value (1.433; 1.343) (Table 5.1).

8. 3.1.3. *Internal quality parameters*

No interaction was evident between the area and cold storage duration as main effects regarding the internal quality parameters during the second season and for the citric acid % and TSS % during the first. During both seasons, fruit harvested from the Western Cape had a significantly lower citric acid content compared to that of Mpumalanga and Eastern Cape, which did not differ from each other (Table 4.1). No significant difference was found between the TSS % of the fruit between the different production areas in

the second season, however, in the first, the Western Cape had a higher content followed by Mpumalanga and Eastern Cape (Table 4.1). Juice % obtained from fruit harvested in the Western Cape was significantly higher than the other areas during 2018, however the lowest out of the three areas during 2017 (Table 4.1; Table 4.2). A decrease in citric acid %, TSS/TA ratio, and juice % was recorded following the cold storage and subsequent shelf-life period. However, the TSS % remained the same (Table 4.1). When evaluating the VIP values of the PLS plot and the correlation values with CI, percentage citric acid at harvest and after storage has a significant contribution to the CI susceptibility, as shown by the relatively high VIP values (Table 5.1).

3.2. *Canopy position*

In both seasons, the fruit harvested in Mpumalanga from the outer part of the canopy developed less CI % and CIS compared to the fruit from the inner part of the canopy (Fig. 6). However, no significant differences during the two seasons were found between the CI % and CIS of fruit harvested from the different canopy positions in neither the Eastern Cape nor Western Cape (Fig. 6). The PLS analysis for this trial also revealed the maximum- and average temperature slopes as the factors having the most influence on the first two components generated by the regression which explained 75% of the variation in CI (Y) (Fig. 7). The maximum temperature and average temperature slopes also correlated with CI during this trial and a higher maximum and average temperature slope. Thus a more rapid decrease in temperature during the 60 days prior to harvest, led to a lower CI development (Table 8.1; Table 8.2). The minimum temperature intercept also showed a significant positive correlation to CIS, showing that the higher the minimum temperature at harvest, the higher the severity of the injury sustained will be (Table 8.2).

3.2.1. *Rind colour as an external quality parameter for canopy position*

A significant interaction regarding the rind colour (Hunter *a/b* ratio) between the sampling date (cold storage) and the canopy position of the fruit was evident in fruit harvested from Mpumalanga and the Western Cape during both seasons. Such an interaction was not evident in fruit harvested from the Eastern Cape, regardless of season (Table 6.1; 6.2). The rind colour was significantly improved by cold storage as all fruit displayed a more intense yellow colour, with less green undertone (demonstrated by the less negative Hunter *a/b* ratio) following shelf life than was observed at harvest. Outside fruit displayed a more intense yellow colour than fruit harvested from the inside in the 2017 season, but no such difference was evident in Eastern Cape fruit during the next season (Table 6.1). The rind colour from the outside fruit after harvest during the 2017 season showed a more distinct yellow colour compared to the inside fruit at harvest in both Mpumalanga and Western Cape fruit. However no such observation was made in the next season (Table 6.2). The PLS analysis and correlation matrix suggest that the rind colour of the fruit at harvest correlated negatively to CI% and CIS, suggesting that a lower, more negative, hunter *a/b* ratio, thus a greener fruit, at harvest correlated to higher CI symptom development (Table 8.2).

3.2.2. *Internal quality parameters for canopy position*

In the fruit harvested from the Mpumalanga area, no interaction was evident regarding the internal quality parameters in 2017 with canopy position and cold storage duration as the main effects. However, during the 2018 season, an interaction was reported for citric acid % and TSS/TA ratio. In 2017, Mpumalanga fruit harvested from the inside had a higher citric acid and juice % compared to the outside fruit (Table 7.1). In 2018, a higher citric acid content was also evident in inside fruit at harvest; however after cold storage, the citric acid content in the outside fruit remained higher (Table 7.2). The TSS % and TSS/TA ratio in fruit harvested from Mpumalanga was higher in the pulp of fruit harvested from the outside, regardless of season or interaction between main effects (Table 7.1; Table 7.2). No interaction was evident in the internal quality parameters, excluding juice %, in the 2017 season of fruit harvested from the Eastern Cape, in 2018

however, an interaction was recorded for the citric acid % and TSS/TA ratio. When evaluating fruit harvested from different canopy positions in the Eastern Cape, the citric acid content of the inside fruit was higher compared to the outside fruit regardless of season (Table 7.1; Table 7.2). The TSS/TA ratio, however, showed that the outside fruit had a higher ratio regardless of season (Table 7.1; Table 7.2).

The Western Cape area fruit also showed the interaction between the cold storage duration and canopy position regarding the citric acid % and TSS/TA ratio in 2017. The fruit had a higher citric acid % after storage when harvested from the inside of the canopy, however, at harvest, the outside fruit showed a higher citric acid % (Table 7.2). The TSS/TA ratio from this area in the inside fruit was higher compared to the outside fruit at harvest. No such finding was evident during the second season (Table 7.2). In general, the canopy position did not have a major or consistent impact on these quality parameters discussed during this trial (Table 7.1). TSS/TA ratio after storage showed the highest correlation to the CI% and CIS parameters, suggesting that this parameter was an indication of CI development during this trial (Table 8.1; Table 8.2).

3.3. *Different sampling periods*

The CI susceptibility varied over the 12-week fruit sampling period, in both seasons with significant differences occurring between the start, middle, and end of the sampling periods (Fig. 8). In general, a pattern emerged with lower susceptibility at the start and end of the season, but more pronounced CI susceptibility shown two weeks' prior the commercial harvesting time, with some overlap with the peak commercial harvest (Fig. 8). For rind colour in general, the Hunter *a/b* ratio decreased with increasing maturity as it extended over the sampling period, revealed fruit with a more yellow colour (Fig.9A, B). This trend was less evident in 2017 but manifested fully in 2018. Full yellow colour development in fruit, for both seasons, however only occurred in the period following commercial harvest. For pigment concentration, a significant interaction was evident in both 2017 and 2018, between the storage duration and the week of harvest, as main effects (Fig. 9C, D, E, F). For both seasons, chlorophyll content was overall at its peak just prior to historic commercial harvest, or at harvest, with a gradual decline with increased maturity, although not consistently so (Fig. 9C, D). The chlorophyll content generally decreased with cold storage for most of the harvest dates, with lower values recorded after shelf life than at harvest. The carotenoid concentration was lower during commercial harvest or just thereafter, with slightly higher earlier and later in the season, particularly so for the 2017 season. Carotenoid levels decreased consistently during storage, except for harvesting date 4 (week 24) in 2018, where no difference between fresh and stored fruit was detected (Fig. 9E, F). In the PLS analysis for this trial, the factors having the strongest influence on the first two components generated are fruit size, and maximum temperature intercept, the two components generated by the analysis explained 85% of the variation in CI(Y) (Fig. 12). The maximum temperature slope and intercept and the Rainfall > 20mm, as in other trials, was also identified as contributing to variation (Table 10.1;10.2) and a significant correlation was also noted between these factors and the CI% sustained by the fruit (Table 10.2). A positive correlation was seen between the maximum temperature at harvest (Intercept) and the CI% sustained, thus a higher temperature at harvest might result in higher CI % sustained (Table 10.2; Fig. 12). The negative correlation of the maximum temperature slope with CI% suggests that a more rapid decrease in maximum temperature during the 60 days before harvest, will lead to less CI% sustained by the fruit after storage (Table 10.2). The rainfall >20mm in the 60 days during harvest show a negative correlation with CI% sustained by the fruit, however not so strong for the CIS sustained (Table 10.2; Fig. 12), which is indicative of higher the rainfall during the 60 days prior to harvest, reduce the CI% of the fruit.

3.3.1. *Rind sugar and polysaccharide content.*

Significant interactions for both 2017 and 2018 seasons occurred for the rind sugars and polysaccharides between different sampling periods and cold storage duration as main effects (Fig. 10). For both seasons'

rind sugars appeared to increase with maturity until the commercial harvest window, or just thereafter. In 2017, the sugar content in general, increased during storage, except for the final fruit sampling period, where a slight decrease during storage and subsequent shelf-life was recorded (Fig. 10). Similarly, in 2018, sugars generally increased with storage, except at commercial harvest, sampling period 3, where a decline was observed (Fig. 10). For polysaccharides, in 2017, significant trends were not obvious, as substantial variation occurred between harvest date and with cold storage and shelf life (Fig. 10C). In 2018, however, polysaccharide content decreased during storage and shelf life and was overall lower during the commercial harvest window at harvest and higher at the start and end of the sampling period (Fig. 10D).

3.3.2. *Water-and calcium soluble pectin content*

Similar to that reported for rind sugar- and polysaccharide content above, significant interactions were evident for the pectin content of the flavedo for the main effects of harvest date and cold storage duration. For 2017 and 2018, both water-soluble and calcium-soluble rind pectins were lowest during the start and toward the end of fruit development, with its peak values recorded during the commercial harvest window or just thereafter (Fig. 11). Water-soluble pectin content either remained unaffected or decreased significantly during storage, in both seasons, except for harvest date 4 in 2017, when an increase in water-soluble pectin was evident with storage (Fig. 11 A; B). Calcium soluble pectin increased during storage in the 2017 season (Fig. 11 C) but decreased during storage in 2018 (Fig. 11 D).

9. 3.3.1. *Internal quality parameters*

No interaction was evident between the main effects of harvest dates and cold storage duration with regards to the internal quality parameters, TSS% in fruit pulp and TSS/TA ratio of the juice, recorded in both seasons (Table 9.1). Citric acid % and juice % had an interaction during both seasons (Table 9.2). In general, the citric acid % as well as the juice %, at harvest, increased with the harvest date and reached a peak during the commercial harvest period or just thereafter (Table 9.2; sampling period 3), with a decline observed only towards the last two weeks of harvest (Table 9.2). No trend could be witnessed for these parameters during storage (Table 9.2). TSS% remained unaffected by cold storage duration (Table 9.1). The TSS% and TSS/TA ratio had an inverse pattern compared to the citric acid percentage as these parameters were lowest during commercial harvest in 2017 (Table 9.1). The correlation matrix of the PLS analysis for this trial shows that fruit size at harvest has high VIP value (Fig. 12; Table 10.1), suggesting that this parameter might have served as predictors for possible CI susceptibility. The positive correlation with CI% and CIS, indicate that a larger fruit size at harvest is related to higher CI% and CIS sustained (Table 10.2). According to the PLS analysis for this trial, the most significant contribution to fruit CI susceptibility was made by fruit size, which was not witnessed in any of the other trials.

3.4. *Cultivar differences and CI susceptibility*

During the 2017 season in Mpumalanga, 'Lisbon' had the lowest CI, whilst 'Eureka', 'Genoa' and the '2PH' seedless lemons sustained similar CI % and CIS similar, but higher compared to 'Lisbon' (Fig. 13A). However, in 2018, the lowest CI incidence was for '2PH' seedless lemon. Significantly higher CI was reported for 'Lisbon', but did not differ significantly from 'Genoa', although being significantly lower than 'Eureka' (Fig 13B). For this season, 'Genoa' and 'Eureka' lemon fruit displayed comparable CI%. In the Eastern Cape production area in 2017, 'Limoneira' was reported with higher CI than 'Genoa' and 'Lisbon' (Figure 13C) and in 2018, the severity of 'Limoneira' was again significantly larger than the other two (Fig. 13D). No difference in CI was reported between the two seedless cultivars 'Eureka' and '2PH seedless' harvested from the Western Cape in either season (Fig. 13E, F). The PLS analysis plot indicates that rainfall and temperature and rind colour after storage had a significant influence on CI development (Table 12.1;

Fig. 15), however only the rainfall >20mm in the 60 days prior to harvest shows a significant negative correlation with CI%, suggesting that the higher rainfall before harvest leads to lower CI% (Table 12.2).

Rind colour. In the cultivars harvested from the Mpumalanga region, 'Lisbon' had the greenest rind colour followed by 'Genoa', 'Eureka' and '2PH' seedless in the 2017 season, in the next season, however, 'Eureka' had the greenest rind colour and differed significantly from the other cultivars harvested in this area (Fig. 11). No significant difference was evident between the different cultivars harvested from the Eastern Cape and the Western Cape (Fig. 11). Rind colour after storage shows a negative correlation with CI development, suggesting that a lower Hunter *a/b* ratio, thus a greener fruit, would've sustained higher CI symptoms (Table 14.2).

10. 3.4.1. *Internal quality parameters*

In 2017, citric acid % was higher in the 'Lisbon' followed by 'Eureka' and 'Genoa' with the lowest in '2PH' seedless (Table 11.1). However, for the 2018 season, the lowest citric acid % was recorded for the '2PH', whereas 'Genoa' had the highest (Table 11.1). Significantly lower TSS% was measured in 'Eureka' fruit during both seasons, compared to the other cultivars. '2PH' seedless fruit had the higher TSS/TA ratio, compared to 'Eureka' and 'Lisbon', but not 'Genoa' in 2017, and again in 2018 (Table 11.1), where 'Eureka' had the lowest ratio. Juice % was lowest in 2PH fruit in 2017, but not in 2018 where no difference between cultivars was recorded (Table 11.1). For this region, internal parameters were either unaffected by storage duration or lower values were obtained for citric acid % and in the TSS/TA ratio for the first season (Table 11.1). No significant interaction could be found between the different cultivars harvested from the Eastern Cape, namely 'Limoneira', 'Genoa' and 'Lisbon' regarding citric acid %, TSS%, TSS/TA ratio and juice % after harvest in 2017 (Table 11.2). In 2018 'Limoneira' fruit had a significantly lower TSS% as well as TSS/TA ratio (Table 11.2). This cultivar had, however, the highest juice % compared to the other (Table 11.2). The seedless cultivars harvested in the Western Cape did not show much difference in internal quality parameters over both seasons. In 2017 the '2PH' seedless had a higher citric acid percentage than the Eureka seedless, whereas in 2018 the opposite was evident (Table 11.3).

3.5. *Susceptibility variation within an orchard*

CI% that developed in fruit were not significantly affected by the various block position within the 12ha orchard, even though a possible numerical difference is still observed between the blocks i.e. 20% vs. 40% (Fig. 16A). No interaction was found for fruit rind colour, when considering block position within an orchard and storage duration as main effects. Fruit rind colour varied significantly depending on the block position, where the fruit produced in block 5 possessed a more intense yellow colour (least negative Hunter *a/b* ratio) compared to fruit harvested from any other block positions (Fig. 16B). Fruit rind was a less green and more yellow colour after storage and subsequent than at harvest (Table 13). In terms of the internal quality of the fruit harvested from the various blocks in a single orchard, no interactions were observed, with block position and cold storage duration as main effects (Table 14). The citric acid content was significantly lower in fruit picked at blocks 2 and 3 in the orchard, whereas blocks 4 and 5 produced fruit with a higher citric acid % compared to the other blocks in the orchard. Significant differences in juice percentage were obtained from fruit harvested from the respective blocks, with blocks 1 and 2 producing less juice than the other three remaining blocks. The TSS % was higher in fruit harvested from blocks 4 and 5, whereas blocks 2 and 3 (the two blocks on the riverside of the orchard) showed the highest TSS/TA ratio out of the different blocks (Table 14). Cold storage did not have any effect on the citric acid % and TSS/TA ratio of this fruit, however TSS and juice percentage increased during storage (Table 14).

4. DISCUSSION

4.1. Chilling injury.

Over the two seasons, the Mediterranean area of the Western Cape had the lowest chilling injury (CI) consistently compared to the two summer rainfall areas. The coastal Eastern Cape production area, which displayed the highest CI during the first season and second-highest during the second season of the study (Fig. 3), experience rainfall throughout the year and is known for its high summer temperatures. Temperature fluctuations, due to being exposed to cold coastal fronts, between seasons in this area might contribute to variation in CI sensitivity between seasons. Fruit harvested from the coastal regions of East-Florida in North America showed a lower incidence of postharvest pitting and CI compared to fruit harvested from the central regions of Florida (Dou, 2005) which is in accordance with the results in this study.

Siboza et al. (2014) found CI to vary within different farm locations/-regions in one area. In this study, no differences in CI susceptibility of fruit from different blocks within one farm was found at the 5 or 10% confidence level. Yet the significant variation detected in fruit colour between blocks suggests that differences in microclimate or soil variation within one orchard may result that fruit from the same orchard, experiencing the same commercial pre-harvest practices, may have rinds with different external qualities and physiological conditions, and may, therefore, have a different predisposition to tolerate environmental stresses that fruit may be exposed to during its development.

The Western Cape typically had lower minimum temperatures than Mpumalanga and less CI. It could be speculated that the exposure of the fruit to lower minimum temperatures as it reaches maturity (stage III of fruit development) might enhance the ability to tolerate extended periods of postharvest cold storage (Fowler and Thomashow, 2002). However, temperatures below or approaching the threshold indicated as 0°C may increase CI susceptibility. This was evident in fruit from the Eastern Cape in 2017, when temperatures close to 0 °C was experienced during the 60 days prior to harvest, which could result in the higher CI compared to the 2018 season when higher minimum temperatures occurred. The Western Cape also had lower minimum temperatures (closer to 0 °C) during the first season, where higher CI was sustained. Although CI as a physiological disorder is caused by postharvest cold storage, susceptibility and symptom development are known to be aggravated by exposure to stress conditions, prior to harvest such as non-optimum temperature (Kays, 1999; Joubert, 2016). It was evident from the partial least squares (PLS) regression analysis in all the trials, that a more rapid decrease in maximum temperature during 60 days prior to harvest does increase the tolerance of fruit to CI and it was evident in the canopy position trial that a lower minimum temperature at harvest gives rise to a lemon fruit more tolerant to CI development during postharvest cold storage. This was clearly illustrated by data from the Eastern Cape, with a higher temperature in 2017, resulting in less CI was evident at harvest compared to 2018. It can be suggested that the stress caused by a rapid temperature reduction and a lower temperature at harvest might induce the expression of certain enzymes involved in keeping reactive oxygen species (ROS), one of the primary causes of CI, at low levels, which might prevent the ROS accumulation during cold storage and thus prevent the development of CI (Galindo *et al.*, 2007; Sibozza and Bertling, 2013). Thus, observed differences in the incidence of CI between the summer and winter rainfall areas could be due to specific climatic factors affecting susceptibility in the respective production areas. However, this complex aspect of fruit development requires further in-depth research. In addition to temperature, rainfall has also shown to contribute to CI incidence as higher rainfall experienced in the 60 days prior to harvest, led to a more CI tolerant of fruit. Furthermore, a higher rainfall at harvest has been shown to induce various postharvest disorders due to a higher turgor caused by the rainfall (oleocellosis), leading to mechanical damage or diseases including sour rot (Eckert and Eaks, 1989). Pre-harvest factors, other than climates, such as soil type characteristics and choice of rootstock, may also influence fruit growth and development and thus the susceptibility of fruit to postharvest rind disorders (Augustí *et al.*, 2003; Cronjé, 2013). However, no such

published research has been found reporting the possible impact of rootstock on chilling susceptibility of citrus fruit and warrant further research. Most of the lemon fruit trees included in this study from the different areas was grafted on 'Rough Lemon', a vigorous rootstock with wider xylem vessels compared to 'Carrizo' rootstock, resulting in higher yield (Rodríguez-Gamir *et al.*, 2010). The 'Lisbon' lemon cultivar, most tolerant to CI during this trial, is grafted on a 'Carrizo' rootstock as opposed to the 'Rough Lemon' rootstocks like the other cultivars which suggest a new research direction. The soil types vary in the different production areas in South Africa (Fey, 2010). Most of the soil texture of the Western Cape production areas were mostly represented by sandy soils, which usually have a low organic content. These soils are extremely well-drained and therefore high, leaching of various mineral nutrients result in a high need for fertilizer to optimize fruit production as any deficit in minerals may lead to a weaker fruit more prone to physiological disorders (Alva and Paramasivam, 1998). More research on the interaction of soil-plant-climate is thus required to elucidate how pre-harvest conditions might influence the rind condition and thus the ability of the fruit to tolerate postharvest conditions.

In addition to variation between areas and orchards, variation in susceptibility of physiological disorders have been shown to occur within a citrus tree due to change in light levels physically affecting the flavedo (Cronje *et al.*, 2011). When comparing CI of lemon fruit from different positions in the tree canopy in the Mpumalanga production area, the fruit grown on the inside of the canopy had higher CI than on the outside. Therefore, variation within the tree canopy can also contribute to the difference in CI susceptibility that manifests in lemons. This variation due to canopy position and CI susceptibility was also shown in grapefruit (although the opposite due to a cultivar specific response), where the fruit positioned on the inside of the tree canopy, developing high lycopene in the flavedo, providing the rind with more resistance to CI than fruit harvested from the outside due to the lycopene products in the rind (Lado *et al.*, 2015). The absence of difference between canopy positions in the two production areas noted could be due to the difference in the canopy and vegetative development as the Mpumalanga area is known to be more vigorous. Thus, no conclusion could be drawn at this stage regarding the canopy effect on CI for 'Eureka' lemon. It can most likely be ascribed to the canopy shape of a 'Eureka' lemon tree as the bearing is less inclined to shade the fruit from the inside of the canopy compared to other citrus varieties and the bearing of fruit terminally on long shoots.

Lemon fruit harvested at different times during the final stages of fruit development or maturity showed distinct differences in CI, with the highest incidence during mid-season and the lowest when the fruit was harvested either early or late in the season. An important commercial finding is that fruit harvested four weeks prior to commercial harvest had 15% lower chilling injury than fruit harvested at commercial harvest. Six weeks after commercial harvest the injury percentage again decreased, with more than 20% in 2017 and as high as 50% in 2018 showing a distinct decrease in CI % as the season and fruit maturity progressed following the commercial harvest. Lower CI % late in the season might be due to an increase in rind maturity, leading to a more stress-tolerant fruit. Green, immature 'Fortune' mandarins were found to be more resistant to CI than midseason orange coloured fruit (Lafuente *et al.*, 1997). Still, this higher CI susceptibility, seen in midseason fruit could possibly be linked to induced stress experienced by fruit during a particular maturity stage which in turn might have led to a weaker rind rendering the fruit rind more vulnerable to pre-harvest or postharvest environmental stresses (Lafuente *et al.*, 1997). 'Fortune' mandarin fruit harvested during the coolest months, displayed the most CI prevalence, due to the accumulation in the field plus the cold room of hours below a chilling threshold. Thus, the influence of low temperatures during fruit development on the rind cell layers, which, when subjected to stress conditions, may have led to a physiologically weaker rind more prone to disorders (Vercher *et al.*, 1994). The results from this study on CI of lemons were in accordance with the above-mentioned studies on 'Fortune' mandarins from Valencia, Spain, as midseason fruit, had the highest CI prevalence as opposed to fruit harvested earlier or later in the season. However, contradicting results from this study show, that the temperature was lower later during a season with low

CI. A positive correlation between the average temperature and CI incidence shows that the higher CI tolerance in fruit from cooler environments, the higher the CI. These results also did not concur with Schirra et al. (1998) which reported that grapefruit cultivars when sampled in Italy at different maturities was significantly more tolerant to cold storage conditions in mid-season, compared to early and later in the season, as produced in a Mediterranean winter rainfall area. As our study is a first report on the effect of fruit maturity on the susceptibility to CI in lemons, it is important for future studies also to be conducted in more production areas, to validate our initial findings. It is possible that the relationship between temperatures could involve two aspects. The first one for temperatures above a chilling inducing temperature ($>4^{\circ}\text{C}$) which increase tolerance due to gradual accumulation. The second mechanism could be when change occurs when exposed for continual hours below the threshold, $<4^{\circ}\text{C}$.

Differences in CI occurred between lemon cultivars cultivated which concur with Schirra et al. (1998). From this study the sensitivity of the cultivar susceptibility to CI development can be ranked in general: Limoneira > Eureka > Genoa > Seedless varieties (Eureka seedless and '2PH' seedless) > Lisbon. Limoneira, which is a selection from Lisbon, had the highest CI % during both seasons in our study in this area. This cultivar typically has a relatively thick rind, its juice- and acid percentage as well as the number of seeds closely resemble that of Lisbon lemon (CRI, 2012). As this cultivar is also regarded as susceptible to sunburn and wind damage of the rind (Saunt, 2000), it is mostly used for processing due to its relatively high amount of rind oil content. Eureka lemon, a cultivar of Italian descend, mostly produces fruit in terminal clusters, rendering it more exposed to environmental stress than would be the case for other lemon cultivars which bear more fruit towards the inside of the canopy. The rind of the 'Eureka' fruit is relatively thin, whilst the internal fruit quality is characterized by a high juice and acid percentage (Saunt, 2000). 'Genoa', which was ranked third with respect to CI, are known to have only one main period for the flowering set, whilst the fruit size is considerably larger compared to that of 'Lisbon' and 'Eureka' lemons (CRI, 2012). The rind is thin and smooth, with acceptable juice percentage that can be obtained from the fruit pulp. 'Lisbon' developed lower CI than 'Eureka' and 'Genoa', regardless of production area, confirming the industry perception that this fruit cultivar is more cold tolerant than the standard 'Eureka' cultivar. 'Lisbon', of Australian origin, typically has denser foliage than that of a 'Eureka' lemon tree, whilst its fruit is grown more on the inside of the canopy, where it is being protected from environmental stresses (Saunt, 2000). When the two seedless cultivars, 'Eureka' seedless, an irradiation-induced mutation of 'Eureka' fruit, and '2PH' seedless, were harvested in the Western Cape production region, a higher injury overall was reported for the first season compared to the second season. The CI sustained overall by these cultivars was much lower during the second season compared to other cultivars harvested in other areas, despite the lower internal quality of these cultivars. The time of fruit maturity of the '2PH' lemon fruit is earlier than the standard 'Eureka' lemon fruit and it has a lower juice percentage as well as juice acid content than the normal 'Eureka' lemon. As this fruit cultivar was developed in Queensland, Australia, it is thus accustomed to the hot and humid climatic conditions, whereas the 'Eureka' seedless variety is thought to prefer a cooler production area (Citrogold, 2018). This suggests that the origin of a cultivar might play a pertinent role in the tolerance of its fruit to certain stressful conditions as they have been selected for its adaptability to withstand these stresses more effectively than other cultivars. Future research is required that will allow for the evaluation and comparison of the different commercial cultivars in two distinct climatic areas to evaluate the ability of the respective cultivars to tolerate a range of environmental stresses and the impact on postharvest disorders.

4.2. Physiological rind condition.

4.2.1. Rind colour. Previous research and results from this study provide sufficient evidence that CI susceptibility is affected by the environmental conditions prior to harvest, the developmental stage of the fruit during the harvest period, the inherent tree growth patterns and the genetics of a specific cultivar. These factors may all directly or indirectly influence the physiological condition of the rind that in turn, affects the rind strength and thus the tolerance of the rind to CI. The physiological condition of the rind, in turn,

depends on the presence of various constituents of the rind, including sugars and the pigments responsible for the rind colour, namely chlorophyll, and carotenoids.

The rind colour of citrus fruit ranges from a bright yellow colour in lemon fruit to a distinctive orange colour of mandarins and oranges and a red colour in grapefruit and pummelo varieties (Alquézar, 2008) and are known to be influenced by position in the canopy, temperature during development, storage conditions, and fruit maturity (Goldschmidt, 1988; Botes, 2018). The more intense yellow rind colour of fruit from the Western Cape compared to fruit from the Eastern Cape and Mpumalanga confirmed that rind colour development of the fruit varies between areas of production. This finding adds support to the climate influence on the rind colour and specifically low temperature before commercial harvest window. These findings coincide with previous studies that low temperature is required for rapid chlorophyll degradation and subsequent colour development (Young *et al.*, 1969; Goldschmidt, 1988; Rodrigo *et al.*, 2013). Furthermore, the larger difference (18°C) between the minimum and maximum temperatures in the Western Cape compared to Mpumalanga (13°C) is suggested to have contributed to the more intense yellow rind colour measured in fruit harvested from the Western Cape. Mandarin fruit harvested in the Western Cape have also shown better colour development in a season with a larger difference between the minimum and maximum temperature was recorded (Botes, 2018; Prins, 2018). This finding further supports the suggestion that a wider range between minimum and maximum temperature, such as mild day temperatures, but with cool night temperatures, occurring in the Western Cape, may be responsible for better yellow colour development in citrus fruit (Erickson, 1960; Barry and Van Wyk, 2006). It should be noted that the Western Cape production area was the last to be harvested in the trial, suggesting that might also have led to a better colour development of the rind at harvest. However, this period is the commercial harvest window when juice % is acceptable in this area. In this study, lemon fruit from the Western Cape production area, with the lowest CI incidence and exhibiting a more intense yellow colour during both seasons also had a trend of higher carotenoid content than the other areas during 2017 (Table 2.1). However, in the second season, the Eastern Cape had the highest carotenoid content, but not a more intense yellow rind colour than the Western Cape, which could indicate the impact of chlorophyll masking colour development. This variation between the rind colour and a number of pigments could be due to the lower overall concentration of carotenoids present in the lemon fruit compared to other more intense orange coloured citrus fruit namely mandarins or oranges (Kato *et al.*, 2004).

Results from our study showed both carotenoids and chlorophyll to decrease during cold storage, irrespective of production areas or treatment, however, the rind colour still increases in intensity, which does not concur with Van Wyk *et al.*, 2009 whom reported a colour reduction in 'Clementine' mandarin. Carotenoid pigments are known to have antioxidant abilities; it is thought to protect the fruit from postharvest stress. Rind carotenoids in fruit from the Eastern Cape was significantly higher during 2018 than during 2017 which might suggest a protective action. Of interest is that CI was significantly lower in 2018, supporting this hypothesis (Alquézar *et al.*, 2008). Results from our study where the influence of sampling period on CI was considered evidence that lemon colour development occurred rather due to the degradation of chlorophyll, as there is not an exponential accumulation of carotenoid pigments during maturation unlike other citrus cultivars, i.e. 'Nadorcott' mandarin, where the accumulation of carotenoids during maturity continues exponentially, giving rise to the characteristic orange rind colour of the fruit and experiencing an inverse trend of carotenogenesis and chlorophyll breakdown during the maturation, leading to a high number of carotenoids in the rind of the fruit at harvest and a low chlorophyll content, resulting in the characteristic colouration of the fruit at harvest (Botes, 2018). This suggests an unmasking colour effect in lemon fruit, and not necessarily an increase in carotenoids during the season, similarly to what is observed during the banana fruit ripening, the total rind colour is thus only evident when the chlorophyll is at its minimum (Von Loesecke, 1929). Yet, differences in CI is still evident with different sampling periods, and carotenoid levels suggest more factors playing a role in CI tolerance other than only carotenoid content.

Furthermore, more mature fruit, as defined by a lower acid and higher sugar content in the pulp, contained less chlorophyll, but a comparable carotenoid content in the rind than that reported for less mature fruit. This finding may explain the resistance to the CI of immature fruit during cold storage than more mature fruit. A similar finding was also reported in the pigment content of the citrus variety *C. grandis* Irm. 'Goliath', which also attains a yellow rind colour at harvest (Gross, 1983). The carotenoid content of this cultivar does not increase during storage; however, it parallels the chlorophyll concentration during development and decreases during maturation, with the highest carotenoid attained by the fruit being the same as the highest chlorophyll concentration during maturation (Gross, 1983). As the pummelo shows within species correlation with the lemon variety, the similarity of the carotenoid patterns might be due to genetic origin (Barret and Rhodes, 1976). In the pigment studies of the Golden Delicious apple, it was also evident that the total carotenoid was at its lowest during mid-season and increased thereafter when de novo synthesis of the violaxanthin carotenoid initiates giving rise to the final fruit colour (Gross, 1978), this finding illustrates the arbitrary contribution of the carotenoid content in the final fruit rind colour at harvest. As a relationship exists between fruit rind colour and the photosynthetic active radiation (PAR) levels reaching the fruit in the different parts of the tree canopy (Cronjé *et al.*, 2013). Fruit borne on the inside of the tree might have a greener rind colour due to deficient light levels reaching these fruit. However, where light levels are reduced by shade nets (18-20%), the rind colour and pigment content remained unaffected in mandarin fruit (Botes, 2018). Differences in fruit rind colour content with canopy position were also reported in 'Star Ruby' grapefruit where fruit grown under the shaded parts of the canopy tend to develop a more intense pink/red colour because of a higher lycopene accumulation (Lado *et al.*, 2015). It is therefore concluded that not all citrus species and cultivars behave similarly in colour development in the orchard and during cold storage. Different citrus species display variation in reaction on different bearing position and canopy shape, with lemon trees displaying a more open shape, as opposed to other citrus varieties like mandarins, tending to have a more clustered canopy shape. Because of its particular bearing habit, lemon fruit on the inside of the canopy is less likely to be completely shaded as is often the case with other citrus varieties of denser foliage as in the case with the mandarin variety. Citrus species, including lemons and grapefruit that is known to be the most chilling sensitive varieties contains 3-4 times fewer carotenoids in the flavedo part of the rind compared to that of a mandarin or orange fruit (Gross *et al.*, 1983). As carotenoids express antioxidant activity fruit of citrus species containing more of these pigments enjoys better protection to rind damage (Fishman and Chikovani, 1988). In this study PLS analysis suggests that fruit with a greener rind colour will be more susceptible to CI development

Total rind sugar. In addition to pigments, rind sugars, consisting of alcohol-soluble sugars and water-soluble polysaccharides, and extractible pectin content influence rind condition. When comparing different areas, during the first season, a higher total rind sugar content was evident in fruit produced in the Eastern Cape, which also had the highest CI. In contradiction, during the second season, fruit from the Western Cape had the highest sugar rind content, yet this area displayed the lowest CI. Increasing sugar content in the flavedo during the cooler months of fruit development of grapefruit have shown a positive relationship with reduced CI as sugar was metabolized, providing protection to the rind (Purvis and Rice, 1983; Holland *et al.*, 1999). Yet, a similar correlation with sugar rind content and reduced CI was not consistently evident during this study, as the mid-season fruit with a higher sugar content compared to the early-harvested fruit, however, displayed a higher CI. A similar increase in sugar rind content was only evident during 2017, and for 2018, an increase only became evident at commercial maturity after which it declined. This inconsistency greatly discredits the favorable relationships recorded in 2017 and indicate the possible difference between lemons and other citrus cultivars.

During postharvest storage, an increase in rind sugar and polysaccharide was observed in fruit sustaining more CI during the different sampling period trial. It is suggested that this could be due to the inhibition of certain sugar-related enzymatic systems, leading to the excessive accumulation of metabolic compounds,

subsequently resulting in the death of cells during cold storage, followed by the expression of CI symptoms (Eaks, 1960). Studies on other fruit types, such as grapes, similarly suggest a significant increase in sugars in the fruit during cold storage (Selvaraj *et al.*, 1973). Yoshioka and Honda (1970) reported the accumulation of sugars due to its synthesis by gluconeogenesis or amylolysis. Of interest is that Kozukue *et al.* (1978) observed the sugar content of eggplant rind to only increase during the initial two days of low-temperature storage, where after the sugar content decreased. This increase in sugar content that was reported early during cold storage could possibly be ascribed to the normal production of sugars in the rind, whereas when stress conditions increased, these sugars were metabolized for energy production to prevent cold damage to the eggplant fruit (Kozukue *et al.*, 1978).

4.2.3. Total extractible pectin. The pectin content of the fruit rind was overall lower in lemon fruit rind after storage than at harvest, suggesting degradation or conversion to other metabolites during the postharvest cold storage. Balandrán-Quintana *et al.* (2002) have shown for pectic oligomer compounds in the fruit rind of zucchini (*Cucurbita pepo* L.) to promote ethylene synthesis during early exposure to cold storage conditions. Ethylene, in this instance, was suggested to have prevented electrolyte leakage and thus inhibited the subsequent CI symptom development in the zucchini rind. Siboza *et al.* (2014) reported that cold storage of lemons led to electrolyte leakage and CI symptom development on the fruit rind. In addition, peach and nectarine fruit, wooliness, a mealy-like condition resulting in a lack of juiciness and dry textured fruit, are regarded as symptoms of CI caused by cold storage temperatures (Fruk *et al.*, 2014). The change in peach pectin metabolism and decrease the amount of extractible pectin content was characterizing the mealy fruit (Ben-Arie and Lavee, 1971; Brummell *et al.*, 2004). Thus, the generally lower pectin content of the lemon fruit rind following cold storage periods in our study might similarly be due to a decline in extractable pectin components of the rind due to cold damage. This decrease in pectin substances is likely to alter the cell-to-cell adhesion of the cell wall, thus affecting the wall fluidity, which in turn may promote the development of CI symptoms (Brummell *et al.*, 2004).

It was found that the extractible pectin substances were highest at commercial harvest compared to before and after the commercial harvest window. The trend of the pectin content thus corresponds to coincide with the CI of the fruit harvested at different maturities. During fruit maturation and senescence, the structure of the flavedo and albedo rind cells change along with the epicuticular wax, all of which might have an influence on the susceptibility of the fruit to postharvest physiological disorders (Storey and Treeby, 1994; Alférez and Zacarías, 2013).

4.3. Internal quality.

With regards to the internal quality of the lemon fruit, the fruit in 2017 in the Western Cape, had lower citric acid content in the pulp compared to fruit from the other production areas. This reduced citric acid content could be ascribed to this area being the last to be harvested, causing the acid content to decrease over the extended period on the tree. Of interest is that the seedless cultivars also had a lower citric acid content in the pulp compared to that of fruit from seeded cultivars. The citric acid content of fruit harvested from the outside of the tree canopy was also found to have lower citric acid content, regardless of the season. Citric acid content increased during maturity until four weeks after commercial harvest, where after a decline was reported. Generally, citric acid content consistently reduced during cold storage. The citric acid content of the fruit is the only internal quality parameter that has shown to have a relationship with CI and the PLS analysis shows that a positive relationship does exist between the citric acid content and the CI development in fruit harvested during different weeks. Other than the citric acid content, the analysis indicates that these pre-harvest factors impact less on internal fruit quality compared to the rind, confirming the rind and pulp of the lemon fruit to be physiologically separate entities (Tadeo *et al.*, 2008) and indicate that a compromised fruit rind due to environmental stress, is no predictor of internal fruit quality.

5. Conclusion

An extensive range of factors is proposed to influence lemon fruit tolerance to CI. These factors include the time of harvest and fruit maturity in addition to production area which can impact on the physiological advancement/maturity of the fruit rind and its ability to withstand postharvest stress and therefore susceptibility to CI. Climate is shown to have the largest influence on differences in rind physiological condition and suggests that the temperature leading up to harvest is of utmost importance in fruit ability to tolerate cold storage conditions postharvest. When the average temperature shows a rapid decline and low temperature at harvest, it is suggested that enzymes are synthesized in the fruit rind and are involved in the precautionary action of stress development and thus increases fruit tolerance to postharvest rind stress. Rind constituents like the rind sugar and pectin content can influence the fruit CI symptom development, and the amount of carotenoid pigments in the fruit rind contribute to the protection of the fruit rind against CI due to the antioxidant properties of these pigments, but to a lesser extent than other citrus types. It therefore remains crucial to produce lemon fruit under optimum conditions to reduce the susceptibility of chilling sensitive fruit. The focus in future studies should be placed on the nature of the interactions of the prevailing climate close to harvest as well as microclimate differences in an orchard on the fruit rind physiology and its ability to tolerate a period of cold storage.

6. References

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7. Tables

Table 1. (pg. 79-81). Experimental sites and cultivars used to evaluate production area, canopy position, different sampling periods, along with within orchard variation and cultivar differences as possible factors that may affect the susceptibility of lemon fruit to CI.

Production area	Farm	Block	GPS Coordinates	Row direction	Year planted	Cultivar	Rootstock	Planting density (m)	Trial
Groblersdal	Schoeman Boerdery	Z2	25°03'55.2S 29°25'22.9E	NW to SE	1998	Eureka	Rough Lemon	6 x 3	Production area & Canopy position & Cultivar
Groblersdal	Schoeman Boerdery	Z7	25°03'58.1S 29°25'36.5E	NW to SE	1998	Eureka	Rough Lemon	6 x 3	Production area
Groblersdal	Schoonbee Landgoed	SL2	25°03'48.2S 29°24'23.1E	N to S	2004	Eureka	Rough Lemon	6 x 3	Production area
Groblersdal	Schoonbee Landgoed	SL3	25°03'27.6S 29°24'31.6E	N to S	1998	Eureka	Rough Lemon	7 x 3	Production area
Groblersdal	Engelbrecht Trust	S36-A	24°59'02.4S 29°19'48.5E	N to S	2011	Eureka	MXT	6 x 3.5	Production area
Groblersdal	Engelbrecht Trust	S19	24°59'16.0S 29°18'57.5E	NW to SW	1996	Eureka	X-638	6 x 3.5	Production area
Groblersdal	Schoeman Boerdery	Z27	25°06'23.3S 29°26'09.9E	N to S	1999	Genoa	X-638	6 x 3	Cultivar
Groblersdal	Schoeman Boerdery	Z15	25°04'4.3S 29°25'44.4E	NW to SE	1998	Lisbon	Carrizo Citrange	6 x 3	Cultivar
Groblersdal	Schoeman Boerdery	Z16	25°04'42.4S 29°25'46.6E	NE to SW	2014	2PH	X-638	6 x 3	Cultivar

Area	Farm	Block	GPS Coordinates	Row direction	Year planted	Cultivar	Rootstock	Planting density (m)	Trial
Sundays River Valley	San Miguel	40	36°26'20.2S 25°42'32.2E	N to S	2000	Eureka	Rough Lemon	6 x 2	Production area
Sundays River Valley	San Miguel	39	33°26'17.4S 25°42'28.4E	N to S	2000	Eureka	Rough Lemon	6 x 2	Production area
Sundays River Valley	Habata Boerdery	1	33°36'39.2S 25°42'14.8E	E to W	1993	Eureka	Rough Lemon	8 x 6	Production area
Sundays River Valley	Habata Boerdery	6	33°36'48.1S 25°41'58.1E	E to W	1994	Eureka	Rough Lemon	8 x 6	Production area
Sundays River Valley	Habata Boerdery	14	33°35'45.5S 25°38'20.8E	E to W	1995	Eureka	Rough Lemon	8 x 6	Production area
Sundays River Valley	Habata Boerdery	22	33°37'10.4S 25°41'59.1E	NE to SW	2000	Eureka	Rough Lemon	6 x 4	Canopy position
Sundays River Valley	San Miguel	41	33°37'10.4S 25°41'59.3E	N to S	2001	Limoneira	Rough Lemon	6 x 2	Cultivar
Sundays River Valley	Halaron	2	33°29'32.2S 25°40'38.2E	NE to SW	2005	Lisbon	Carrizo Citrange	6 x 3	Cultivar
Sundays River Valley	Habata Boerdery	54	33°37'08.5S 25°41'08.9E	NE to SW	2004	Genoa	Rough Lemon	6.5 x 2	Cultivar
Citrusdal	Mouton	39	32°29'37.5S 18°58'37.2E	N to S	1993	Eureka	Rough Lemon	6 x 4.5	Production area
Citrusdal	Mouton	52	32°30'59.9S 18°59'32.7E	E to W	1982	Eureka	Rough Lemon	6 x 4.5	Production area & Canopy position

Area	Farm	Block	GPS Coordinates	Row direction	Year planted	Cultivar	Rootstock	Planting density (m)	Trial
Citrusdal	ALG	K20	32°43'54.51S 19°03'42.30E	N to S	1950	Eureka	Rough Lemon	6 x 5	Production area
Citrusdal	ALG	N20	32°43'09.67S 19°03'16.43E	N to S	1980	Eureka	Rough Lemon	6 x 5	Production area
Citrusdal	Olivier broers	1	32°51'04.4S 19°05'35.2E	E to W	2012	Eureka	Rough Lemon	6 x 4	Production area
Citrusdal	Olivier broers	2	32°50'43.2S 19°05'16.5E	E to W	2014	Eureka	Rough Lemon	6 x 4	Production area
Citrusdal	Mouton	60	32°30'27.6S 18°59'22.7E	N to S	1996	Eureka	Rough Lemon	6 x 3	One-block variation
Citrusdal	Kweekkraal	-	-	-	-	2PH seedless	Rough Lemon	-	Cultivar
Citrusdal	Kweekkraal	-	-	-	-	Eureka seedless	Rough Lemon	-	Cultivar
Somerset West	Cavali	-	34°00'55.9S 18°49'06.8E	NW to SE	2012	Eureka	Rough Lemon	5 x 3	Harvesting date

Table 2.1 Rind colour (expressed as Hunter *a/b* ratio) together with chlorophyll- and carotenoid pigment concentration ($\mu\text{g}\cdot\text{g}^{-1}$ dry weight (DW)) of 'Eureka' lemon fruit at harvest and after 32 days in cold storage and subsequent 7-day shelf life period. The fruit was harvested from three different production areas (Mpumalanga, Eastern Cape, and Western Cape) during the 2017 and 2018 seasons.

<u>Season</u>	2017		2018		
		Carotenoid concentration ($\mu\text{g}\cdot\text{g}^{-1}$ DW)	Rind colour (Hunter <i>a/b</i> ratio)	Carotenoid concentration ($\mu\text{g}\cdot\text{g}^{-1}$ DW)	Chlorophyll concentration ($\mu\text{g}\cdot\text{g}^{-1}$ DW)
<u>Production area</u>					
Mpumalanga		33.67b ^z	-0.29b	38.13b	113.37 ^{NS}
Eastern Cape		38.41ab	-0.32c	48.15a	119.10
Western Cape		43.81a	-0.20a	43.45ab	103.28
<u>Cold storage duration</u>					
0		45.81a	-0.37b	50.25a	160.37a
32 + 7 days shelf-life		31.44b	-0.18a	35.21b	71.50b
<u>p-value</u>					
<i>Production area</i>		0.0000	0.0004	0.0209	0.3751
<i>Cold storage duration</i>		0.0255	0.0000	0.0000	0.0000
<i>Cold storage duration x Production area</i>		0.0485	0.1160	0.0571	0.2838

^{NS} Non-significant difference on a 5% level

^z Different letters represents significant differences between the means of the sampling dates within each column on a 5% significance level according to Fishers LSD test at *p*-value <0.05

Table 2.2 Rind colour (expressed as Hunter *a/b* ratio) together with chlorophyll pigment concentration ($\mu\text{g} \cdot \text{g}^{-1}$ dry weight) of 'Eureka' lemon fruit at harvest and after 32 days in cold storage and subsequent 7-day shelf life period. The fruit was harvested from three different production areas (Mpumalanga, Eastern Cape, and Western Cape) during the 2017 season.

<u>Season</u>	2017			
	<u>Cold storage duration</u>			
	0	32+ 7 days shelf life	0	32+ 7 days shelf life
	Rind colour (Hunter <i>a/b</i> ratio)		Chlorophyll concentration ($\mu\text{g} \cdot \text{g}^{-1}$)	
<u>Production area</u>				
Mpumalanga	-0.40d ^z	-0.16a	146.38a	19.98d
Eastern Cape	-0.30c	-0.21ab	119.20b	61.24c
Western Cape	-0.27bc	-0.13a	131.51a	122.14b
<u>p-value</u>				
<i>Production area</i>	0.0291		0.0543	
<i>Cold storage duration</i>	0.0000		0.0002	
<i>Cold storage duration x Production area</i>	0.0256		0.0128	

^z Different letters represent significant differences between the means of the sampling dates within each column on a 5% significance level according to Fishers LSD test at *p*-value <0.05

Table 3.1. The difference in total rind sugar and polysaccharides concentration expressed as mg glucose equivalents per gram flavedo dry weight (DW) (mg. g⁻¹ DW), of 'Eureka' lemon fruit harvested from three different production areas (Mpumalanga, Eastern Cape, and Western Cape) during the 2017 and 2018 seasons. Rind sugars are extracted at harvest and after cold storage at -1 °C for 32 days and subsequent shelf-life period of 7 days.

<u>Season</u>	<u>2017</u>		<u>2018</u>	
	Total free rind sugar (mg·g ⁻¹ DW)		Total free rind sugar (mg·g ⁻¹ DW)	Total rind polysaccharides (mg·g ⁻¹ DW)
<u>Production area</u>				
Mpumalanga	2.37b ^z		2.18b	1.19 ^{NS}
Eastern Cape	2.99a		1.88c	0.6
Western Cape	2.45b		2.60a	0.68
<u>Cold storage duration</u>				
0	2.64 ^{NS}		2.44 ^{NS}	0.71 ^{NS}
32+7days shelf life	2.8		2.19	0.94
<i>p-value</i>				
<i>Production area</i>	0.0116		0.0000	0.6383
<i>Cold storage duration</i>	0.2536		0.6154	0.4126
<i>Cold storage duration x Production area</i>	0.5941		0.0752	0.1794

^{NS}Difference between the means of treatment was non-significant on a 5% significance level

^z different letters denote a difference in treatment means at a 5% significance level according to Fishers LSD test at *p*-value < 0.05

Table 3.2. The difference in total rind polysaccharides concentration, expressed as mg glucose equivalents per gram flavedo dry weight (DW), of 'Eureka' lemon fruit harvested from three different production areas (Mpumalanga, Eastern Cape, and Western Cape) during the 2017 season. Rind sugars are extracted at harvest and after cold storage at -1 °C and subsequent shelf-life period of 7 days.

<u>Season</u>	<u>2017</u>	
	<u>Cold storage duration</u>	
	0	32 + 7 days shelf life
	Total rind polysaccharide (mg·g ⁻¹ DW)	
<u>Production area</u>		
Mpumalanga	0.54d ^z	4.54bc
Eastern Cape	4.25c	7.39a
Western Cape	4.98bc	4.49b
<i>p-value</i>		
<i>Production area</i>	0.0038	
<i>Cold storage duration</i>	0.0186	
<i>Cold storage duration x Production area</i>	0.0000	

^z Different letters denote a difference in treatment means at a 5% significance level according to Fishers LSD test at *p*-value < 0.05

Table 4.1. Internal quality parameters of fruit from three different production areas at harvest and after storage at -1°C for 32 days and subsequent 7 days at shelf life conditions, for seasons 2017 and 2018.

Season	2017		2018			
	Citric acid (%)	TSS ^x (%)	Citric acid (%)	TSS ^x (%)	TSS/TA ratio	Juice %
<u>Production area</u>						
Mpumalanga	6.56a ^z	8.26ab	6.74a		1.22c	37.25b
Eastern Cape	6.68a	8.12b	6.38a	8.16	1.28b	34.38b
Western Cape	5.55b	8.37a	5.68b	8.37	1.48a	40.18a
<u>Cold storage duration</u>						
At harvest	6.28 ^{NS}	8.29 ^{NS}	6.47a	8.27 ^{NS}	0.79a	38.52a
32 + 7days shelf life	6.26	8.20	6.06b	7.30	0.75b	36.02b
<i>p-value</i>						
<i>Production area</i>	0.0000	0.0029	0.0000	0.3731	<0.0001	0.0002
<i>Cold storage duration</i>	0.9169	0.4136	0.0084	0.2954	<0.0001	0.0163
<i>Productions area x</i>	0.7874	0.8064	0.9339	0.3273	0.1998	0.0729

^{NS}Difference between the means of treatment was non-significant on a 5% significance level

^xTSS refers to the amount of total soluble sugars in the fruit pulp, measures as °Brix and expressed as %

^z Different letters denote a difference in treatment means at a 5% significance level according to Fishers LSD test at *p*-value < 0.05

^y Total soluble sugar: Titratable acidity (Citric acid percentage) in pulp

Table 4.2. Internal quality parameters, juice percentage (%) and TSS/TA ratio of fruit from three different production areas at harvest and after storage at -1°C for 32 days and subsequent shelf-life of 7days for season 2017.

<u>Season</u>	2017			
	<u>Cold storage duration</u>			
	0	32+ 7days shelf life	0	32+ 7 days' shelf life
	TSS/TA ratio ^Y		Juice (%)	
<u>Production area</u>				
Mpumalanga	1.14f ^z	1.22e	49.45b	45.42c
Eastern Cape	1.39c	1.33d	43.51c	58.05a
Western Cape	1.46b	1.58a	44.90c	44.54c
<i>p-value</i>				
<i>Production area</i>		<0.0001		0.0000
<i>Cold storage</i>		0.0042		0.0009
<i>Cold storage duration x Production area</i>		<0.0001		0.0000

^Different letters denotes difference in treatment means at a 5% significance level according to Fishers LSD test at p -value < 0.05

^YTotal soluble sugar: Titratable acidity (Citric acid percentage) in pulp

Table 5.1. Variable Importance in Projection (VIP) of the PLS analysis of the 10 variables with the highest values of the production area trial. Higher value expresses higher importance in the expression of the PLS plot in fig. 5.

Variable	VIP value (t1 axis)	VIP value (t2 axis)
Maximum temperature slope	1.740	1.608
Average temperature slope	1.724	1.593
Citric acid % after storage	1.618	1.513
Carotenoid rind content after storage	1.552	1.451
Maximum temperature intercept	1.454	1.355
Citric acid % at harvest	1.436	1.341
Polysaccharides after storage	1.433	1.343
Rain<5mm	1.376	1.322
Rind colour after storage	1.238	1.143
TSS % after storage	1.201	1.198
Fruit size after storage	1.181	1.263
Juice % after storage	1.151	1.070
Fruit size at harvest	1.094	1.129
TSS/TA ratio after storage	1.087	1.105
Days with temperatures <4°C	1.064	1.191

Table 5.2. Correlation matrix of the PLS analysis, showing the variables with the highest (>0.05) correlation to the CI% and CIS during the production area trial.

Variable	CIS		CI%	
	R	p	R	p
Maximum temperature slope	-0.773	0.071	-0.809	0.048
Average temperature slope	-0.763	0.077	-0.804	0.051
Citric acid % after storage	0.719	0.108	0.753	0.084
Carotenoid content of rind at harvest	-0.662	0.152	-0.747	0.088
Maximum temperature intercept	0.677	0.139	0.647	0.165
Citric acid % at harvest	0.669	0.146	0.639	0.172
Rain (mm)<5mm	0.604	0.204	0.646	0.165
Polysaccharide rind content after harvest	0.576	0.232	0.723	0.105
Rind colour after storage	0.513	0.298	-0.610	0.199

Table 6.1. Rind colour (expressed as Hunter *a/b* ratio) of fruit harvested from different positions of the tree canopy, the inside and outside (0-30cm into the canopy) in 2017 and 2018 in the Eastern Cape. Rind colour was measured at harvest as well as after 32 days in cold storage at -1°C and subsequent shelf life period of 7 days. No interaction between the main effects, namely canopy position, and cold storage duration, regarding the rind colour of the fruit.

Season	2017	2018
	Rind colour (Hunter <i>a/b</i> ratio)	Rind colour (Hunter <i>a/b</i> ratio)
<u>Canopy position</u>		
Inside	-0.33b ^z	-0.39 ^{NS}
Outside	-0.30a	-0.41
<u>Cold storage duration</u>		
0	-0.38b	-0.46a
32+ 7 days shelf life	-0.30a	-0.36b
<u><i>p</i>-value</u>		
<i>Canopy Position</i>	0.0062	0.2262
<i>Cold storage duration</i>	0.0000	<0.0001
<i>Canopy position</i> x <i>Cold storage duration</i>	0.1562	0.4490

^{NS}Differences between treatment (areas or sampling date) means was non-significant on a 5% significance level

^z Different numbers show a difference in treatment means according to Fishers LSD when $p < 0.05$

Table 6.2. Rind colour (expressed as Hunter *a/b* ratio) of fruit harvested from different positions of the tree canopy, the inside and outside (0-30cm into the canopy) in 2017 and 2018 in Mpumalanga and the Western Cape. Rind colour was measured at harvest as well as after 32 days in cold storage at -1°C and subsequent shelf-life period of 7 days.

<u>Season</u>		2017		2018	
		<u>Cold storage duration</u>			
		0	32 + 7days shelf life	0	32 + 7days shelf life
<u>Area</u>		Rind colour (Hunter <i>a/b</i> ratio)		Rind colour (Hunter <i>a/b</i> ratio)	
<u>Mpumalanga</u> <u>a</u>	<u>Canopy position</u>				
	Inside	-0.41d ^z	-0.29b	-0.51d	-0.26a
	Outside	-0.37c	-0.21a	-0.40c	-0.36b
	<i>p-value</i>				
	<i>Canopy position</i>		<0.0001		0.5277
	<i>Cold storage</i>		<0.0001		<0.0001
	<i>Canopy position x Cold storage</i>		0.0220		<0.0001
<u>Western</u> <u>Cape</u>	<u>Canopy position</u>				
	Inside	-0.32b	-0.13a	-0.13a	-0.18b
	Outside	-0.31b	-0.12a	-0.11a	-0.21b
	<i>p-value</i>				
	<i>Canopy position</i>		0.0180		0.8555
	<i>Cold storage</i>		<0.0001		<0.0001
	<i>Canopy position x Cold storage</i>		0.0178		0.0460

^z Different numbers show a difference in treatment means according to Fishers LSD when $p < 0.05$

Table 7.1. (pg. 90-91). Internal quality parameters of fruit harvested from different the inside and outside of the tree canopy in various production areas during the 2017 and 2018 season at harvest and after 32 days in cold storage at -1°C and subsequent shelf-life period of 7 days. No significant difference between the main effects regarding these parameters

Area		2017				2018			
		Citric acid (%)	TSS (%) ^x	TSS/TA ^y	Juice (%)	Citric acid (%)	TSS (%)	TSS/TA	Juice (%)
<u>Mpumalang</u> ^a	<u>Canopy position</u>								
	Inside	6.41a ^z	7.29a	1.11b	37.38a	6.86b			40.47 ^{NS}
	Outside	6.17b	7.53b	1.18a	29.04b	7.77a			41.77
	<u>Cold storage duration</u>								
	0	7.02a	7.75b	1.10b	51.20 ^{NS}	7.44a			41.41 ^{NS}
	32+7days shelf life	6.29b	7.41a	1.18a	33.21	7.19b			40.83
	<i>p-value</i>								
	<i>Canopy position</i>	0.0004	0.0257	<0.0001	0.1574	<0.0001			0.0605
	<i>Cold storage</i>	<0.0001	0.0015	<0.0001	<0.0001	0.0110			0.4006
	<i>Canopy position x Cold storage</i>	0.6455	0.9110	0.3765	0.3362	0.9575			0.07642
<u>Eastern Cane</u>	<u>Canopy position</u>								
	Inside	6.94a	8.33 ^{NS}	1.20b		8.79 ^{NS}			29.70 ^{NS}
	Outside	6.52b	8.62	1.32a		9.00			32.53
	<u>Sampling date</u>								
	0	6.72 ^{NS}	8.49 ^{NS}	1.27 ^{NS}		8.83 ^{NS}			31.74 ^{NS}
	32+7days shelf life	6.73	8.46	1.26		8.97			30.49
	<i>p-value</i>								
	<i>Canopy position</i>	0.0036		0.0027					0.1934
	<i>Cold storage</i>	0.9974		0.9432					0.5615
	<i>Canopy position x Cold storage</i>	0.8274	0.9930	0.8690		0.8034			0.8399

Area	2017			2018		
<u>Western Cane</u>	<u>Canopy position</u>					
	inside	8.18 ^{NS}	45.60 ^{NS}	5.92a	7.49 ^{NS}	37.18 ^{NS}
	outside	8.30	47.72	5.47b	11.80	40.45
	<u>Cold storage</u>					
	duration					
	0	8.19 ^{NS}	46.24 ^{NS}	5.95a	1.95 ^{NS}	39.30 ^{NS}
	32+7days shelf life	8.29	47.08	5.44b	7.66	38.32
	<i>p-value</i>					
	<i>Canopy position</i>	0.0807	0.2665	<0.0001	0.2867	0.2890
	<i>Cold storage</i>	0.1431	0.3993	<0.0001	0.4154	0.7410
	<i>Canopy position x Cold storage</i>	0.1083	0.3410	0.7159	0.3271	0.2410

^{NS} Differences between treatment means was non-significant on a 5% significance level

^Y Total soluble sugar: Titratable acidity (Citric acid percentage) in pulp

^Z Different numbers show difference in treatment means according to Fishers LSD when $p < 0.05$

^XTSS refers to the amount of total soluble sugars in the fruit pulp, measures as °Brix and expressed as %

Table 7.2. Internal quality parameters of fruit harvested from different the inside and outside of the tree canopy in various production areas during the 2017 and 2018 season at harvest and after 32 days in cold storage at -1°C and subsequent shelf-life period of 7 days. ^{NS}No significant difference between the main effects regarding these parameters

Season	2017						2018							
Area	Eastern Cape		Western Cape		Western Cape		Mpumalanga		Mpumalanga		Eastern Cape		Eastern Cape	
	Juice %		The citric acid (%)		TSS/TA ratio ^Y		Citric acid (%)		TSS/TA ratio		Citric acid (%)		TSS/TA ratio	
Canopy position	<u>Cold storage duration</u>													
	0	32	0	32	0	32	0	32	0	32	0	32	0	32
Inside	28.72a	6.09b	6.56a	1.35ab	1.25a	6.78a	5.74d	1.03c	1.17b	7.51a	6.80b	1.16b	1.31a	
Outside	42.68b	40.73a	6.14b	1.30c		6.36b	6.06c	1.24a	1.26a	7.00b	6.81b	1.28a	1.32a	
<i>p</i> -value														
Canopy Position	0.0260	0.2776	0.0950	0.4886	<0.0001	0.0015	0.0032							
Cold storage	<0.0001	0.0939	0.5596	<0.0001	<0.0001	<0.0001	0.0001							
Canopy position x Cold storage	0.0030	0.0007	0.0003	<0.0001	0.0002	0.0012	0.0355							

^Z Different numbers show a difference in treatment means according to Fishers LSD when $p < 0.05$

^Y Total soluble sugar: Titratable acidity (Citric acid percentage) in pulp

Table 8.1. Variable Importance in Projection (VIP) of the PLS analysis of the variables with the highest values of the canopy position trial. Higher value expresses higher importance in the expression of the PLS plot in fig.7

Variable	VIP value (t1 axis)	VIP value (t2 axis)
TSS/TA ratio after storage	1.670	1.607
Maximum temperature intercept	1.601	1.435
Average Temperature Intercept	1.554	1.394
TSS/TA ratio at harvest	1.388	1.251
Days with temperature <13°C	1.376	1.249
Rind colour (Hunter a/b ratio) at harvest	1.344	1.247
Minimum temperature intercept	1.308	1.197
Rain (mm) < 5mm	1.251	1.121
Maximum temperature slope	1.042	0.943
Average temperature slope	1.010	0.909

Table 8.2. Correlation matrix of the PLS analysis, showing the variables with the highest correlation (>0.05) to the CI% and CIS during the canopy position trial.

Variable	CIS		CI%	
	R	p	R	p
TSS/TA ratio after storage	0.785	0.002	0.772	0.003
Maximum temperature intercept	0.770	0.003	0.721	0.008
Average temperature intercept	0.767	0.004	0.677	0.016
TSS/TA ratio at harvest	0.699	0.011	0.589	0.044
Day with temperatures < 13°C	-0.691	0.013	-0.586	0.045
Minimum temperature intercept	0.662	0.019	0.551	0.063
Rind colour at harvest	-0.651	0.022	-0.600	0.039
Rain (mm) < 5mm	0.576	0.050	0.592	0.043

Table 9.1. Internal quality parameters of 'Eureka' lemon fruit sampled at different weeks. Each number refers to the two-weekly sampling interval from week 16 to week 26 during both seasons 2017 and 2018. The third sampling period refers to the commercial harvest window. No significant interaction between the main effects regarding these parameters.

Season	2017		2018	
	TSS (%) ^X	TSS/TA ratio ^Y	TSS (%)	TSS/TA ratio
<u>Sampling period</u>				
1	8.26b ^Z	1.41a	8.02 ^{NS}	1.21 ^{NS}
2	8.23b	1.36b	11.85	1.81
3	8.18bc	1.25b	7.96	1.24
4	8.25b	1.29b	7.44	1.20
5	8.00c	1.24c	7.82	1.22
6	8.68a	1.38ab	7.83	1.28
<u>Cold storage duration</u>				
0	8.24 ^{NS}	1.32a ^Z	7.92 ^{NS}	
32	8.29	1.39b	9.04	1.43b
<i>p-value</i>				
<i>Sampling period</i>	<i><0.0001</i>	<i><0.0001</i>	<i>0.3118</i>	<i>0.3455</i>
<i>Cold storage duration</i>	<i>0.4878</i>	<i>0.0002</i>	<i>0.3670</i>	<i>0.2568</i>
<i>duration x Sampling period</i>	<i>0.3584</i>	<i>0.2956</i>	<i>0.4547</i>	<i>0.4826</i>

^Z Different values refers to the difference between the averages on a 5% significance level as calculated by Fishers LSD

^{NS} Means of the different stages or storage duration do not differ on a 5% significance level

^XTSS refers to the amount of total soluble sugars in the fruit pulp, measures as °Brix and expressed as %

^Y Total soluble sugar: Titratable acidity (Citric acid percentage) in pulp

Commercial harvest period is shown in bold

Table 9.2. Internal quality parameters of 'Eureka' lemon fruit sampled at different weeks. Each number refers to the two-weekly sampling interval from week 16 to week 26 during both seasons 2017 and 2018. The third sampling period refers to the commercial harvest window.

<u>Season</u>	<u>2017</u>				<u>2018</u>			
	<u>Cold storage duration</u>				<u>Cold</u>			
	<u>storage duration</u>							
	0	32	0	32	0	32	0	32
	Citric acid (%)		Juice (%)		Citric acid (%)		Juice (%)	
<u>Sampling period</u>								
1	5.76fg ^z	5.54ef	34.10g	35.64fg	6.54ab	6.73bc	35.28de	32.24b
3	6.12cde	5.89def	44.77c	36.84rfg	6.52cd	6.44bc	40.06e	30.38a
5	6.54a		37.88ef	70.25a	6.76ef	6.1a	39.26bc	34.76a
7	6.42ab	5.99ab		66.40b	6.49f	5.983c	40.37e	30.57a
9	6.49ab	6.43abc	41.38d	37.51ef	6.54de	6.27bc	33.06f	28.31cd
11	6.20bcd	6.28	36.31fg	35.27fg	6.17f	6.05ef	31.69d	
<i>p-value</i>								
<i>Sampling period</i>	<0.0001		<0.0001		<0.0001		<0.0001	
<i>Cold storage</i>	<0.0001		<0.0001		<0.0001		<0.0001	
<i>Cold storage duration x</i>	0.0223		<0.0001		<0.0001		<0.0001	

^z Different values refers to the difference between the averages on a 5% significance level as calculated by Fishers LSD

Table 10.1. Variable Importance in Projection (VIP) of the PLS analysis of the variables with the highest values of the different harvest period trial. Higher value expresses higher importance in the expression of the PLS plot in fig. 13B.

Variable	VIP value (t1 axis)	VIP value (t2 axis)
Fruit size at harvest	1.977	1.681
Maximum temperature slope	1.852	1.597
Maximum temperature intercept	1.848	1.588
Juice % at harvest	1.744	1.673
Rain (mm) > 20mm	1.632	1.387
Cumulative rainfall	1.517	1.349
Average temperature intercept	1.516	1.365
Juice % after storage	1.340	1.172
Fruit size after storage	1.300	1.157
Rind pectin content after storage	1.282	1.106
Rain (mm) < 5mm	1.233	1.200
Days with temperature < 13°C	1.161	1.173
Rind sugars after storage	0.971	1.431
Minimum temperature slope	0.931	1.003

Table 10.2. Correlation matrix of the PLS analysis, showing the variables with the highest correlation (>0.05) to the CI% and CIS during the different sampling period trial.

Variable	CIS		CI%	
	R	p	R	p
Fruit size at harvest	0.684	0.014	0.780	0.003
Maximum temperature slope	-0.686	0.14	-0.692	0.013
Juice % at harvest	0.628	0.029	0.667	0.018
Maximum temperature intercept	0.619	0.032	0.747	0.005
Rain (mm) > 20mm	-0.505	0.094	-0.695	0.012

Table 11.1. Internal quality parameters of various lemon cultivars harvested, as available, in Mpumalanga production area at harvest and after 32 days in cold storage at -1°C and subsequent shelf life of 7-days during 2017 and 2018. The parameters at harvest and after storage and subsequent shelf-life were evaluated separately with only the cultivar evaluated as influencing factor.

	<u>2017</u>								<u>2018</u>							
	Cold storage duration								Cold storage duration							
	0	32	0	32	0	32	0	32	0	32	0	32	0	32	0	32
<u>Cultivar</u>	Citric acid (%)		TSS (%) ^x		TSS/TA ratio ^y		Juice %		Citric acid (%)		TSS (%)		TSS/TA ratio		Juice (%)	
'Eureka'	7.28a ^z	6.21b	7.7b	7.87b	1.05b	1.27b	45.41	43.35	7.11 ^N	5.87b	7.5c	7.11c	1.05b	1.21c	34.13bc	
'Lisbon'	6.14b	6.70a	8.6a	8.73a	1.40a	1.30b	35.01	44.69	6.61	6.05b	8.2b	8.00b	1.24c		39.35b	36.22
'Genoa'	-	6.21b	8.3ab	8.88a	-	1.43a	41.55	45.02	6.72	6.45a	8.6a	8.16b	1.28c	1.32b	44.70a	36.58
'2PH'	-	5.80c	-	8.6a	-	1.48a	-	35.13	6.15	5.51c	8.7	8.7a	0.71a	1.58a	36.62b	38.05
								b								
<i>p-value</i>																
<i>Cultivar</i>	0.000 1	0.000 1	0.005 4	0.000 16	0.0325	0.000 1	0.001 1	0.036 1	0.254	<0.000 1	<0.000 1	<0.0001	<0.000 1	<0.00 01	0.0002	0.6378

^z Different values refer to the difference between the averages on a 5% significance level as calculated by Fishers LSD

^{NS} Means of the different stages of storage duration do not differ on a 5% significance level

^xTSS refers to the amount of total soluble sugars in the fruit pulp, measures as °Brix and expressed as %

^y Total soluble sugar: Titratable acidity (Citric acid percentage) in pulp

Table 11.2. Internal quality parameters of various lemon cultivars harvested, as available, in the Eastern Cape production area at harvest and after 32 days in cold storage at -1°C and subsequent shelf life of 7-days during 2017 and 2018. The parameters at harvest and after storage and subsequent shelf life were evaluated separately with only the cultivar evaluated as influencing factor.

Season	2017								2018							
	<u>Cold storage duration</u>								<u>Cold storage duration</u>							
	0	32	0	32	0	32	0	32	0	32	0	32	0	32	0	32
Cultivar	Citric acid (%)		TSS (%) ^X		TSS/TA ratio ^Y		Juice percentage (%)		Citric acid (%)		TSS (%)		TSS/TA ratio		Juice percentage	
	'Limoneira'	6.21 ^{NS}	6.28 ^{NS}	8.1 ^{NS}	7.42a	1.30 ^{NS}	1.34 ^{NS}	41.42	42.82 ^{NS}	7.00	6.32 ^{NS}	7.7c	7.79b	1.10	1.23b	31.4
'Lisbon'	6.43		8.4		1.31	1.30	38.52	40.92	7.16	6.47	8.4b	8.38a	1.17	1.30a	47.0	31.5
'Genoa'	6.90	6.46	8.5	8.48a	1.23	1.32	38.75	40.46	6.85	6.36	8.6a	8.51a	1.26	1.34a	38.9	6.35
<i>p-value</i>																
<i>Cultivar</i>	0.154	0.14	0.065	0.001	0.236	0.374	0.000	0.3792	0.00	0.051	0.00	<0.00	0.00	<0.00	0.00	0.00
	4	13	4	95	5	6	1		43	9	14	01	01	01	41	06

^Z Different values refer to the difference between the averages on a 5% significance level as calculated by Fishers LSD

^{NS} Means of the different stages of storage duration do not differ on a 5% significance level

^XTSS refers to the amount of total soluble sugars in the fruit pulp, measures as °Brix and expressed as %

^Y TSS/TA ratio. Total soluble sugar: Titratable acidity (Citric acid percentage) in pulp

Table 11.3. Internal quality parameters of various lemon cultivars harvested, as available, in the Western Cape production area at harvest and after 32 days in cold storage at -1°C and subsequent shelf life of 7-days during 2017 and 2018. The parameters at harvest and after storage and subsequent shelf life were evaluated separately with only the cultivar evaluated as influencing factor.

Season	2017								2018							
	Cold storage duration								Cold storage duration							
	0	32	0	32	0	32	0	32	0	32	0	32	0	32	0	32
	Citric acid (%)		TSS (%) ^X		TSS/TA ratio ^Y		Juice %		Citric acid (%)		TSS (%)		TSS/TA ratio		Juice (%)	
Cultivar																
'2PH	7.2 ^{NS}	5.7		7.09		1.23 ^N	39.6	39.02 ^{NS}	5.0	4.87a	6.7 ^N		1.34a	1.47b	30.2	
'EurekaS' ^A	7.1	5.2	7.1		1.40	1.30	69.6	39.00	5.4	4.47b		6.57	1.22b	1.31a	32.2	
<i>p-value</i>																
<i>Cultivar</i>	0.24	0.003	0.22	0.17	0.2655	0.1	<0.000	0.87	0.025	<0.00	0.06	0.08	<0.00	<0.00	0.00	0.71

^Z Different values refer to the difference between the averages on a 5% significance level as calculated by Fishers LSD

^{NS} Means of the different stages of storage duration do not differ on a 5% significance level

^XTSS refers to the amount of total soluble sugars in the fruit pulp, measures as °Brix and expressed as %

^Y Total soluble sugar: Titratable acidity (Citric acid percentage) in pulp

^A EurekaS- Eureka seedless cultivar

Table 12.1. Variable Importance in Projection (VIP) of the PLS analysis of the variables with the highest values of the different cultivar trial. Higher value expresses higher importance in the expression of the PLS plot in fig.15.

Variable	VIP value (t1 axis)	VIP value (t2 axis)
Rain (mm) >20mm	1.725	1.497
Maximum temperature intercept	1.460	1.251
Days <4°C	1.318	1.313
Minimum temperature slope	1.307	1.218
Average temperature slope	1.302	1.162
Citric acid % after storage	1.232	1.184
Maximum temperature slope	1.201	1.132
Rind colour (Hunter a/b ratio) after storage	1.174	1.006
Rain (mm) <5mm	1.167	1.170

Table 12.2. Correlation matrix of the PLS analysis, showing the 10 variables with the highest correlation to the CI% and CIS during the different cultivar trial.

Variable	CIS		CI%	
	R	p	R	p
Rain (mm) >20mm	-0.439	0.068	-0.468	0.050

Table 13. Rind colour (expressed as Hunter *a/b* ratio) of ‘Eureka’ lemon fruit harvested from various positions/blocks from one orchard situated in Citrusdal in the Western Cape in the 2018 season. Rind colour was measured at harvest as well as after 32 days in cold storage at -1°C and subsequent shelf-life period of 7 days. No interaction between the main effects, namely Picking a position and cold storage duration, regarding the rind colour of the fruit.

	Rind colour (Hunter <i>a/b</i> ratio)
<u>Picking position</u>	
1	-0.27b ^z
2	-0.28b
3	-0.22a
4	-0.24a
5	-0.20a
<u>Cold storage duration</u>	
0	-0.37a
32	-0.19b
<i>p-value</i>	
<i>Picking position</i>	<0.0001
<i>Storage duration</i>	0.0023
<i>Picking position x Cold storage duration</i>	0.7872

^z Different values refer to the difference between the averages on a 5% significance level as calculated by Fishers LSD

Table 14. Internal quality parameters of ‘Eureka’ lemon fruit picked from various positions/blocks in one orchard, located in Citrusdal, Western Cape, harvested in 2018. The fruit were evaluated both at harvest and after storage at -1°C for 32 days and subsequent 7-day shelf life period. No significant interaction between the picking position and cold storage duration as main effects regarding quality parameters.

	Citric acid (%)	TSS (%) ^x	TSS/TA ratio ^y	Juice (%)
<u>Picking position</u>				
1	5.29b ^z	8.05b	1.51c	54.07b
2	4.98c	8.17b	1.63b	53.85c
3	5.07c	8.42a	1.68a	55.79a
4	5.52a	8.45a	1.54c	55.93a
5	5.44ab	7.87b	1.44d	55.81a
<u>Storage duration</u>				
0	5.25 ^{NS}	7.99b	1.54 ^{NS}	45.87b
32	5.27	8.19a	1.56	55.11a
<i>p-value</i>				
<i>Picking position</i>	<0.0001	<0.0001	<0.0001	0.0132
<i>Cold storage duration</i>	0.7210	0.0071	0.1562	0.0516
<i>Picking position x Cold</i>	0.7875	0.0578	0.2432	0.1212

^z Different values refer to the difference between the averages on a 5% significance level as calculated by Fishers LSD

^xTSS refers to the amount of total soluble sugars in the fruit pulp, measures as °Brix and expressed as %

^y Total soluble sugar: Titratable acidity (Citric acid percentage) in pulp

^{NS} Means of the different stages of storage duration do not differ on a 5% significance level

8. Figures

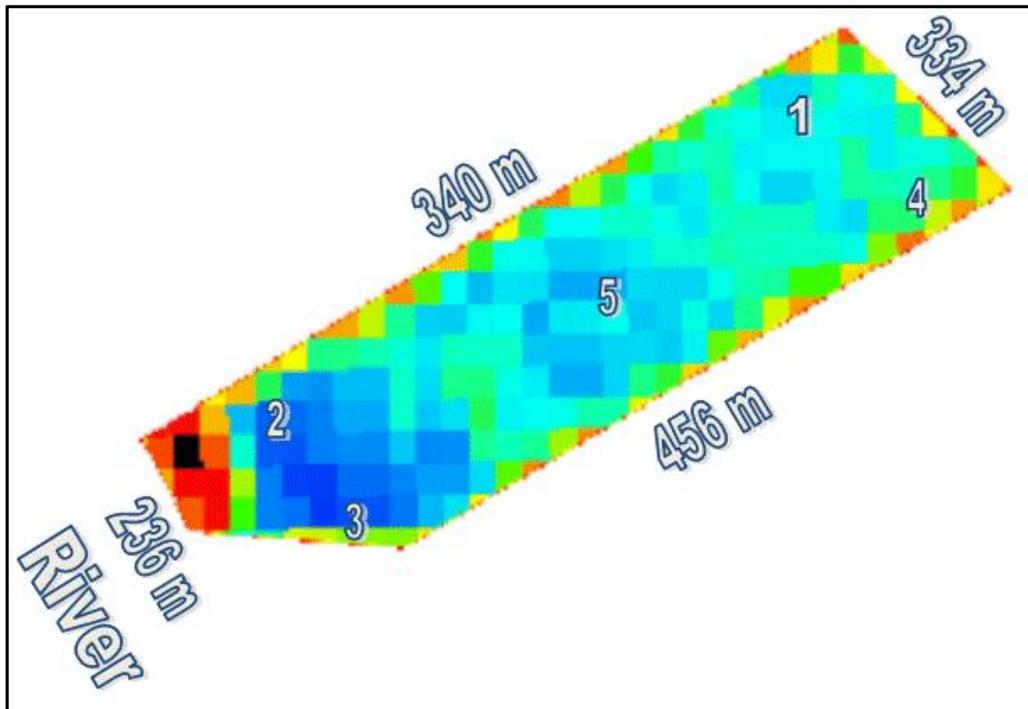


Figure 1. A Fruitlook image of a 12ha 'Eureka' lemon orchard located in the Citrusdal production area within the Western Cape, used to study the effect of different block positions within the same orchard on the development of CI on lemon fruit. Each number represents a different block within the orchard, with an indication of spacing (m) between respective blocks, whereas colour coding corresponds to biomass production with blue indicating a high and green a low biomass per surface area. (Image:<https://www.fruitlook.co.za/>)

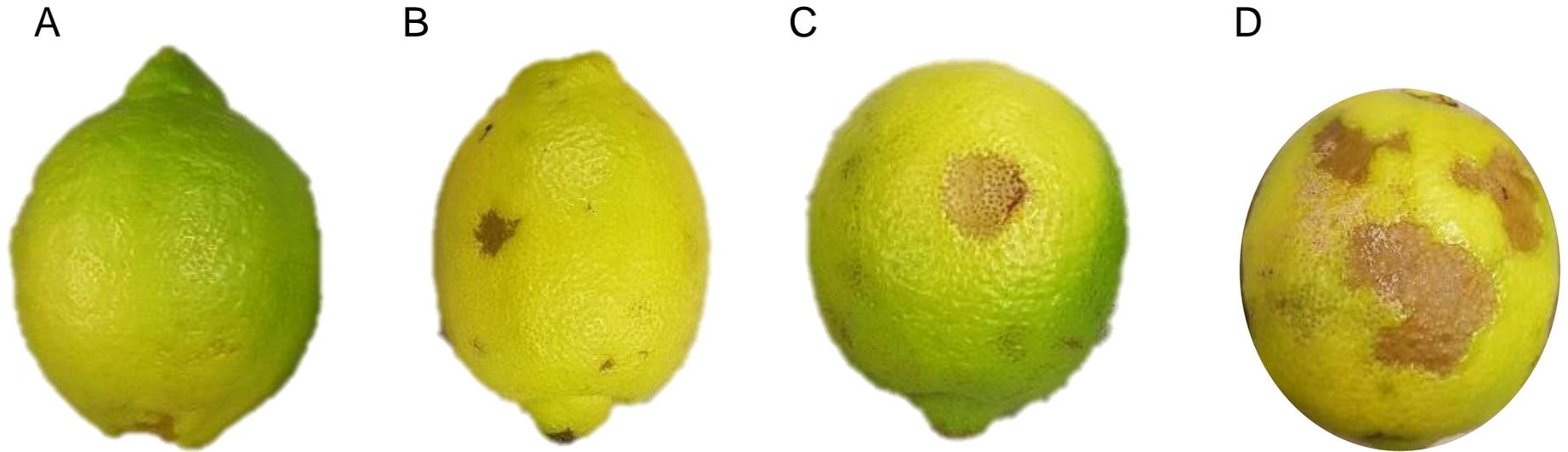


Figure 2. Increasing levels of chilling injury severity sustained by 'Eureka' lemon fruit. Fruit was scored on a scale from 0-3, based on the severity of the injury, where (A) implies fruit sustained no injury and is considered commercially viable at rating 0; in (B) slight chilling injury is sustained, but is still commercially viable at rating 1; in (C) chilling injury symptoms are evident upon coalescing, resulting in large brown spots on the rind, rendering the fruit unmarketable and obtain a rating score of 2; at (D) fruit are completely covered in brown necrotic spots, to warrant a rating of score of 3

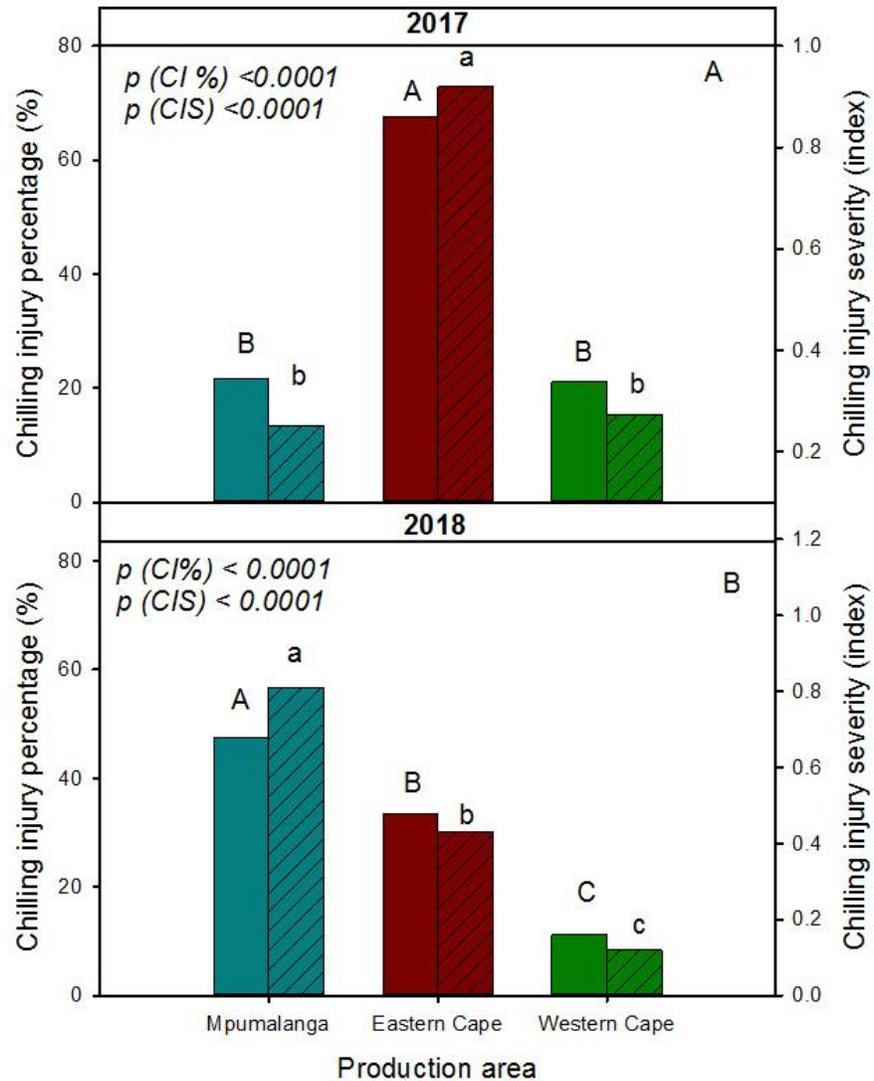


Figure 3. Chilling injury percentage (CI %) (solid bar) and severity (CIS) (striped bar) of fruit harvested from three, distinct climatic areas over the 2017 (A) and 2018 (B) seasons. Mpumalanga represents production areas in North-Eastern South Africa where a semi-tropical climate prevails; Eastern Cape refers to the coastal citrus production of the Sundays River Valley; whereas the Western Cape indicate the Mediterranean production region of the Citrusdal area. Each bar represents the average values of the injury sustained in six orchards (replicates) (n=6). Different letters indicate a difference between the treatments (production area) on a 0.05 significance level. Capital letters A, B, and C indicate the significant difference in the CI % between areas (treatments), whereas a, b and c indicate the significant difference in the CIS of each area.

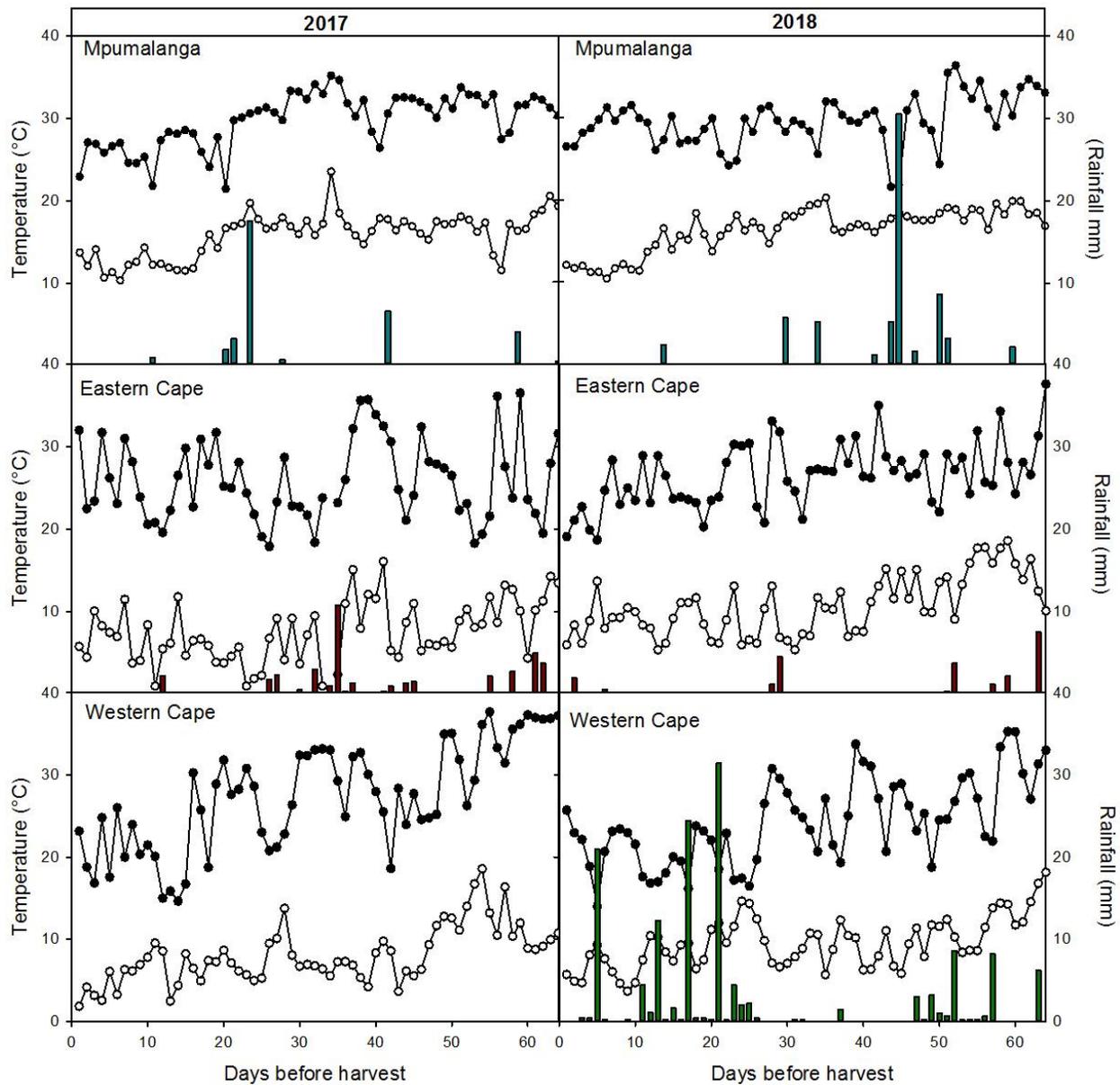


Figure 4. Maximum and minimum temperature (°C) and average rainfall (mm) in three different citrus production areas, namely Mpumalanga, Eastern Cape, and Western Cape, over a 60-day period prior to harvest. Temperature is depicted by line graphs on the left y-axis and rainfall is indicated by bar graphs on the right y-axis. No rainfall data is presented for the Western Cape area during 2017, due to unavailability in data.

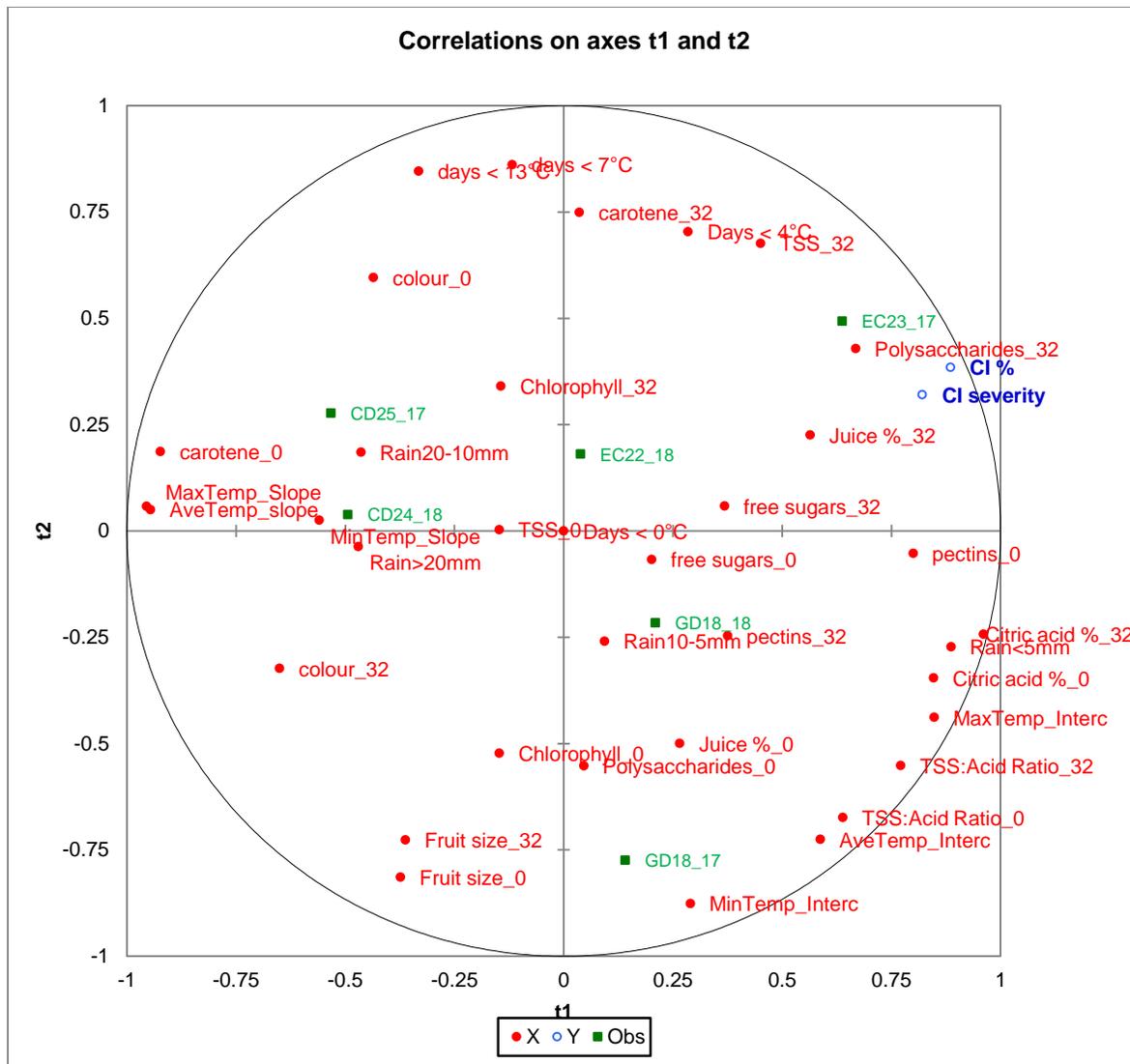


Figure 5. Plot of the first two dimensions of Partial Least Squares (PLS) regression analysis on various internal and external, as well as different climatic parameters (X matrix, indicated in red) on the CI severity and CI percentage (Y matrix, indicated in blue) of 'Eureka' lemon fruit harvested from 6 different orchards (n=6) in three different climatic distinct areas, respectively over two seasons, 2017 and 2018.

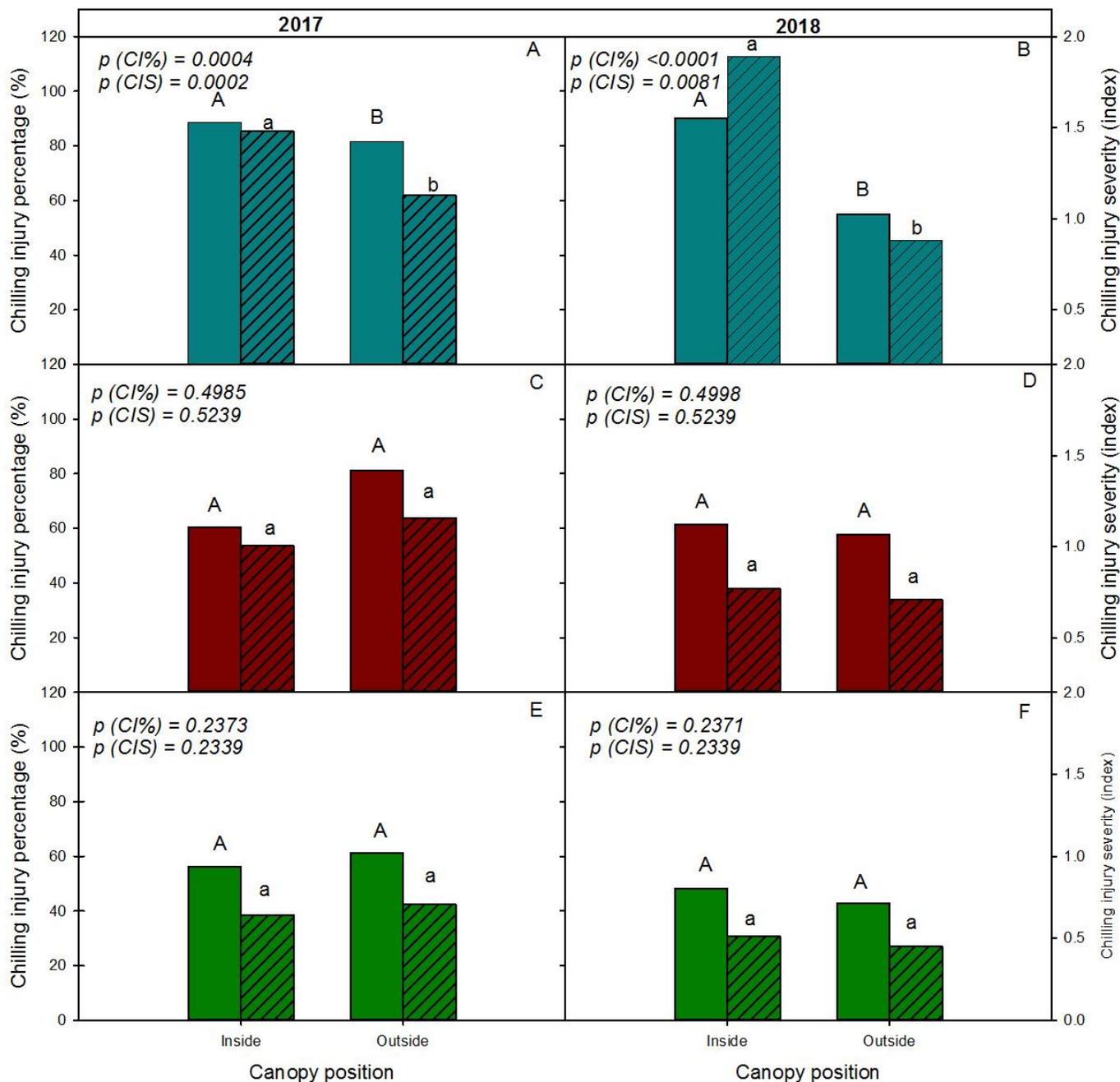


Figure 6. Chilling injury percentage (CI%) (solid bar) and chilling injury severity (CIS) (striped bar) of fruit harvested from the inside and the outside of the tree canopy respectively from orchards based either Mpumalanga (A; B); the Eastern Cape (C; D); and the Western Cape (E; F) during the two seasons of 2017 (A; C; E) and 2018 (B; D; F). Capital letters; A and B, indicate the significant difference in the CI % between treatments (canopy position), whereas a, b and c indicate the significant difference in the CIS between treatments.

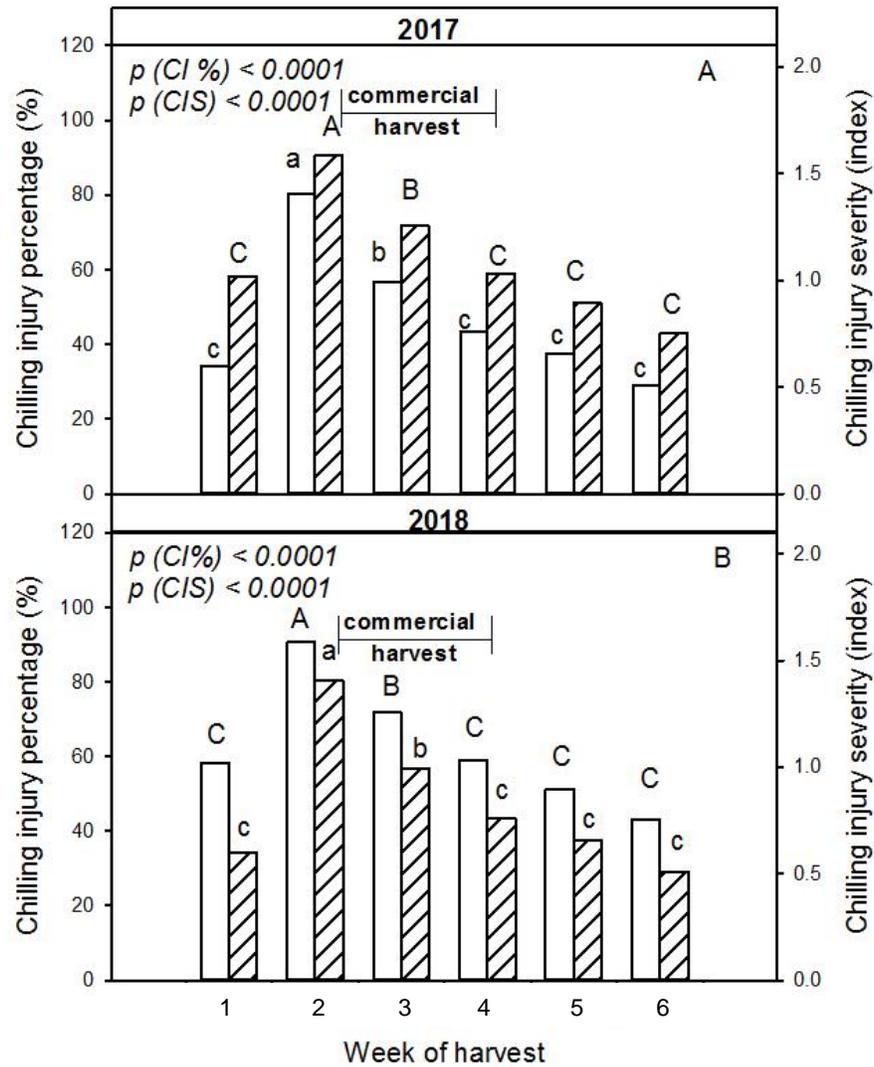


Figure 8. Chilling injury percentage (CI %) (solid bar) and severity (CIS) (striped bar) of 'Eureka' lemon fruit sampled at two-weekly intervals during the final stage of fruit development in 2017 (A) and 2018 (B) in the Stellenbosch/Somersset West area (Table 1) from April to July. The fruit were harvested in two-weekly intervals over a twelve-week period, with the commercial harvesting window of the production area coinciding with the 3rd harvesting date, as indicated by the horizontal bar. After harvest, fruit was cold-stored at a delivery air temperature of -1°C for 32 days and evaluated for CI after a subsequent shelf life period of 7 days. Capital letters A, B, and C indicate the significant difference in the CI % between treatments (sampling period), whereas a, b and c indicate the significant difference in the CIS of treatment.

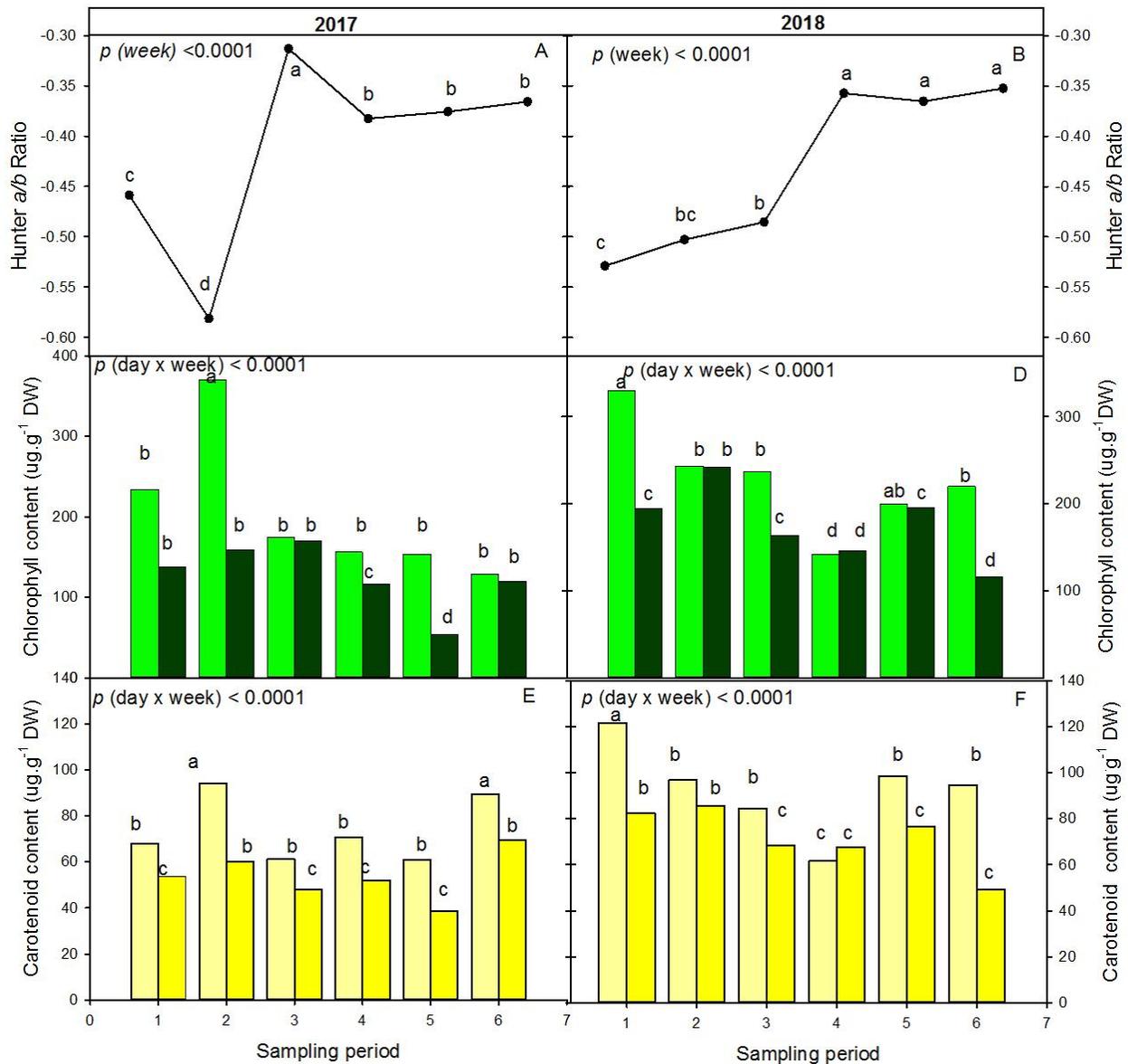


Figure 9. Rind colour (A; B) at harvest, reported as a Hunter a/b ratio, and chlorophyll and carotenoid concentration, both expressed as $\mu\text{g}\cdot\text{g}^{-1}$ dry weight, of 'Eureka' lemon fruit when harvested at two-weekly intervals during a 12-week development period in 2017 (A; C; E) and 2018 (B; D; F). Harvesting starting four weeks prior to the historical commercial harvesting date of each orchard to coincide with the third harvesting date (indicated with a horizontal bar) and continued for six weeks thereafter. In both seasons, chlorophyll content (C; D) and carotenoid concentration (E; F) was determined prior to storage (left bar) and after a cold storage period followed by a shelf life simulation period of seven days (right bar). The different letters refer to a difference between treatments on a 5% significance level.

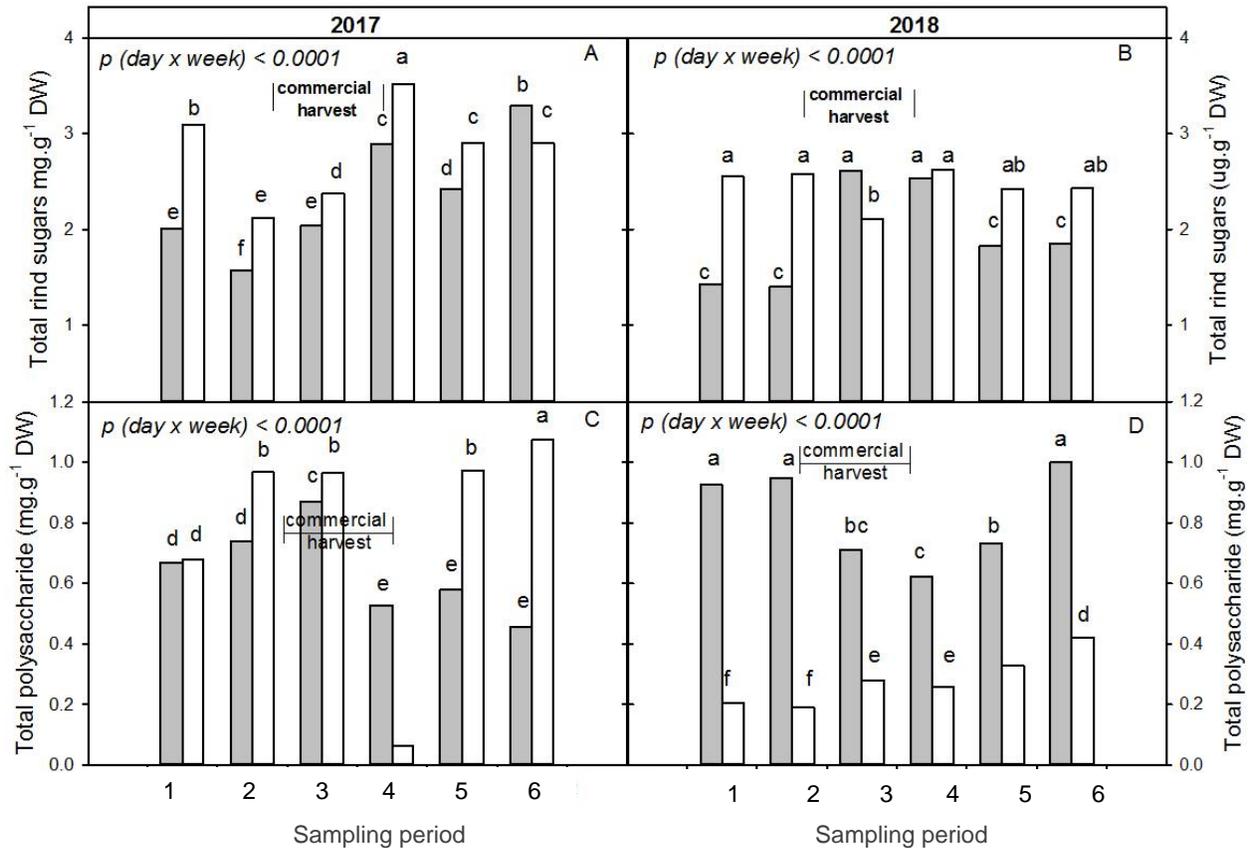


Figure 10. Rind sugar content ($\text{mg}\cdot\text{g}^{-1}$ dry weight) (A; B) and water-soluble polysaccharides (C; D) determined from the rind of 'Eureka' lemon fruit when sampled during the final stage of fruit development in 2017 (A; C) and 2018 (B; D). Fruit were harvested at two-weekly intervals over a twelve-week period, where the historical commercial harvest date coincided with the third picking period as indicated by a horizontal bar. The grey and white bar respectively represent the sugar and polysaccharide content of the fruit rind at harvest and following cold storage at -1°C for 32 days and a 7-day shelf life simulation period respectively. Significant interactions ($p < 0.0001$) between harvesting date (week) and storage duration (day) is evident in both seasons, for both sugar and polysaccharides. The different letters represent significant differences between treatments on a 5% significance level.

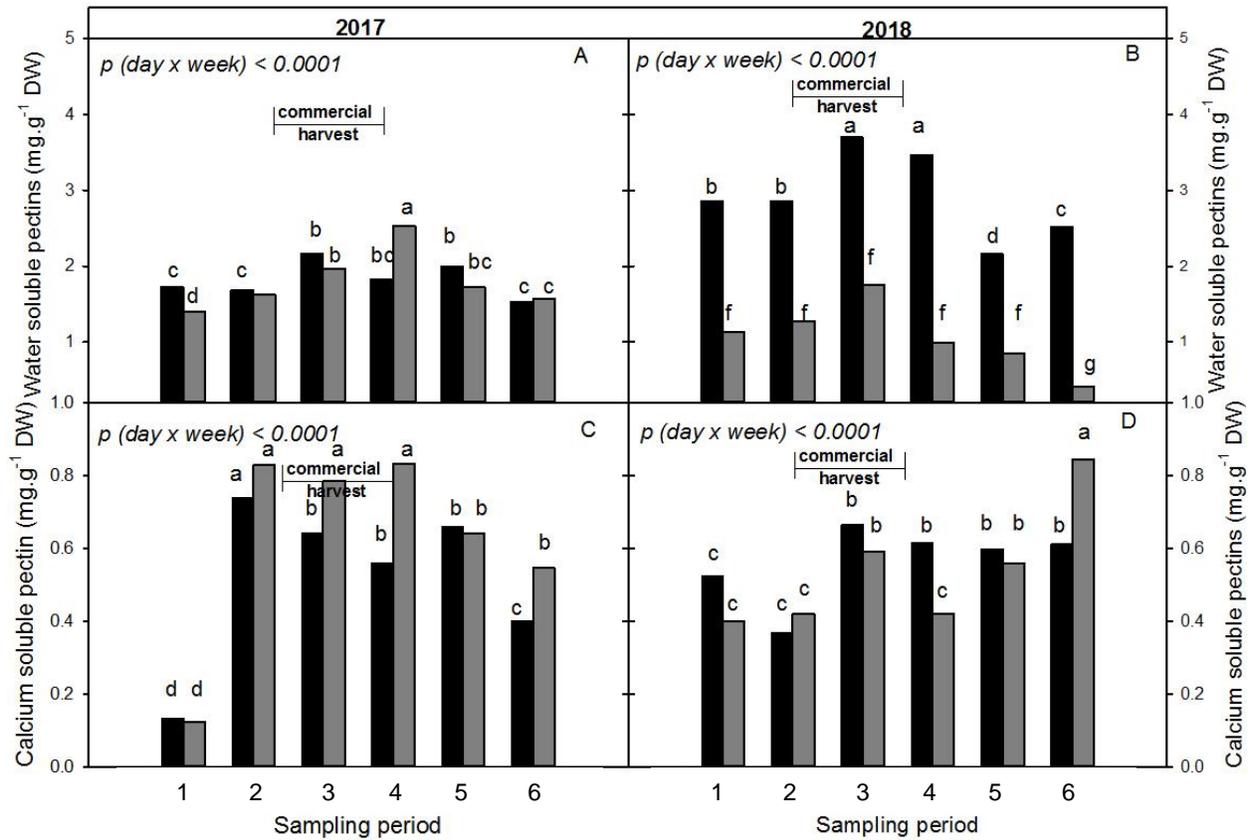


Figure 11. Water (A; B)- and calcium soluble (C; D) pectin content ($\text{mg}\cdot\text{g}^{-1}$ dry weight) of 'Eureka' lemon fruit rind flavedo sampled at two-weekly intervals over a twelve-week period during the last stage of fruit development in 2017 (A; C) and 2018 (B; D) where the historical commercial harvest date coincided with the third picking period as indicated by a horizontal bar. The black and grey bar respectively represents the pectin content of the fruit rind at harvest and following cold storage at -1°C for 32 days and a 7-day shelf life simulation period. Significant interactions ($p < 0.0001$) between harvesting date and storage duration (day) is evident in both seasons for both pectin categories. The different letters represent significant differences between treatments on a 5% significance level.

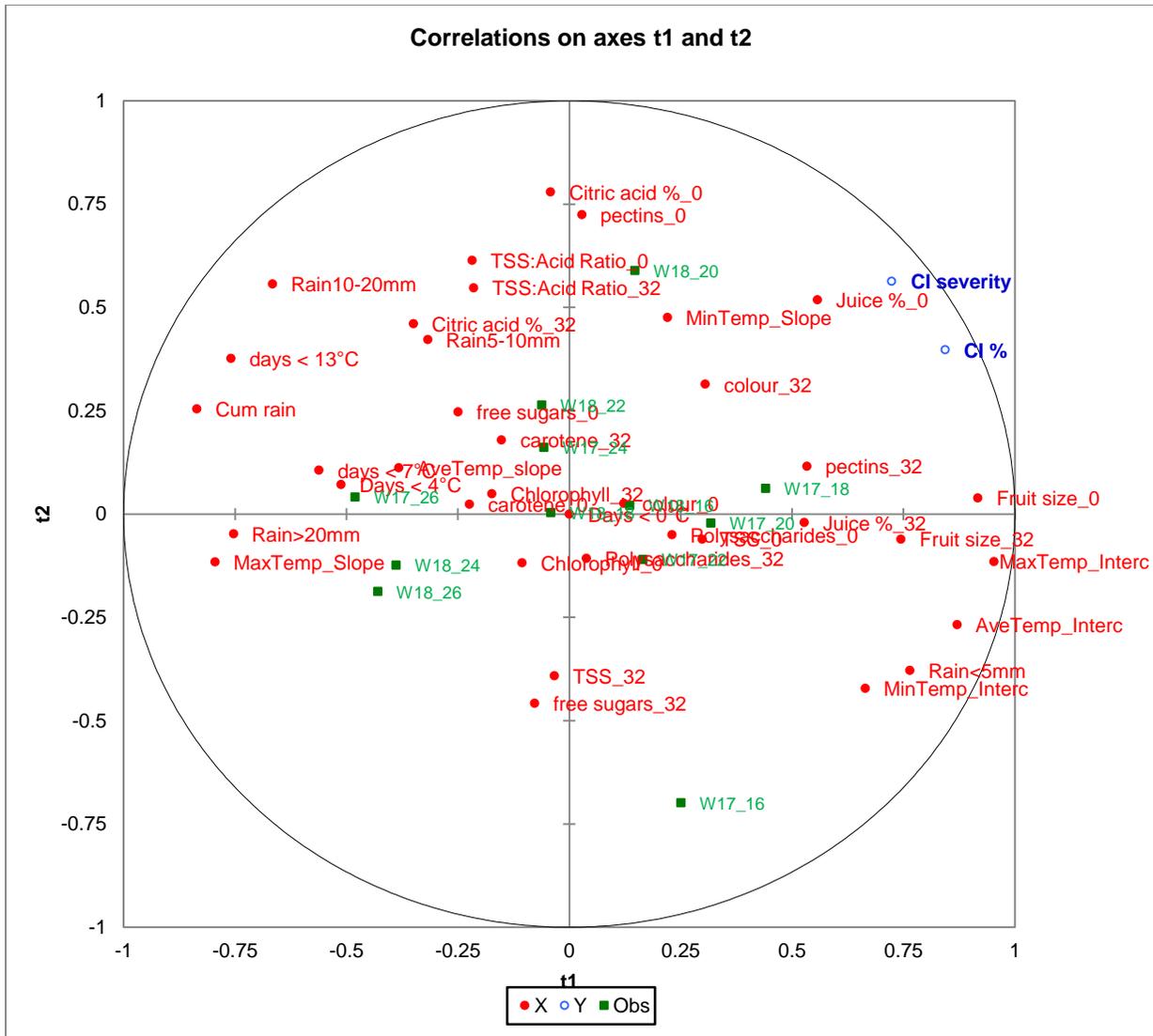


Figure 12. Plot of the first two dimensions of Partial Least Squares (PLS) regression analysis on various internal and external, as well as different climatic parameters (X matrix, indicated in red) on the CI severity and CI percentage (Y matrix, indicated in blue) of 'Eureka' lemon fruit harvested from 10 different trees at two-weekly intervals during the 2017 and 2018 season.

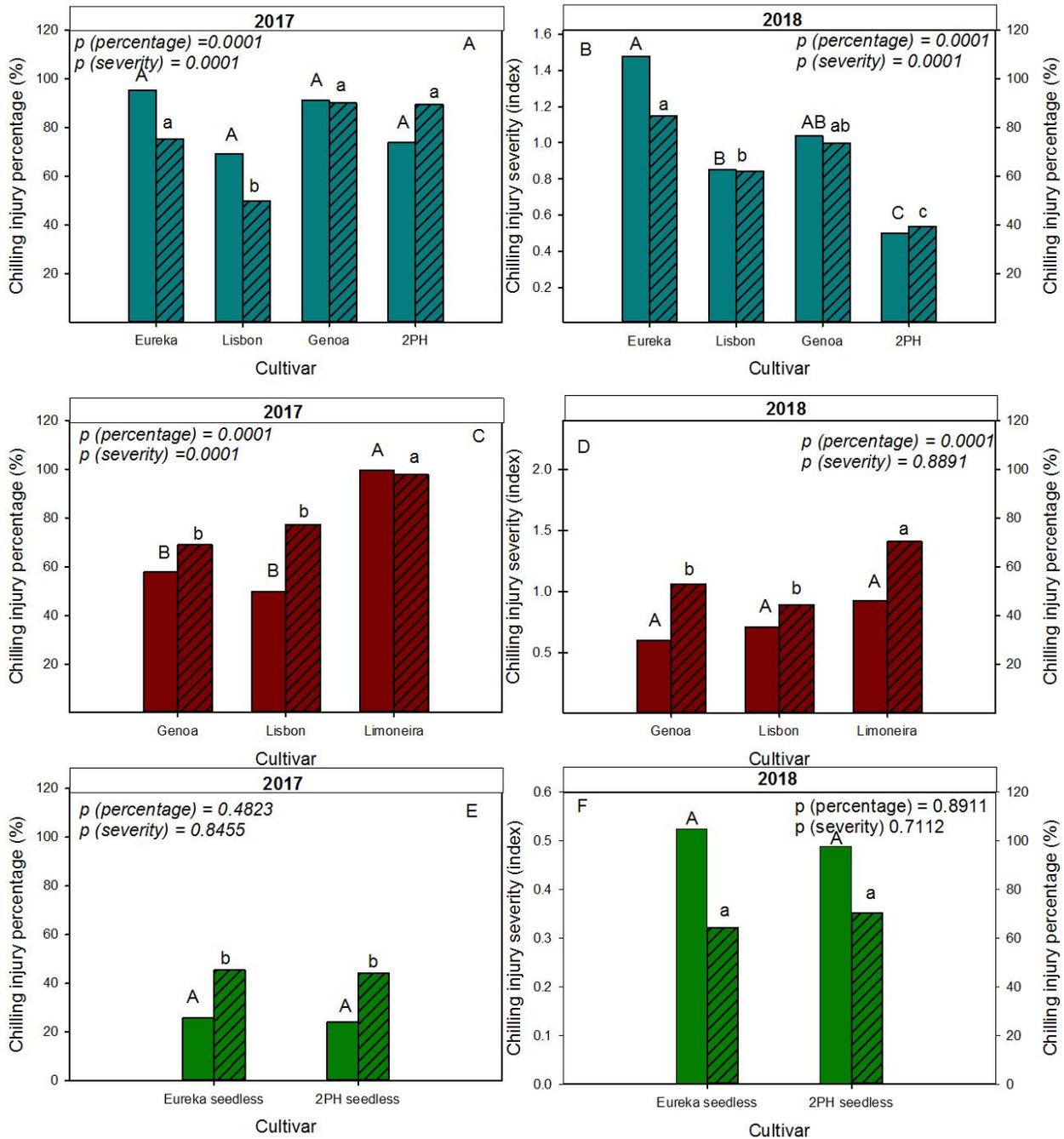


Figure 13. Chilling injury percentage (CI %) (solid bar) and chilling injury severity (CIS) (striped bar) of different lemon cultivars harvested in the Mpumalanga (A; B); Eastern Cape (C; D) and Western Cape (E; F) production areas respectively in 2017 (A; C; E) and 2018 (B; D; F). CI was determined following cold storage of fruit at a delivery air temperature of -1°C for 32 days and a subsequent shelf life period of seven days at ambient temperatures.

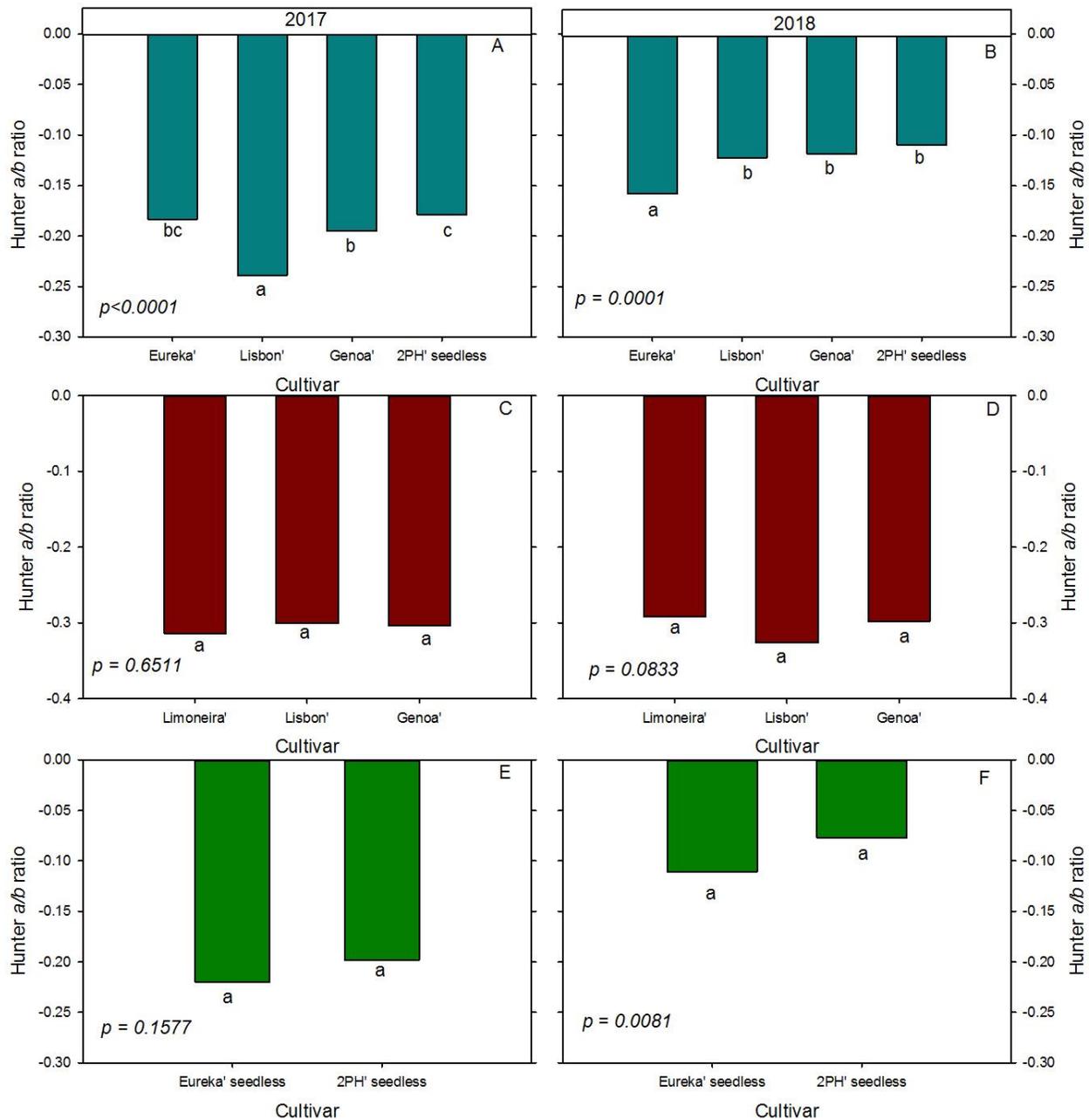


Figure 14. Rind colour (A; B) after cold storage and subsequent 7-day shelf life simulation period, reported as a Hunter *a/b* ratio, of various lemon fruit cultivars harvested throughout South Africa. In the Mpumalanga area, 'Eureka', 'Genoa', 'Lisbon' and 2PH seedless were harvested (A; B). 'Limoneira', 'Lisbon' and 'Genoa' was harvested from the Eastern Cape (C; D), and two seedless cultivars namely Eureka seedless and 2PH seedless were harvested in the Western Cape. These various cultivars were harvested and evaluated throughout the 2017 (A; C; E) and 2018 (B; D; F) seasons. The different letters refer to a difference between treatments on a 5% significance level.

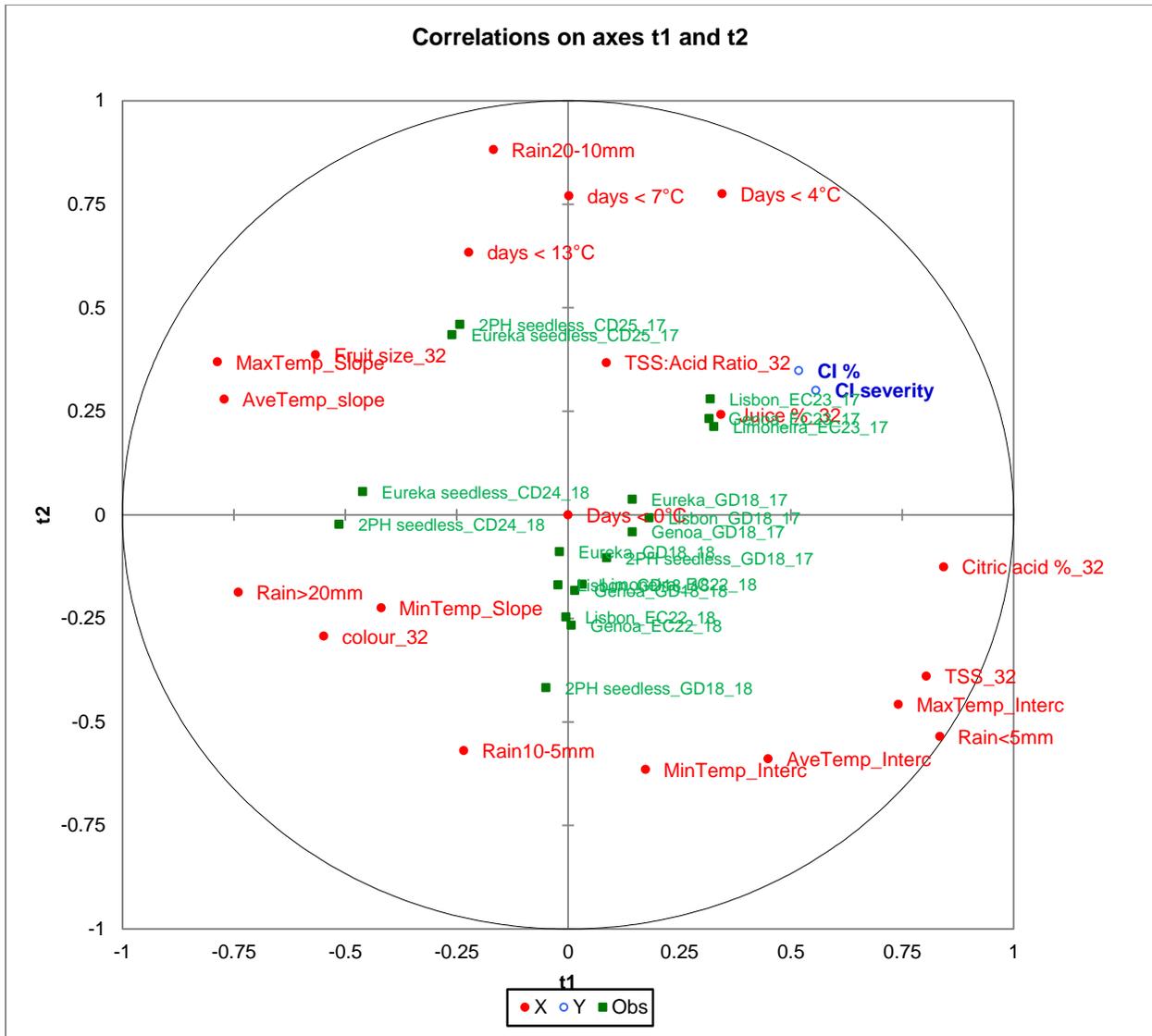


Figure 15. Plot of the first two dimensions of Partial Least Squares (PLS) regression analysis on various internal and external, as well as different climatic parameters (X matrix, indicated in red) on the CI severity and CI percentage (Y matrix, indicated in blue) of various lemon cultivars over two seasons, 2017 and 2018.

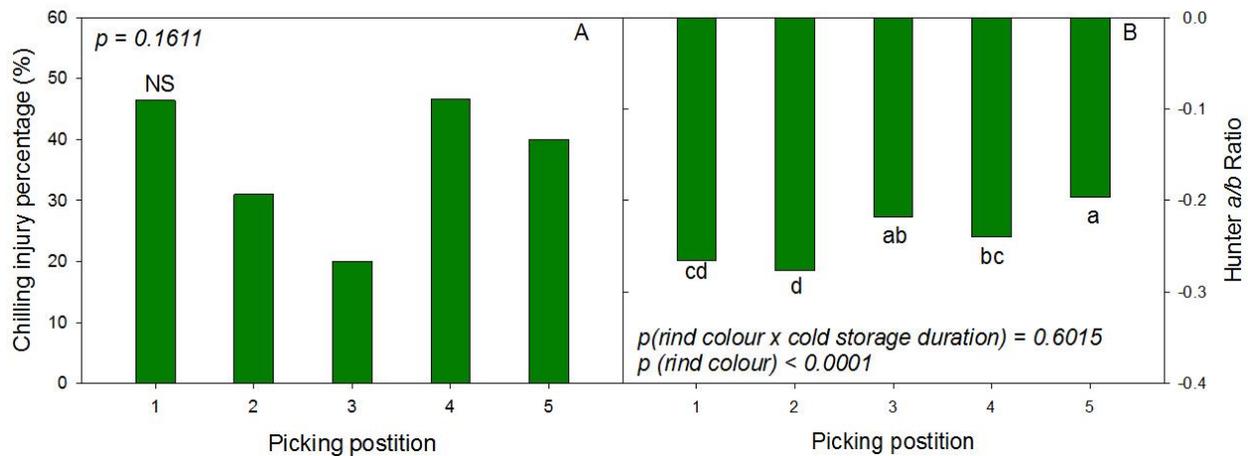


Figure 16. Chilling injury percentage (CI %; A) and rind colour (Hunter *a/b* ratio; B) of fruit harvested from six different blocks from one 12 ha orchard located in Citrusdal in the Western Cape. Numbers 1 to 4 refers to corner blocks of the orchard, whereas fruit from block 5 was harvested from within the center of the orchard. CI was determined after a cold storage period of 32 days and a subsequent shelf-life period of seven days at ambient temperatures. No significant difference between the different blocks in the orchard was found with regards to the CI % of the fruit ($p = 0.1611$). Rind colour (B) was determined at harvest (Day 0) and after cold storage and subsequent shelf-life period of seven days at ambient temperatures. No interaction ($p = 0.6015$) was found between cold storage duration and harvest block with regards to the rind colour. Significant differences were noted between the orchard blocks regarding the rind colour ($p < 0.0001$). The different letters refer to a difference between treatments on a 5% significance level.

The efficacy of postharvest application in the reduction of chilling injury incidence in lemon fruit (*Citrus limon* (L.) Osbeck)

ABSTRACT

To effectively maintain citrus fruit quality and ensure acceptability to the consumer, various postharvest control measures throughout the supply chain should be considered and implemented. Cold storage of fruit is one of the most effective measures used to preserve fruit quality. However, lemon fruit are a known chilling sensitive citrus type, and therefore storage of these fruit below 7°C should be avoided. The sensitivity of lemon fruit to cold conditions present a challenge when exporting lemon fruit to countries with phytosanitary requirements, the as long term cold storage at temperatures of between -0.6 °C and 2 °C is likely to result in blemishes of the fruit rind, a condition classified as chilling injury (CI). This physiological disorder manifests as brown, sunken, necrotic areas in the rind due to cell membrane damage leading to a cascade of secondary events. The incidence of the disorder is known to be dependent on the duration of storage at a threshold temperature. The objective of this study was to determine how the temperature and storage duration interaction affects lemon fruit as well as to evaluate postharvest methods for its efficacy to control symptom development. Lemon fruit, when stored at temperatures of 2°C or lower showed a clear increase in CI incidence, enhanced during prolonged storage. However, the incidence of CI was reduced with an increase in the solid content of the various wax types, suggesting that wax applications with a higher solid percentage provide better protection to fruit rind against postharvest cold stress. The results indicate that shipment at 2°C, irrespective of the postharvest wax treatments, are still not commercially viable, whereas storage at higher temperature ranges of between 4°C and 7°C, when used in combination with wax applications has the potential to significantly reduce CI to within commercially acceptable levels.

1. Introduction

The importance of maintaining citrus fruit quality during the cold chain is key to optimize the return on investment for producers sustainably. Citrus fruit are generally considered less sensitive to internal quality changes during the postharvest storage and transport compared to other fruit and vegetables (Chalutz *et al.*, 1985). However, maintaining optimum temperature throughout the postharvest chain is still crucial to prevent postharvest decay and loss of fruit quality, particularly with respect to moisture loss and disorder development. An important consideration with cold storage is that citrus fruit, due to their tropical and subtropical origin, are generally known to be sensitive to low temperatures when stored for extended durations (Wu *et al.*, 2018). The threshold temperature whereby damage may occur significantly varies among citrus types and cultivars, yet lemon and lime fruit are considered being of the most sensitive, sustaining CI at higher temperatures during storage, compared to mandarins and oranges. As a result, lemon fruit is mostly recommended to be stored at temperatures above 10-12°C (Chalutz *et al.*, 1985; Lado *et al.*, 2019).

Due to existing phytosanitary pests present in South Africa, certain countries require sterilization treatments to eliminate any False Codling Moth and Fruit fly larvae in the fruit during shipment (Biolatto *et al.*, 2005). The temperatures and duration of these treatments are however, far below the threshold storage condition considered suitable for lemons. Export regulations to the United States of America requires citrus fruit to be shipped at a pulp temperature of -0.55°C for 22 days (d), whereas China demands a period of 24 d at -0.6°C. These disruptive cold sterilization protocols necessarily result in making it very high level of CI sustained by the exported lemon fruit, effectively excluding South African produced lemons from these markets due to financial and quality pressures (PPECB, 2019).

Lemons subjected to temperatures below the threshold often exhibit a physiological disorder known as chilling injury (CI). Associated the symptoms of CI is evident as small, brown pits on the surface of the fruit rind with the tendency to coalesce and form bigger depressions on the fruit surface because of cell membrane damage, rendering affected fruit unmarketable. The severity of the disorder is a function and interaction of the storage temperature as well as the duration to which the fruit are exposed to the low temperatures (Lyons, 1973; Biswas, 2017). Both pre-and postharvest factors during which fruit are exposed to during the citrus supply chain, significantly impact on the final quality of the fruit exported. These factors include climatic changes during fruit growth, such as the prevalent temperature and relative humidity, postharvest handling practices, including packaging, and various aspects of transportation (Ladaniya, 2004). Citrus fruit destined for fresh fruit markets are manually harvested, where after they are subjected to several postharvest protocols and treatments in the packhouse which include degreening; washing; and, fungicide- and wax application prior to packing and exports. However, processes such as postharvest washing, with the purpose to clean the fruit and remove possible latent decay organisms from the rind, also remove the natural protective waxy layer of citrus fruit rind is resulting in rapid fruit dehydration (Hall and Sorenson, 2006). This unintentional removal of natural rind surface waxes also interferes with the natural resistance of fruit against various physiological disorders, including that of fruit rind disorders and decay. Various studies on citrus have been done, mostly on grapefruit and orange, illustrating the positive impact of wax coatings to prevent moisture loss (Hagenmaier and Baker, 1993). Furthermore, wax-coated grapefruit have been shown to be also more tolerant to CI development than non-coated fruit (Dou, 2004). These coatings, such as carnauba and beeswax, are most often referred to as waxes, mainly because of their composition even though they do not necessarily consist of wax. Commercially there are several different types of waxes available, where each are developed for a specific purpose which may include: after degreening; during the packing process to reduce moisture loss and lower respiration rate; as a carrier for fungicides, or to improve the general appearance and shine of the citrus fruit rind (Hall, 1981; Mannheim and Soffer, 1996; Petracek *et al.*, 2000; Ladaniya, 2004). The two common types of waxes used in the

South African industry are solvent- and water-based waxes (Hall, 1981). The solvent wax typically consists of various types of resin dissolved in a petroleum solvent, as opposed to water-based waxes, where the fruit is required to be completely dry before application, where the wax is easily removed from the fruit after storage (Hall, 1981, Hall and Sorenson, 2006). Water-based waxes can be further differentiated into two types of which the first type refers to resin solution waxes or simply, resin waxes, that are composed of alkali-soluble resins like shellac, or resin secreted by an insect or wood resin, that is then dissolved by an alkali to which either alcohols, glycerine or propylene glycol are added to promote the dissolving of the resin (Hall, 1981). Organic acids and oils might also be added to act as wetting agents to these water-based waxes. The second group of water-based wax refers to emulsion waxes and consists of either a natural wax-like carnauba or a synthetic wax type such as oxidized polyethylene (Hall, 1981). For optimum fruit protection to be extended by emulsion waxes the fruit must have a clean surface that may range from damp to dry, but with no free water present prior to the application of the water-based wax, keeping the fruit resistant to re-wetting (Hall, 1981). The main purpose of a wax application is to provide uniform coverage to the fruit throughout the packing process (Hall and Sorenson, 2006). The method of application varies significantly between different wax types; therefore, care should be taken to select the correct applicator (nozzle type and position), brushes and pack line to ensure uniform coverage. In addition, several factors must be considered before commencing fruit waxing to ensure that a suitable wax type is selected and may include: the cost of the wax; the market acceptability; coating durability and fruit respiration (gas exchangeability), amongst others (Hall, 1981; Hall and Sorenson, 2006). Citrus varieties have different responses to wax applications, such as the development of off-flavors by some mandarin and grapefruit varieties (Ladaniya, 2004). Wax coatings, when used as a carrier for fungicides to prevent postharvest decay, can either be mixed along with the wax or applied before waxing, depending on the mode of action of the respective fungicide (Njombolwana, *et al.*, 2013). Immobile, protective fungicides, such as Imazalil (IMZ), are more effective against infections like green mold when applied before waxing, as these types of fungicides become bound to the rind of the fruit once applied and is not able to transfer to the infection site (Njombolwana *et al.*, 2013). Thiabendazole (TBZ), a fungicide used curatively or protectively against the green mold causing fungicide, *Penicillium digitatum*, has shown to reduced CI in citrus fruit due to an unexplained mechanism (Schirra and Mulas, 1995; Schirra *et al.*, 2000; Hordijk *et al.*, 2013; Kellerman *et al.*, 2014). Although TBZ decreased CI susceptibility when applied to the fruit via a wax coating, this fungicide tends to precipitate, thus offering less protection to physiological disorders and postharvest decay and are therefore considered to be more effective when applied prior to waxing (Kellerman *et al.*, 2014; Ehlers, 2016). Another postharvest treatment effective in controlling CI development is the application of the two plant hormones, Salicylic acid, and Methyl Jasmonate which have been shown to effectively control the development of this disorder when applied as a postharvest dip treatment (Siboza *et al.*, 2012). It is believed that these treatments increase the enzymes in the fruit needed to scavenge the ROS species involved in causing CI (Siboza *et al.*, 2017). In addition to postharvest treatments, the physiological rind condition at harvest is an important factor affecting increasing resistance against CI. The carotenoid pigments, responsible for the distinctive rind colour of citrus, offers protection to the fruit against disorders such as CI. Tolerance to CI was higher in red coloured, lycopene-containing, 'Star Ruby' grapefruit as opposed to higher injury susceptibility witnessed in 'Marsh' grapefruit, that contained no lycopene, suggesting that lycopene as a carotenoid pigment, acts as antioxidants to prevent fruit from developing CI (Lado *et al.*, 2015). In addition to pigments, sugar constituents of the rind have also shown protective action against CI, with temperature playing a key role in the sugar metabolism of the fruit as treatment of citrus fruit with hot water enhanced the accumulation of sucrose in the fruit (Holland *et al.*, 2005). Following postharvest treatment and prior to loading of fruit into refrigerated containers or commencing transport by reefer ships, the fruit must be precooled. This heat transfers from the fruit commodity to the medium of cooling effectively reduce or prevent fruit decay, inhibit water loss, and reduce ethylene production (Ladaniya, 2004). However, even during this pre-cooling period, citrus fruit might already sustain CI if the process is not controlled, for instance if the temperature reduction is too fast (McGlashan *et al.*, 2017). As

the extent to which cold storage below 10 °C for long durations affect the incidence of CI of lemon fruit has not yet been studied, the aim of this paper was to evaluate the CI development of lemon fruit stored at different temperatures for various durations. In addition, the efficacy of solid content of wax coatings to reduce CI incidence was quantified.

2. Materials and methods

2.1. Cold storage temperature regimes

2.1.1. Fruit variety, areas and plant material

'Eureka' (*Citrus limon*) lemon fruit were harvested from a commercial orchard in Robertson, Western Cape, South Africa. This trial was carried out over the 2017 and 2018 seasons in weeks 25 and 26 respectively, which falls in the peak lemon harvesting window for this area. No postharvest chemical treatments were applied at harvest. Fruit were immediately transported to the laboratory and cold rooms of the Department of Horticultural Science at Stellenbosch University, as described in Chapter 3 (pg. 40-41).

2.1.2. Treatment and experimental layout.

Cold storage treatments. Three temperature treatments were used during the first season and five during the second, with four different storage durations (time) during both seasons. The trial was laid out as a complete randomized block design (CRBD) for which 10 replications were used per treatment combination (Temperature x Storage Duration), with each replication (n=10) that consisted of 10 fruit each. Thus, a total of 400 randomly allocated fruit were stored per temperature treatment, and 100 fruit were evaluated per different storage duration of 12, 22, 32, and 42 days respective. For the day 0 evaluation at harvest, ten replications of five fruit each were used. The delivery air temperature in the different cold rooms were controlled at -1 °C, 2 °C and 7 °C during 2017 and at -1 °C, 0 °C, 1 °C, 2 °C and 7 °C for the 2018 season. In 2018, an additional experiment was conducted where a commercial treatment of Endura-Fresh™ wax (18% solids, JBT Food, Tech, Brackenfell, SA) mixed with Thiabendazole (TBZ) (2000 ppm), was applied prior to storage, to determine the impact of a standard commercial packhouse treatment. The wax was sprayed via three nozzles onto the fruit whilst moving on rotating brushes. After waxing, the fruit were dried and put into cold storage for 0, 12, 22, 32 and 42 days at three different delivery air temperatures of -1 °C, 2 °C and 7 °C respectively. Once storage was completed, the pre-allocated fruit were transferred to ambient temperature (+/-20°C) for seven days to simulate shelf life conditions, during which CI symptoms were allowed to develop fully.

2.1.3. Data collection

Chilling injury evaluation.

As described in Chapter 3 (43.), each fruit were evaluated for CI incidence and severity after seven days at shelf-life conditions by being assigned a number relating to the severity of injury sustained (0-3) (Fig. 1) As CI % and CI index/severity produced similar trends and results, only CI % was reported.

Internal and external fruit quality evaluation.

After harvest, day 0- and CI evaluation, the internal and external quality were quantified as described in Chapter 3 (pg. 44) for external quality by means of fruit weight, diameter and rind colour, whilst internal quality was evaluated with respect to citric acid % and total soluble sugars in the pulp.

Flavedo rind analysis.

To determine the impact of the temperature duration treatments on the rind, carbohydrate- and pigment contents of the flavedo were analyzed as described in Chapter 3 (pg. 44-48).

Respiration rate. To study the physiological reaction of lemon fruit to the interaction between various temperature and duration treatments, the respiration rate was determined during the second season. At the start of the cold storage period, five fruit were placed in each of five airtight plastic containers in each of the cold rooms set at temperatures of ambient, 1°C, 2°C, and 7°C respectively. The gas was sampled within the cold room to ensure the continuity of the cold chain, at each of the set intervals of 12, 22, 32, 42d respectively. Prior to sampling, the containers were closed for 24h after which 2mL air was extracted from the headspace of the containers through a rubber septum fitted in the lid with a syringe and then injected into an Infrared Gas Analyzer (Model S-151; Qubit Systems Inc., Kingston, Ontario), where after respiration rate of the fruit was calculated as mL CO₂ kg⁻¹·h⁻¹ (Kays and Paul, 2004).

2.1.4. *Statistical analysis*

The data were evaluated using a two-way factorial ANOVA in Statistica 13 VEPAC (TIBCO Software Inc., 2018) with temperature and storage duration as main effects. The post-doc test, Fishers least significant difference (LSD) at a significance level of 5% was used to determine the statistical differences between the treatment values. Correlation analysis was done using Statistica 13 VEPAC module (TIBCO Software Inc., 2018) to calculate spearman's coefficient.

2.2. *Experiment 2: Evaluating the effect of post-harvest wax applications on the chilling injury tolerance of lemon fruit*

2.2.1. *Area and plant material*

For both seasons, the 'Eureka' lemon fruit, were obtained from one commercial orchard in the Ashton area and delivered to Unipack packhouse in Ashton, Western Cape, South Africa. The fruit were harvested at commercial maturity, during weeks 25 and 28, in 2017 and 2018 respectively. On arrival at the packhouse, the fruit were drenched with a chlorine solution and then kept at ambient temperature for one day to prevent fruit decay until the wax coating application was performed.

2.2.2. *Treatments and experimental design*

The fruit used were divided into 9 treatments, each with 10 replications (n=10), consisting each of 10 fruit. The unblemished fruit, of similar size (50-60mm) and colour (nr. 4-5 on CRI, 2004 standard colour chart), was assigned to the different wax treatments, according to a complete randomized design. Treatments consisted of chlorine-drenched control and eight different wax treatments (Table 1). The waxing apparatus (John Beam Technology, Riverside California, USA) consisted out of 12 rotating brushes, made from a combination of horsehair and polyethylene, over which three nozzles were lined up horizontally from where the wax was sprayed at a constant volume (1L·ton⁻¹), resulting in an even application onto the brushes, and then subsequently the fruit surface, as the fruit moved over the brushes. The waxed fruit were then dried in a tunnel at approximately 30°C prior removal from the line, where after fruit were packed in carton boxes for transportation to the laboratory. To ensure no carry-over effect between the different wax treatments, a waiting period of ten minutes existed following each switch to the next wax treatment, during which lemon fruit were send over the production line but was not included in the trial. After the application and drying of the different wax treatments, the fruit were transported to the Department of Horticulture at the University of Stellenbosch to commence a storage period of 32d of cold storage at -1°C, where after fruit was moved from cold storage to ambient temperature at 20 ±2°C for seven days to simulate shelf life, to allow for CI symptom development. Data collection, included an assessment of chilling injury incidence, external and internal fruit quality evaluation, as well as flavedo rind pigment analysis as described in Chapter 3 (pg.43-48)

2.2.4. Statistical analysis.

The data analysis proceeded by means of a one-way ANOVA in Statistica 13 VEPAC module (TIBCO Software Inc., 2018) with wax type as the independent variable. The Fishers least significant difference (LSD) posthoc test, at a 5% significance level, was used for mean separation between the treatments. Correlation analysis was done using Statistica 13 VEPAC module (TIBCO Software Inc., 2018) to calculate spearman's coefficient.

3. Results

3.1. Cold storage temperature regimes

3.1.1. *Percentage chilling injury.* During the 2017 season, no interaction occurred between storage duration and temperature as main effects with regards to CI ($p=0.4120$), as a result, the data was analyzed separately (Fig. 2.1A; B). Results clearly indicate that a higher CI % was sustained by fruit stored at lower temperatures, regardless of storage duration (Fig. 2.1B) and an exponential increase in CI% was sustained when the fruit was cold stored for a longer duration (Fig. 2.1A). In 2018, CI development was more severe than in the previous season, whilst in addition, interaction between temperature and storage duration was reported (Fig. 2.1C). At each storage temperature, CI% increased progressively with extended storage duration, which became evident especially after 20 days, even at the higher storage temperature of 2°C and 7°C. The CI% was most pronounced at the lowest storage temperature of -1°C, with approximately 80 % more CI reported than in fruit stored at 7°C. At storage temperatures of 1°C and above, the CI % showed a more gradual trend of increase in although a definite acceleration in the onset of CI was evident after 30 days (Fig. 2.1C). When comparing the seasons, the CI at 40% for fruit stored at -1 °C during 2017 was much lower compared to the 98 % CI experienced in fruit for the 2018 season, at the same temperature (Fig. 2.1).

3.1.2. *Respiration rate.* The respiration rate was very low during cold storage (Fig. 2A). However it increased drastically within 24 h after transfer to ambient temperatures (20 °C \pm 2°C) (Fig. 2B). Throughout cold storage, the lower temperature treatments of -1°C and 2°C resulted in reduced respiration compared to fruit stored at 7 °C. Fruit were kept at ambient temperature of the entire storage period, to serve as a comparative value indicative of progressive non-stored lemon fruit respiration. This fruit showed a constant respiration rate, but with a decreasing trend with increased fruit maturity, compared to that of fruit after seven days of shelf life at ambient temperature. Yet, the following transfer to ambient temperature from the cold rooms, a significant lower respiration rate was reported for fruit stored at 7 °C, compared to that of fruit stored at either -1 °C or 2 °C occurred after 24 hours.

3.1.3. *Rind colour and pigment content.* Storage temperature and duration significantly affected the rind colour (Fig. 4). Fruit stored at higher temperatures (7°C) developed a more intense yellow rind colour as was evident in the lower negative Hunter *a/b* ratio, compared to fruit stored at lower temperatures such as -1°C, where a greener colour was reported. However, in general, a trend was observed of increased yellow rind colour with increased storage duration, for all treatments (Fig. 4A; B). The rind colour development was supported by the pigment content results of the flavedo. A more prominent yellow colour was more due to chlorophyll degradation than carotenoid synthesis (Fig. 4). The chlorophyll content of the rind was observed to decrease with extended storage duration, regardless of temperature, whereas the carotene content remained relatively constant throughout the storage duration, except in 2017 when an unexpected decrease in carotenoid content was seen from the first two weeks (Fig 4E). In the 2018 season, no increase in carotenoid content occurred during storage, whilst at low-temperature treatments, pigment content appears

to decrease toward the end of storage, although not significantly so (Fig. 4F). A weak negative correlation (Spearman's coefficient, $r = -0.372$, $p < 0.0001$) was found between chlorophyll content and storage temperature, suggesting that the lower temperature resulted in higher chlorophyll content, thus a slower chlorophyll breakdown during storage. Alternatively, a weak positive correlation ($r = 0.244$; $p = 0.0140$) between flavedo carotenoid content and temperature, indicate a slightly higher carotene content with increasing storage temperature. Furthermore, another weak positive correlation ($r = 0.2019$; $p = 0.0049$) between the CI % and the chlorophyll content of the fruit rind, suggest chilling injured fruit to exhibit a higher chlorophyll content.

3.1.4. *Rind sugar and polysaccharide content.* An interaction was evident between the main effects, storage temperature, and duration, regarding the sugar and polysaccharide content of the rind. The rind sugar content had an overall decreasing trend during cold storage from day 12 to day 42 at all the respective temperatures (Fig. 5A; B). However, an increase in total sugar occurred during both seasons in the initial cold storage duration. In the first season, the polysaccharides also increased before decreasing during the later stage of cold storage (Fig. 5C), which contrasts with the second season which shows a gradual decline from the start of cold storage (Fig. 5D). No consistent trend due to the temperature and storage treatments emerged in the rind sugar content between the 2017 and 2018 seasons. A weak negative correlation was found (Spearman's correlation coefficient, $r = -0.2215$, $p = 0.02667$) between sugar content of the fruit rind and the cold storage duration where the sugar content of the rind decreased with increase in storage duration. However, a weak positive correlation ($r = 0.1989$; $p = 0.0473$) emerged between rind sugar content to be present in the rind at higher storage temperatures.

3.1.5. *Internal quality.* An interaction was evident in the juice % of fruit evaluated during the 2017 season and in the citric acid %; TSS/TA ratio and TSS % in the fruit pulp sampled in the 2018 season (Table 2.1). Fruit stored at $-1\text{ }^{\circ}\text{C}$ had a lower citric acid percentage than fruit stored at higher temperatures, whilst in general, no clear trend regarding the citric acid % was noted during storage regardless of season (Table 2.1; 2.2). In both seasons, the % total soluble sugars (TSS) in the pulp, was lower in fruit stored at lower storage temperatures than fruit stored at higher temperatures, (Table 2.1; 2.2), no continuous trend could be witnessed during different storage duration regarding this parameter in the 2018 season (Table 2.1; 2.2). No clear trend was evident regarding the TSS/TA ratio regarding season and main effects (Table 2.1; 2.2). No significant difference was evident when evaluating the juice % during 2017 with temperature and storage duration (Table 2.1). During 2018, a decline in juice % was witnessed after 42 days in storage at $-1\text{ }^{\circ}\text{C}$ (Table 2.2). No other difference in juice % regarding storage temperature or duration as main effects was witnessed during 2018.

3.1.6. *Wax coatings with cold storage regimes.* No interaction was evident between the wax coating treatment, the storage duration, and storage temperature as main effects regarding CI%. Thus these parameters were evaluated separately (Fig. 6.1). There was however, an interaction between wax coating treatment and storage duration (Fig 6.1A) and also between wax treatment and storage temperature (Fig 6.1B). The wax-coated fruit sustained significantly less CI at the low storage temperature $-1\text{ }^{\circ}\text{C}$ and retained a higher quality during longer storage durations (Fig 6.1A) compared to non-waxed fruit. A significant interaction was evident between wax treatment, temperature, and storage duration regarding the rind colour of the fruit. The fruit coated with commercial wax had a yellower rind colour after storage than the fruit that did not receive any wax (less negative Hunter a/b ratio) (Fig. 6.2).

3.2. Post-harvest wax applications for control of chilling injury (CI)

3.2.1. *Wax coatings as affecting CI and quality parameters.* Significant differences were achieved with regards to CI when different wax coatings were applied (Fig. 7A; B; Fig. 8). The fruit coated with a higher amount of solid percentage wax developed less CI symptoms during the 2017 season, this trend was also demonstrated in 2018, although not as effectively as in 2017 as during 2018, where the overall CI% was much lower, giving rise to smaller differences between treatments, however, significant differences were still witnessed between the control and lowest wax solid content (14%) and the double application of the higher solid waxes (Fig. 7B). In the first season, there was an 80 % reduction in CI in the 2x 20% NAT+TBZ treatment (double wax-coated fruit) compared to the non-waxed control (Fig. 7A). Fruit treated with 14% wax did not differ from the control treatment that did not receive any wax treatment (Fig. 7A; B). Fruit treated with 18 and 20 % solids wax sustained less CI compared to control fruit with no wax, regardless of season, with the exception of fruit from the 18% Natural wax + TBZ treatment in 2017. When considering the colour development of the fruit, control fruit (No wax) after storage, had the most intense yellow rind colour (least negative Hunter a/b ratio) and as the solid percentage of the wax increased, a decrease in the ratio was seen (Fig. 7C; D). The rind colour of the control fruit was more intense after storage than at harvest (Fig 7C; D). This finding is supported by the pigment content of the fruit rind harvested in 2017, where the chlorophyll and carotenoid content of the fruit with no wax was significantly less than that of the fruit rind with the double wax application (Fig. 9). This suggests that a greener and less yellow rind colour associated with an increase in solids in the wax. However, in the 2018 season, chlorophyll content did not differ significantly between treatments, whilst a higher carotenoid content was recorded in non-waxed fruit compared to the 2x 20%+TBZ coated fruit (Fig 9). Significant higher moisture loss was recorded for the control, non-waxed fruit in 2018 (data not collected during the 2017 season) at the 10% confidence level compared to wax-treated fruit, supporting the observed lower CI lesion development in wax fruit, particularly those with increased solids (Fig. 8).

3.2.2. *Internal quality parameters.* Differences between the different wax treatments regarding the internal fruit quality such as citric acid percentage as well as total soluble sugars of the pulp (%) and TSS/TA ratio of the pulp and juice % exists but not in a definite trend (Table 3)

3.2.3. *Natural vs. synthetic waxes.* In the 2017 season, no significant differences in CI were obtained between fruit coated with synthetic waxes (SYN) and those treated with natural waxes (NAT), in 2018 the fruit coated with synthetic waxes developed a lower CI% (Fig. 10). When considering colour development, the natural waxes resulted in a more desirable colour than that of the fruit with the synthetic wax coating during the 2017 season. However no significant difference was obtained between the treatment categories during the 2018 season (Fig. 10).

4. Discussion

4.1. *Temperature role.* Chilling injury in lemon fruit will increase exponentially with extended storage and lower storage temperature, which is indicative of the interaction of storage duration and temperature on this physiological disorder (Figure 2.1C). It has been reported that citrus fruit will sustain a chilling injury (CI) if stored below 10°C at levels which would not be commercially viable (<10%) in a sustainable export program (Eaks, 1960). However, the positive impact of postharvest wax treatments evaluated during this study does offer lemon producers a mitigating technology to reduce the incidence of chilling injury to acceptable levels. Results on lemon fruit in this study concur with cold storage studies of other susceptible citrus types such as 'Marsh' grapefruit, which also develop a relatively high incidence of CI, seen as pitting when stored at 2 °C. (Chalutz *et al.*, 1985; Lado *et al.*, 2015) Furthermore, and of real interest to the citrus industry, is the

difference in susceptibility between the two seasons also noted in chapter 3 Lemon fruit, harvested from the same orchard, often have noticeable difference in susceptibility between different years, which strongly suggest the important impact of environmental conditions on the rind during fruit development, thus affecting the CI susceptibility of the fruit at harvest (Fergusson *et al.*, 1999; Gonzales-Aguilar *et al.*, 2000). It is thus likely that exposure of citrus fruit during the winter to low temperatures prior to harvest could result in increased CI sensitivity. However, limited studies to date have addressed this aspect in depth.

4.2. *Rind chlorophyll and carotenoid pigments.* Low storage temperatures have an adverse effect on the rind pigmentation of citrus flavedo due to the degradation of carotene pigments during storage, with a resulting paler colour of the cold-stored fruit (Van Wyk *et al.*, 2009; Rodrigo *et al.*, 2013). In addition, higher chlorophyll content noted in the lemon fruit rind stored at lower temperatures, also evident in this study, gives rise to the fruit of a much less attractive, greener rind colour, compared to the fruit stored at higher temperatures. Studies on orange-colored citrus fruit such as 'Or' and 'Odem' mandarins showed the development of a paler rind colour when stored at 3-4°C than with storage at higher 5-8 °C (Tietel *et al.*, 2012). An important finding on 'Palmer Navel' sweet orange was that in this fruit the initial colour prior to storage plays a key role in the eventual colour obtained after storage. This has led to commercial specification on colour at storage, even though some carotenoid degradation and colour loss are expected to take place (Van Wyk *et al.*, 2009). The decline of carotenoid pigments during cold storage documented in citrus and kiwifruit, not only leads to a paler rind colour in the fruit but also to decreased antioxidant activity (Tavarini *et al.*, 2008; Van Wyk *et al.*, 2009; Rodrigo *et al.*, 2013). Carotenoids are known to prevent chilling damage to grapefruit rind, which could explain the reduced content of carotenoids in the flavedo after storage (Lado *et al.*, 2015). However, in our study, the carotenoids and chlorophyll content in the lemon flavedo showed a distinct decline with the onset of and early on during storage, with the exception of carotenoid content in 2018, where after the pigment content showed a relatively constant trend throughout the storage period at all the temperature regimes of -1 °C, 2 °C and 7 °C. This data may suggest the unmasking effect of the yellow pigments responsible for the colour of the lemons, similar than is known about colour development in the banana fruit. Von Loesecke (1929) reported that the total amount of yellow pigments present in the banana rind remains constant during maturation, with a similar amount present in both the ripe and unripe fruit. An unmasking process of the yellow pigments is thus rather possibly responsible for the yellow colouration expected by consumers. Our findings are supported by a study on 'Fino 49' lemons, where rind colouration was due to the chlorophyll degradation (Conesa *et al.*, 2019). Another study also supporting this phenomenon was evident on the pummelo species *Citrus Grandis* 'Goliath' where the carotenoid content of the rind parallels the chlorophyll degradation during storage (Gross, 1983). Furthermore, there is evidence of differences in seasonality that also affected the fruit rind pigments. Chlorophyll and carotenoid content of the rind of fruit harvested during the first season was higher than that of the fruit harvested during the second season. Yet, fruit harvested in the second season had a yellower rind colour, despite a lower carotenoid content. This suggests that the higher chlorophyll in the fruit during the first season masked the carotenoid pigments, therefore displaying a more green colour (more negative Hunter *a/b* ratio) compared to the fruit from the second season (Fig. 2). Temperature is known to have an impact on the pigmentation of fruit rind as areas with a minimum temperature higher than 15 °C, in general, having a more prominent green coloured rind than fruit grown in areas where temperatures regularly drop below 15°C. When though the initial colour of the fruit rind affects the final colouration of the fruit rind, stressful environmental conditions, such as low temperatures, was shown to lead to a paler final colour in lemon fruit (Manera *et al.*, 2012).

4.3. *Total soluble carbohydrate content.* During storage the total rind sugar and polysaccharide content have reported an initial increase during the first 12 days of storage, suggesting the initial accumulation of sugar in the rind via gluconeogenesis, similar to that observed in eggplant fruit (Kozukue *et al.*, 1978). An overall decreasing trend in sugar content occurred throughout the remaining storage period, but with a

more gradual decrease at higher storage temperatures, suggesting that sugars are metabolized via glycolysis to provide the energy for the prevention of damage caused by continuous cold conditions (Perotti *et al.*, 2015). In our study, the degradation of the sugars in the fruit rind during the first season was noticeable steeper than that recorded for the second season. This finding focusses the attention to yet again on the importance of the pre-harvest conditions impacting on the rind physiology on susceptibility to disorders.

4.4. *Respiration rate.* Lemon fruit, like most citrus types, have a low respiration rate which can be further reduced at low temperatures storage (Grierson and Ben-Yehoshua, 1986). Lemon fruit in our study stored at -1°C had an approximately 6 times lower respiration rate ($\text{mgCO}_2\text{-kg}^{-1}\text{h}^{-1}$) compared to fruit stored at ambient temperature (20°C). As storage temperature increased from -1°C to 2°C and again 7°C , the respiration rate increased accordingly. Within 24 h of transfer from cold storage to ambient temperature, a rapid and 2-3-fold increase in respiration rate was witnessed. This agrees with postharvest literature that chilling sensitive fruit subjected to any type of stress have an increased respiration rate, so that the transfer from cold to a warmer temperature, is expected to produce a higher than normal production of CO_2 (Eaks and Morris, 1956; Eaks, 1961; Grierson, 2006). It is during this period that cellular damage leads to oxidation, and visible symptoms emerge on the rind as darker areas of damaged tissue (Eaks, 1960). The higher observed respiration rate after 20h at ambient temperature for fruit stored at -1°C thus correlates with the higher CI recorded, whereas the CO_2 produced at ambient temperature by the fruit stored at either 2°C and 7°C was much more gradual, suggesting less CI. The respiration in fruit stored at -1°C increased to such an extent that at some point it exceeded that of the fruit stored at 7°C by approximately 70 %, suggesting significantly more severe cellular damage in fruit stored at the sub-zero temperature and with a higher energy requirement for tissue repair. This accelerates respiration rate over a longer duration for fruit stored at -1°C is then likely to result in more severe CI symptoms (Kader and Saltveit, 2003). The rind and pulp of citrus fruit are considered to be two separate developing units, each reacting differently to the gas exchange, where the flavedo absorbs up most of the oxygen (O_2), then followed by the albedo and lastly the juice sacs absorbing the least amount of O_2 . This higher oxygen absorbance would support a higher respiration rate, making the fruit rind responsible for contributing most to the total respiration of the fruit (Hussein, 1944). Furthermore, dark green lemons have been reported to have a higher respiration rate compared to mature, lighter green fruit (Bartholomew and Sinclair, 1951). Further evaluation of the respiration rate of fruit subjected to low-temperature storage regimes should extend the period where respiration rate is recorded from 48h to 72h following transfer to ambient temperature. Beyond 72 hours is not recommended as a high level of decay that is expected to develop will compromise results.

4.5. *Fruit quality parameters.* In our study, the citrus pulp and rind showed no relationship between the internal parameters and CI susceptibility, for any treatments, in either of the two seasons. This supports the concept that the internal and external development of the fruit is physiologically distinctly separate units, independent of one another so that the external appearance of the fruit provides no indication of the internal characteristics of the fruit (Tadeo *et al.*, 2008). In our study, citric acid was lower after storage at -1°C compared higher storage temperatures. However no physiological link could be found with higher CI. Low-temperature storage has also been reported to negatively impact on the flavour of 'W. Murcott' and 'Owari' mandarin, mostly due to a decline in titratable acidity (TA) which plays a key role in the flavor development of the mandarin fruit (Eaks, 1961; Obenland *et al.*, 2011). The excessively high acid content of lemons, however, makes some decline in TA less. Previous studies have shown that the TSS of lemon as well as 'Valencia' orange fruit increases at higher storage temperatures (Eaks, 1961; El-Zeftawi, 1976; Obenland *et al.*, 2011), this finding was supported by results obtained from this study on lemon fruit. 'Valencia' oranges stored at 5°C showed a higher juice % than fruit stored at 15°C as higher temperatures are associated with higher moisture loss (El-Zeftawi, 1976). This finding could not be confirmed in our study as fruit stored at a

higher temperature of 7°C had more juice % than fruit stored at -1°C.

4.6. *Wax treatments with cold storage.* Results for CI that emerged on comparing wax and non-waxed fruit showed that fruit treated with an 18% solid wax had far less CI symptom development of 26% at -1 °C, compared to a 42% CI development in non-waxed fruit stored at the same temperature. Untreated control fruit reached 30% CI after only 12 d in storage at -1°C, whereas waxed fruit only reached similar CI incidence levels after 32 d of cold storage. Thus, the commercial application of the correct wax could effectively increase the postharvest storage duration at low temperatures by approximately two weeks. In general, results indicate that wax-treated fruit can potentially be stored for significantly longer durations. At higher storage temperatures of 2°C and 7°C, the wax-treated fruit did not differ in the CI % sustained when compared to the non-treated fruit. This finding suggests that the wax application only suppresses symptom development but does not prevent the actual cellular damage from occurring at suboptimal low temperatures.

4.7. *Wax formulations.* In the current study, differences in CI % was seen with the various wax formulations, where different % solids in the coating would alter the consistency of the wax and subsequently also its gaseous exchange capacity and susceptibility to possible moisture loss. Our results showed that fruit coated with the highest solid wax coating (20 %) developed the lowest CI%, particular so for the first season. It might, therefore, be hypothesized that wax coatings with formulations that consist of a lower solid percentage, consisting of a lower solid percentage, does not offer sufficient protection to replace the natural wax removed from the fruit during the various washing and handling protocols on the pack line. Alternatively, the reduction in CI % in fruit treated with coatings containing a higher wax content could also be the result of the film preventing CI symptom development, similar to can be seen in cucumber fruit with shrink wrap, allowing for storage at temperatures lower than 10 °C (Dhall, 2011). In cucumber, the shrink film protects the fruit from losing moisture and desiccation, therefore preventing CI symptom development. In a similar way, the wax layer possibly acts to prevent moisture loss from the fruit rind, with waxes containing higher % solids offering better resistance against moisture loss (Dou, 2004). The thickness of the wax layer, along with the effective application thereof, has an influence on the gaseous exchange between the fruit and its environment, thereby also influencing possible desiccation of the fruit. As moisture loss in itself is not a direct cause of CI, but merely an indicator and result of symptom development, the relevance of not only selecting the best wax but also the correct application methodology, effectively resulted in a suitable barrier against moisture loss, was seen in the double 20% solid treatment (Purvis, 1984; Cohen *et al.*, 1994).

4.8. *Colour development with waxing.* The wax coating has been shown to reduce rind colour development as seen in 'Oroblanco' fruit, resulting in a lower Hunter *a/b* ratio (greener rind colour) associated with a higher % solid wax (Rodov *et al.*, 2000). A possible explanation is that a thicker coating is likely to more resist CO₂ from exiting the rind, which in turn will inhibit chlorophyll breakdown due to the higher CO₂ inhibiting ethylene at the cellular binding sites, reducing the positive impact on chlorophyll breakdown (Miller, 1946). The lower chlorophyll content in the waxed fruit compared to the non-waxed fruit during the second season indicate that the chlorophyll degradation continues, but that the low carotenoid content leads to a paler fruit colour in treated fruit compared to the non-waxed fruit. Alternatively, the lower carotenoid content of the non-waxed fruit recorded in the first season could be due to carotenoid degradation during oxidative stress under conditions that as cellular damage is likely to increase.

4.9. *Thiabendazole.* The fungicide Thiabendazole (TBZ) is known to have a physiological effect in controlling CI symptom development and has also shown to control pitting development in grapefruit (Lyons, 1973). The fungicide can be effectively incorporated into the wax mixture to prevent the development of CI symptoms in citrus fruit (Schirra *et al.*, 2000; Kellerman *et al.*, 2014). In 'Valencia' oranges TBZ was only

effective in preventing postharvest pitting when applied to the fruit via a postharvest dip, as when the fungicide was added to the wax treatment, the efficacy of the wax treatment was reduced (Ehlers, 2016). When added to the wax, TBZ tends to precipitate on the fruit rind and therefore does not penetrate the rind, thereby providing less effective protection (Kellerman *et al.*, 2014).

5. Conclusion.

Results from this study show that various factors, including those of storage temperature and duration of storage, and postharvest applied coatings, interact during postharvest handling to influence fruit susceptibility to CI. Long periods of cold may negatively impact on the tolerance of the fruit to CI by affecting the physiological condition of the rind through a reduction in pigments such as carotenoid, rind sugars, and moisture loss. To protect the fruit from developing a chilling injury, fruit can be coated with wax types containing high percentages of solids. However this intervention may reduce proper rind colour development in the fruit. The increased production of lemon fruit and access to phytosanitary markets demand low-temperature shipments, with its associated risk of induced chilling injury. This study provides a better understanding of the rind physiology when developing chilling injury. Wax application is identified as an available technology to reduce the negative impact of postharvest stresses on fruit rind and thus fruit quality, although it will not prevent the physiological processes driving this disorder. Furthermore, future studies and postharvest protocols should aim to understand and reduce exposure of the fruit to possible stresses through rigorous control throughout the cold chain

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TABLES

Table 1. Composition and application rate of various wax treatments used to evaluate their efficacy on 'Eureka' lemon fruit to reduce the incidence of chilling injury during long-term cold storage.

Treatment	Wax name (%solid content)	Type of wax	Coating composition	Application (L. ton ⁻¹)	TBZ concentration (mg·L ⁻¹)
1	Control				
2	Endura-Fresh™ Natural (14%)	Natural	Water; vegetable-based wax & resin; vegetable-derived fatty acid salts; shellac	1	
3	Endura-Fresh™ QDP (14%)	Synthetic	Water, polyethylene coating; vegetable-derived fatty acid salt; shellac; petroleum based; sorbitan fatty acids	1	
4	Endura-Fresh™ Natural (18%)	Natural	Water; vegetable-based wax & resin; vegetable-derived fatty acid salt; shellac based	1	
5	Endura-Fresh™ QDP (18%)	Synthetic	Water, polyethylene coating; vegetable-derived fatty acid salt; shellac based; petroleum based; propylene glycol	1	
6	Endura-Fresh™ QDP (18%)	Synthetic	Water, polyethylene coating; vegetable-derived fatty acid salt; shellac based; petroleum based; propylene glycol	1	2000
7	Endura-Fresh™ Natural Supreme	Natural	Water; vegetable-based wax & resin; vegetable-derived fatty acid salt; shellac based	1	
8	Endura-Fresh™	Natural	Water; vegetable-based wax & resin; vegetable-derived fatty acid salt; shellac based	1	2000

9	Natural Supreme Endura-Fresh™ Natural Supreme	Natural	Water; vegetable-based wax & resin; vegetable-derived fatty acid salt; shellac based;	2	4000
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Table 2.1. Internal parameters (% citric acid; TSS/TA ratio; % juice; %TSS) of 'Eureka' lemon fruit from 2017 season when stored at -1°C, 2°C and 7°C for the duration of 12, 22, 32 and 42 days respectively. No significant interaction was evident between the main effects, temperature, and storage duration regarding the parameters in the table below, and the influence of the main effects on these parameters could be analyzed separately. Fruit during the 2017 season were stored at three different temperatures, -1°C, 2°C and 7°C as opposed to fruit during the 2018 season which were stored at five different temperatures (-1°C, 0°C, 1°C, 2°C and 7°C) respectively.

	2017			2018
	Citric acid percentage	TSS/TA Ratio	TSS in pulp (%)	Juice %
<u>Temperature^X</u>				
-1°C	5.90b ^Z	1.22 ^{NS}	7.21b	40.32 ^{NS}
0°C				40.19
1°C				41.46
2°C	6.12a	1.21	7.38a	41.01
7°C	6.19a	1.20	7.45a	41.09
<u>Duration^Y</u>				
0	6.26a	1.27a	7.52a	40.75 ^{NS}
12	6.03b	1.21c	7.29b	41.57
22	6.26a	1.21c	7.54a	40.05
32	6.06b	1.20c	7.25b	40.89
42	5.93b	1.23b	7.30b	40.75
<i>p-value</i>				
<i>Temperature</i>	0.0002	0.1981	0.0003	0.4346
<i>Duration</i>	0.0011	0.0855	0.0002	0.3162
<i>Temperature x Duration</i>	0.4760	0.7970	0.1251	0.2470

^X Average of the various internal quality parameters over various durations at the different storage temperature

^Y Average of the various internal quality parameters in various storage temperatures at the different storage durations.

^Z Different letters denote the differences between the means on a 5% significance level as calculated by Fishers LSD

^{NS} Shows that there are no differences between the treatment means on a 5% significance level

Table 2.2. Internal parameters (% citric acid; TSS/TA ratio; % juice; %TSS) of 'Eureka' lemon fruit from 2017 season when stored at -1°C, 2°C and 7°C for the duration of 12, 22, 32 and 42 days respectively. Significant interaction occurs between the temperature and storage duration regarding these parameters. Fruit during the 2017 season were stored at three different temperatures, -1°C, 2°C and 7°C as opposed to fruit during the 2018 season which were stored at five different temperatures (-1°C,0°C,1°C,2°C and 7°C) respectively.

Storage duration	2017 Juice %					2018 Citric acid (%)					2018 TSS in pulp (%)					2018 TSS/TA ratio				
	0	12	22	32	42	0	12	22	32	42	0	12	22	32	42	0	12	22	32	42
Temperature ^x	39.29a ^z	38.46a	42.84a	37.52a	30.46b	6.34a	5.59c	6.12b	5.64b	5.74b	7.78a	7.39a	7.11c	7.43a	7.45a	0.82b	0.76c	0.86a	0.76c	0.77c
-1°C																				
0°C																				
1°C																				
2°C	39.29a	44.25a	39.53a	39.64a	40.75a	6.34a	5.43c	5.86b	5.96b	6.00b	7.78a	7.26b	7.59a	7.69a	7.18b	0.82b	0.75c	0.77c	0.78c	0.84a
7°C	39.29a	39.94a	37.27a	40.43a	37.15a	6.34a	5.87b	5.87b	5.77b	5.55c	7.78a	7.43a	7.60a	7.27b	7.27b	0.82b	0.79c	0.77c	0.85ab	0.76c
<i>p-value</i>																				
Temperature			0.0012					<0.0001					0.0507							0.0001
Duration			0.0005					0.0856					0.0004							0.0004
Temperature x Duration			<0.0001					<0.0001					<0.0001							<0.0001

^x Average of the various internal quality parameters over different durations at a specific temperature (-1°C,0°C,2°C,7°C)

^y Average of the various internal quality parameters at different storage temperatures during a specific duration in storage (12,22,32,42d)

^z Different letters refers to difference in treatments on a 5% significance level and mean separation with Fishers LSD test.

Table 3. Juice percentage (%) of the pulp of 'Eureka' lemon fruit when treated with different wax types, differing in their % of solids during the 2017 and 2018 season before storage (Day 0) and after cold storage at -1°C for 32 days and subsequent shelf-life period of seven days

Season	2017				2018			
	Citric acid (%)	TSS (%)	TSS/TA ratio	Juice (%)	Citric acid (%)	TSS (%)	TSS/TA ratio	Juice (%)
<i>Wax treatment</i>								
Day 0	5.94a	7.52de	1.27f	39.29d	6.01ab	7.59e	1.25e	34.52e
No wax (Control)	5.31bc	7.77c	1.41d	40.61cd	6.01ab	7.87ab	1.36bc	41.85b
14% NAT	5.82a	7.48de	1.29f	38.51d	5.84cde	7.31f	1.25e	39.00c
14% SYN	5.67ab	7.62cd	1.35e	46.33ab	5.85cde	8.37a	1.43a	34.45e
18% NAT	5.32cde	8.38a	1.58a	43.80abc	5.72e	4.47ef	1.31d	36.24de
18% SYN	5.76a	7.51de	1.30ef	44.04abc	5.79de	7.61e	1.31d	36.85d
18%SYN + TBZ	5.36cd	7.76c	1.45cd	44.95abc	5.82de	7.97bc	1.37b	40.06bc
20% NAT	5.27de	8.03b	1.52ab	42.55bcd	6.05a	8.38a	1.38b	45.60a
20% NAT + TBZ	4.98f	7.36e	1.48bc	47.79a	5.90bcd	7.68de	1.30d	39.38c
2 x (20% NAT + TBZ)	5.13ef	7.76c	1.52ab	47.14ab	5.97abc	7.87cd	1.32cd	36.45de
<i>p-value</i>								
<i>Treatment</i>	<0.0001	<0.0001	<0.0001	< 0.0001	0.0015	<0.0001	<0.0001	<0.0001

^x Average of the juice percentage of the different treatments

^z Different letters denote the differences between treatment means on a 5% significance level as calculated by Fishers LSD

FIGURES

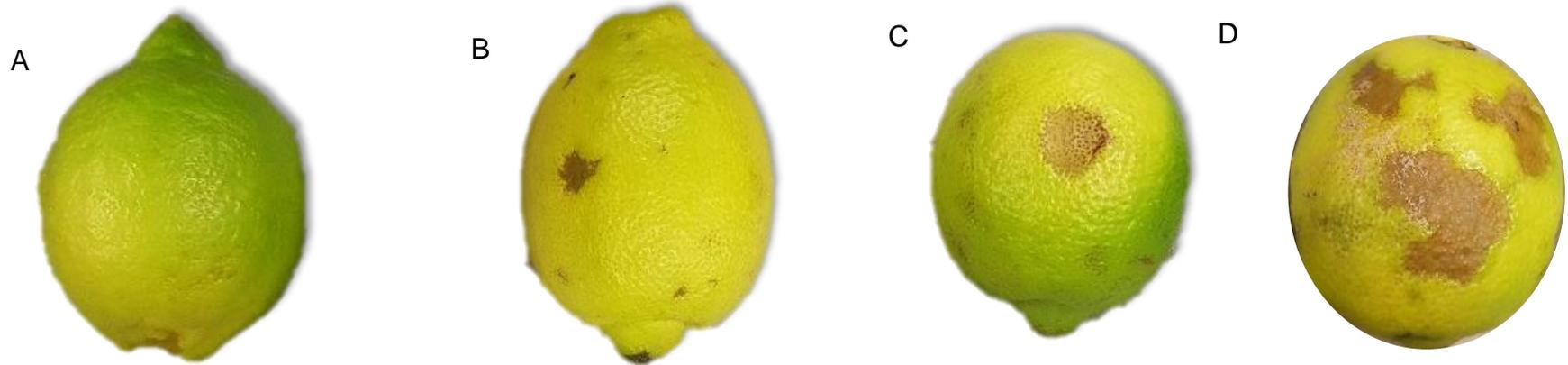


Figure 1. Chilling injury scores assigned to 'Eureka' lemon fruit on the basis of the severity of the injury sustained. Fruit are assigned a number 0-3, depending on the severity of the injury of the individual fruit. A = no injury sustained, fruit is still commercially viable, with an injury score of 0; B= small injury sustained or initiation of an injury visible, but still consumer acceptable and commercially viable, with a score of 1; C= Large area of the flavedo is affected, therefore not commercially viable anymore, with a score of 2; D= Most of the fruit flavedo is affected and the fruit is totally not commercially viable, with a score of 3.

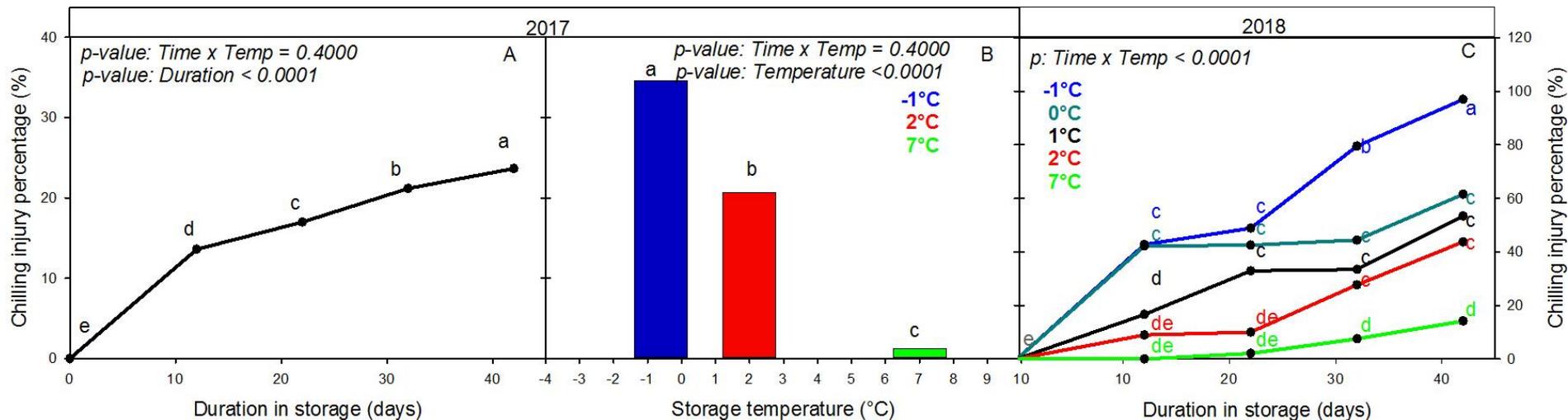


Figure 2.1. Chilling injury percentage (CI %) of the 'Eureka' lemon fruit rind stored at -1°C, 2°C and 7°C during the first season and -1°C, 0°C, 1°C, 2°C and 7°C during second for 0, 12, 22, 32 and 42 days respectively. Graphs A, C, E, and G shows the results from the first season (2017) and graphs B, D, F and H shows the chilling injury percentage of the fruit harvested during the second season (2018). Different letters denote the difference between the treatment means on a 5 % significance level as calculated by Fishers LSD. NS refers to the non-significant differences between the different treatment means on a 5% significance level.

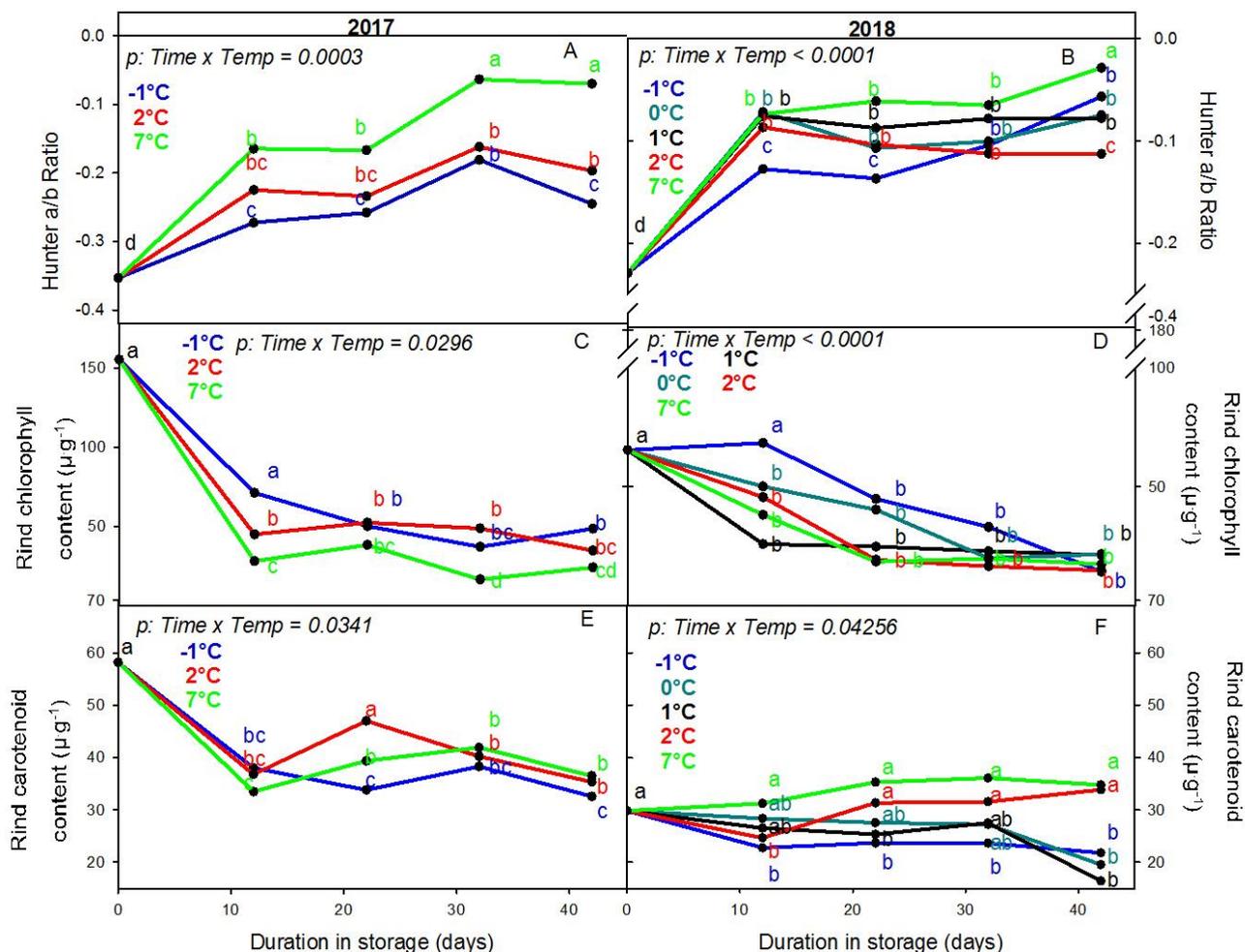


Figure 2.2. Rind colour (Hunter *a/b* Ratio) and pigment content (carotenoid and chlorophyll) expressed as $\mu\text{g}\cdot\text{g}^{-1}$ dry weight (DW) of 'Eureka' lemon fruit following storage at temperatures of -1°C , 2°C and 7°C during the 2017 season (Figs) and that of -1°C , 0°C , 1°C , 2°C and 7°C during 2017 season (Fig) for 0, 12, 22, 32 and 42 days respectively in both seasons. Different letters denote the difference between the treatment means on a 5 % significance level as calculated by Fisher's LSD test. NS refers to the non-significant differences between treatment means on a 5% significance level.

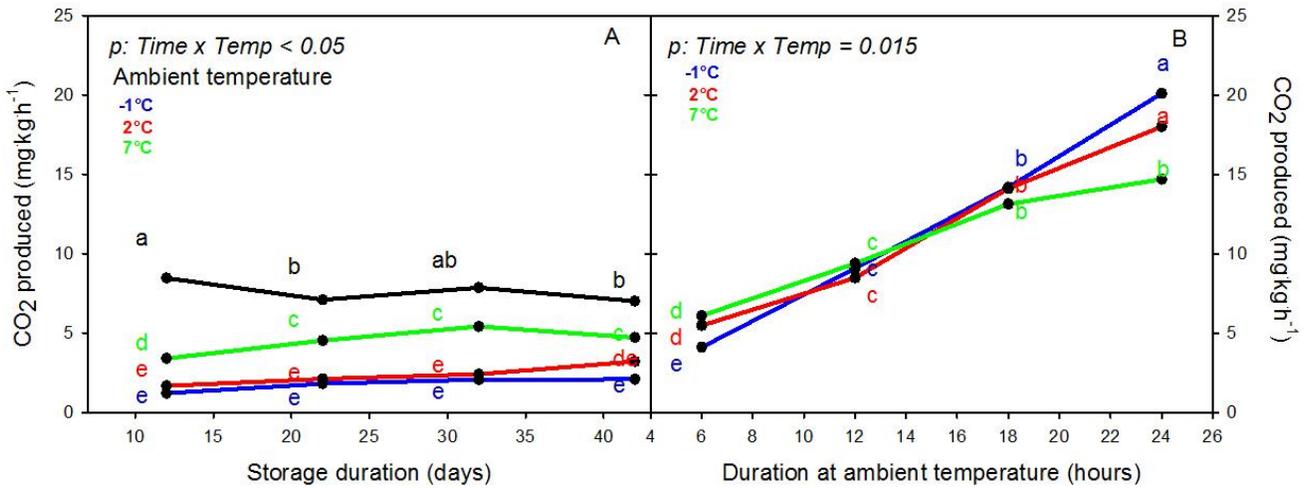


Figure 3. Respiration rate ($\text{mg}\cdot\text{kg}\cdot\text{h}^{-1}$) of 'Eureka' lemon fruit during storage at temperatures of -1°C , 2°C and 7°C for 12, 22, 32 and 42 days (A) respectively and following 6, 12, 18, 24 hours respectively when held at ambient temperature ($20^{\circ}\text{C} \pm 2^{\circ}\text{C}$) after removal from cold storage (B). The different letters represent the difference between the treatment means on a 5% significance level as calculated by Fishers LSD test.

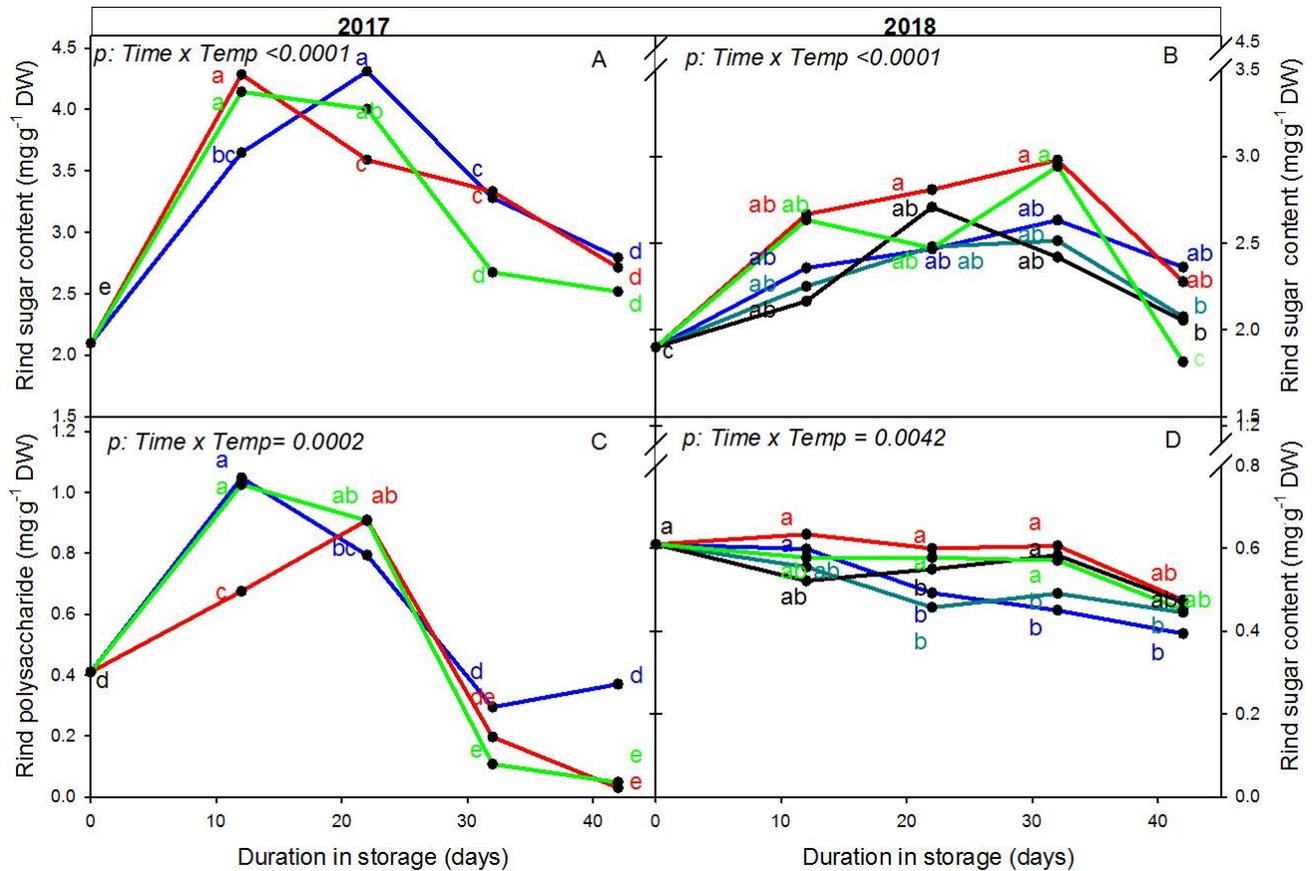


Figure 4. The rind sugar (A; B) (expressed as glucose equivalents in mg·g⁻¹ DW) and polysaccharides (C; D) content of 'Eureka' lemon fruit following storage at -1°C, 2°C and 7°C during the 2017 season (A; C) and -1°C, 0°C, 1°C, 2°C and 7°C during the 2018 season (B; D) for 12, 22, 32, 42 days respectively during both seasons. The different letters denote the difference between treatment means at a 5% significance level as calculated by Fishers LSD test. An interaction was evident between the storage duration and storage temperature as main effects regarding rind sugar and polysaccharide content.

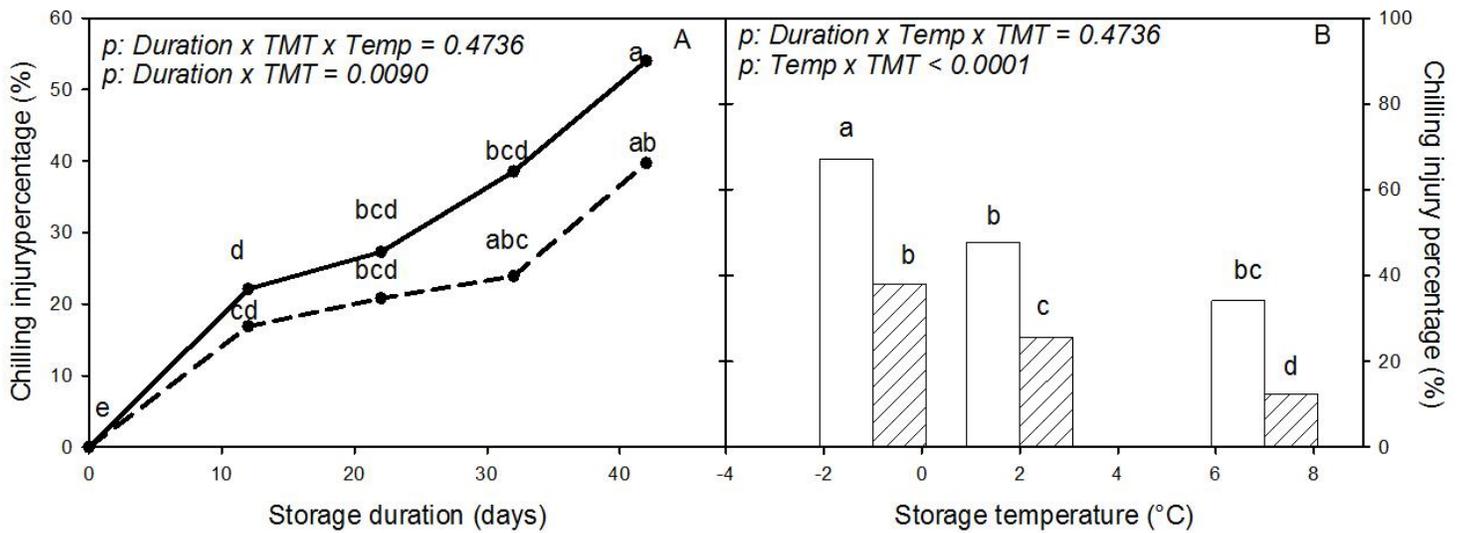


Figure 5.1 Chilling injury percentage (CI%) of waxed fruit (broken line-A; striped bar-B) sustained following various storage durations and a further 7-day shelf life period at ambient temperature of $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ (A) and at different storage temperatures (B). No interaction was evident between the treatment, storage duration and storage temperature regarding CI%. Thus these parameters were evaluated separately in graphs A and B. The different letters on the respective graphs represent the difference between treatment means on a 5% significance level as calculated by Fisher's LSD posthoc test.

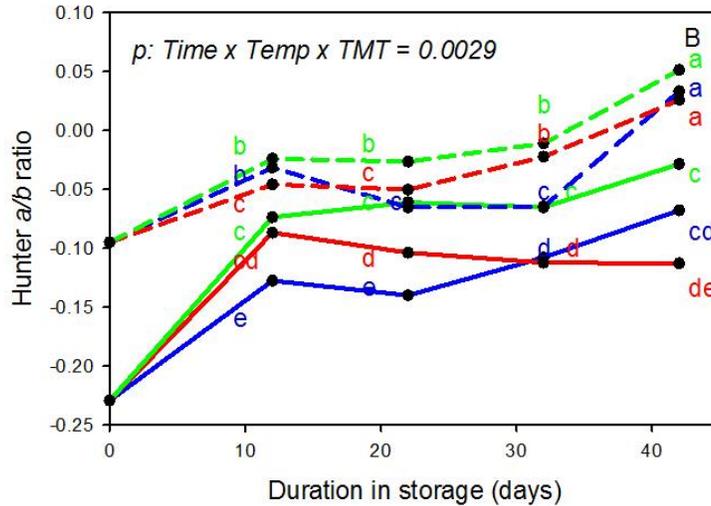


Figure 5.2. Rind colour of 'Eureka' lemon fruit expressed as Hunter a/b ratio of waxed (broken line) and non-waxed (solid line) fruit when stored at -1°C (blue); 2°C (red) and 7°C (green) for 32 days and following a shelf life period of seven days at ambient temperature of $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$. The different letters represent the difference between treatment means on a 5% significance level as calculated by Fisher's LSD posthoc test.

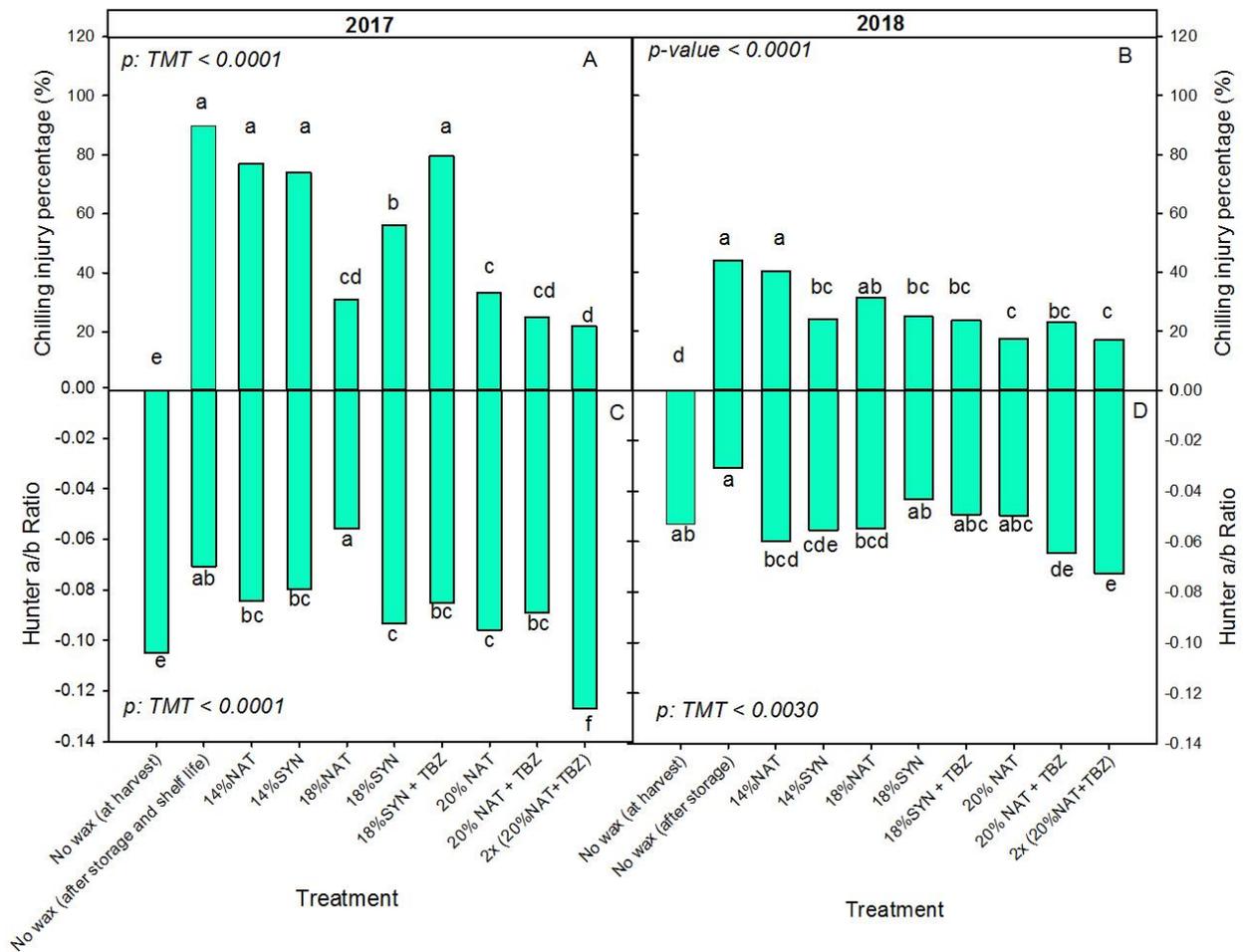


Figure 6. Chilling injury percentage (CI %) (A; B) and rind colour, expressed as Hunter *a/b* ratio (B; C), of 'Eureka' lemon fruit, harvested in 2017 and 2018, where after the fruit was treated with various waxes, differing in solid percentages, and scored for CI as well as rind colour evaluation following cold storage at -1°C for 32 days and a seven-day shelf life period at ambient temperature (20°C +/-2°C). The different letters represent the difference between the treatment means on a 5% significance level as calculated by Fishers LSD posthoc test.

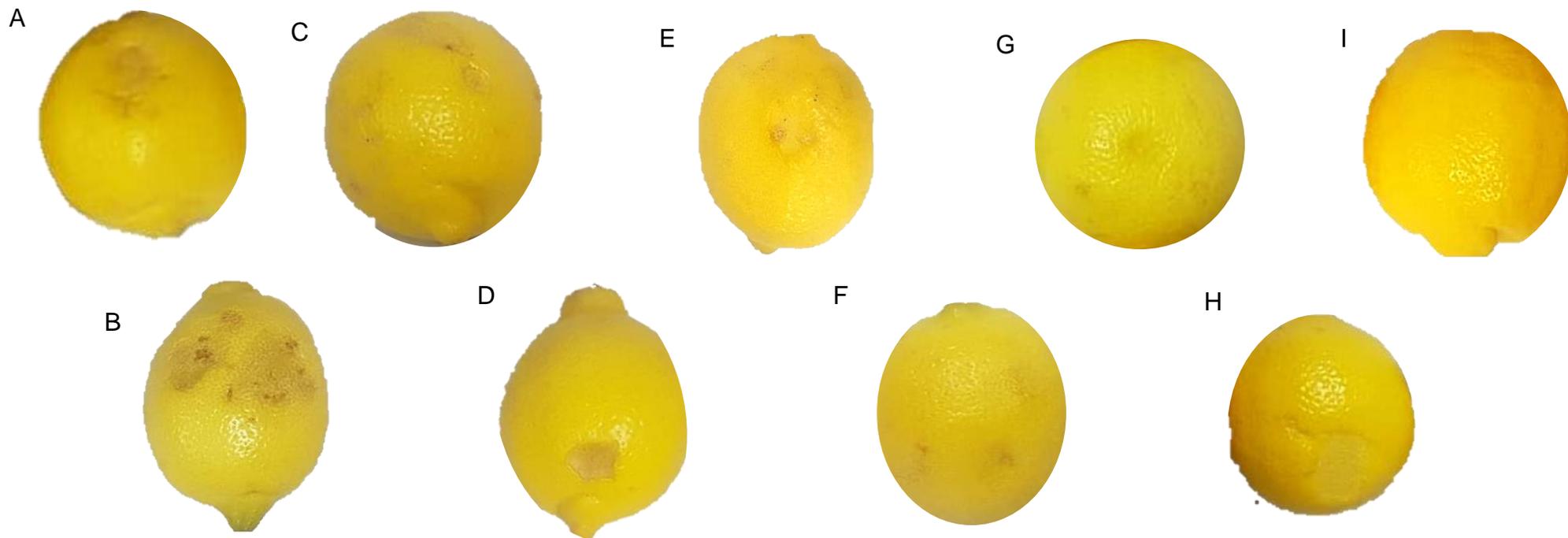


Figure 7. Chilling injury percentage (CI %) of 'Eureka' lemon fruit treated with different wax coatings, consisting of various solid percentages (A- No wax; Low solid waxes, B-14%NAT and C- 14%SYN; high solid waxes, D- 18% NAT, E- 18% SYN, F- 18% SYN+ TBZ, G- 20% NAT; H- 20%NAT+TBZ and I- a double application of high solid wax) consisting of different solid percentages and cold-stored for 32 days at -1°C . Pictures showcase the symptoms developed following cold storage for 32 days at -1°C and subsequent seven days at ambient temperature. The solid content of the waxes thus increases from A to I, with A showing the control which did not receive any treatment, and I illustrating the double wax application.

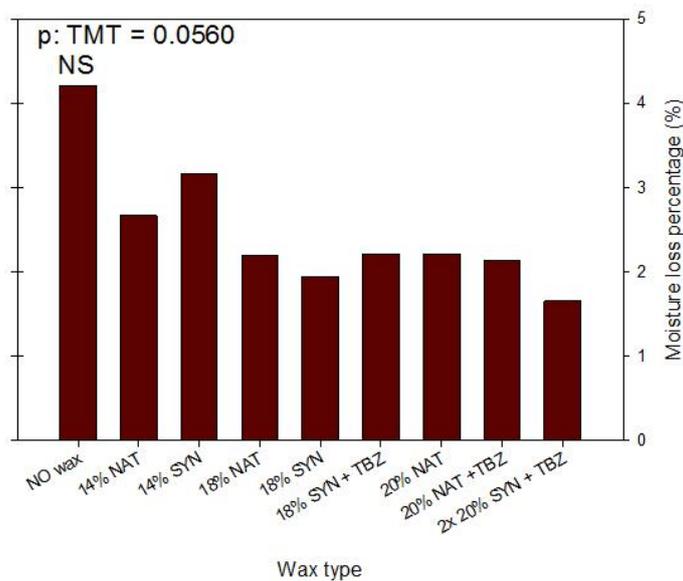


Figure 8. Moisture loss of treated fruit in 2017 is presented. This was measured following cold storage period and a subsequent shelf-life period of seven days at ambient temperature, except for the control fruit which did not receive any was treatment. NS refers to no difference between treatment means on a 5% significance level as evaluated by Fisher's LSD posthoc test.

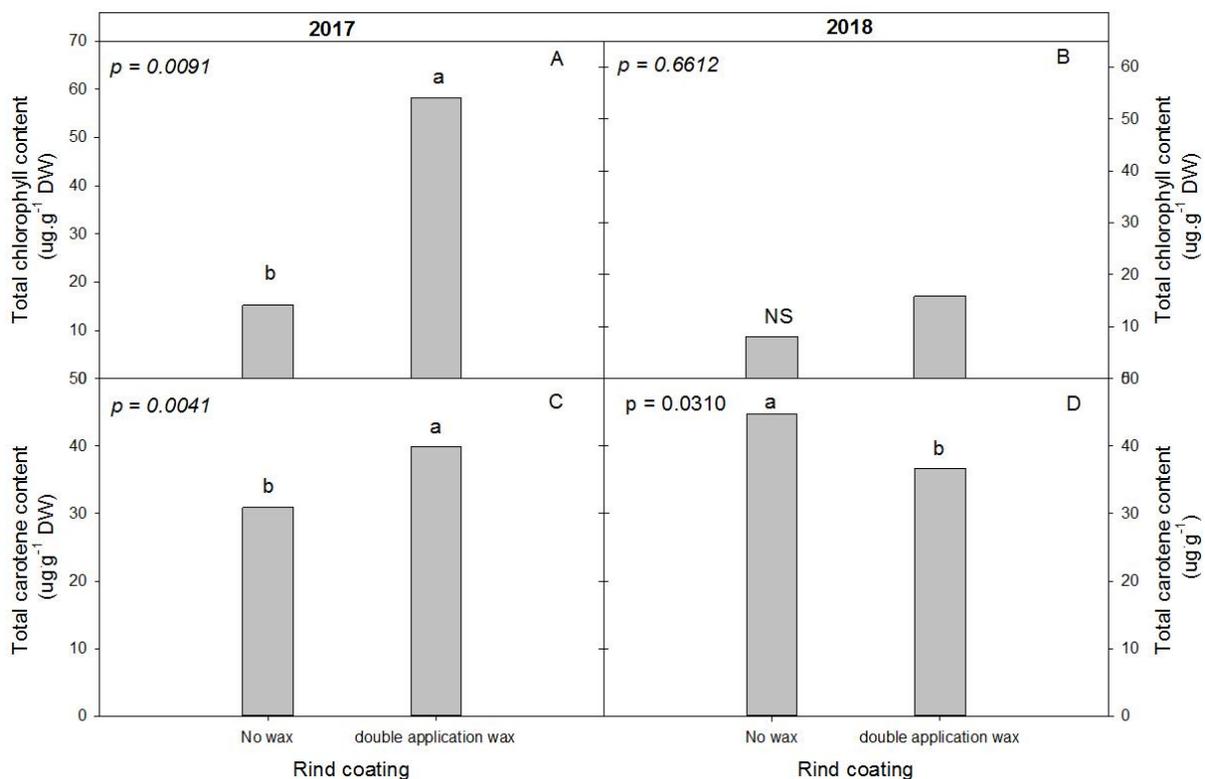


Figure 9. 'Eureka' lemon fruit rind pigment content of carotene and chlorophyll expressed as $\mu\text{g}\cdot\text{g}^{-1}$ dry weight (DW) of fruit that received either a double application of the high solid wax treatment or no rind coating prior to storage at -1°C for 32 days. Flavedo pigment analysis was done after subsequent shelf-life conditions for seven days, following the cold storage period. Different letters represent the statistical differences between treatment means as calculated by Fisher's LSD posthoc test.

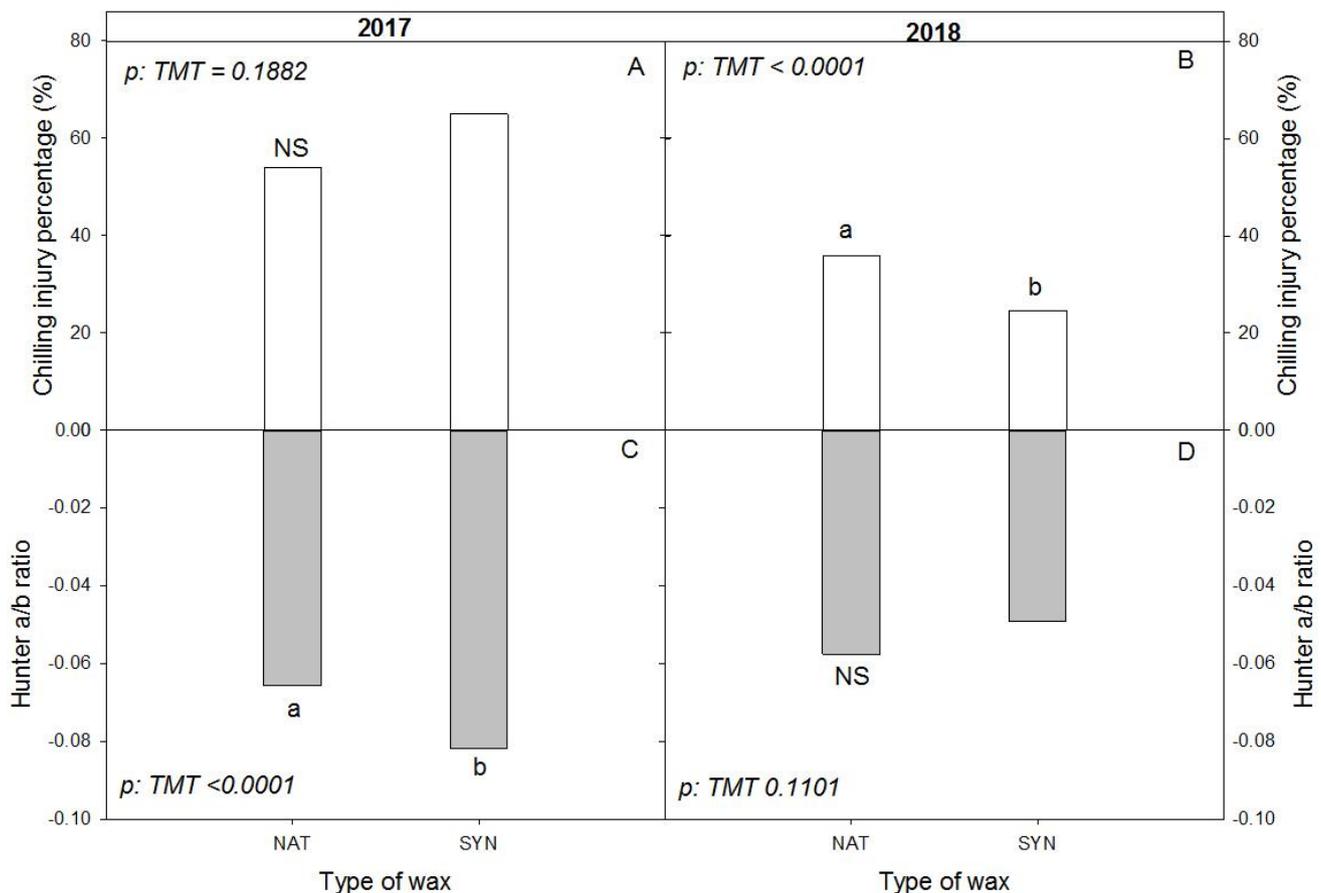


Figure 10. Chilling injury percentage (%) and rind colour (Hunter a/b ratio) of 'Eureka' lemon fruit treated with natural and synthetic wax types during the 2017 and 2018 seasons and stored at -1°C for 32 days and for a further subsequent 7 days at ambient temperature $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Different letters represent the difference between the treatment means on a 5% significance level as calculated by Fisher's LSD posthoc test.

Conclusion and final recommendation

Understanding the contribution of factors influencing the fruit rind physiology and the tolerance to CI is important, however from a horticultural industry point of view, it is vital to develop methods to prevent the development of the disorder below critical commercial levels. In terms of postharvest treatments, wax application at higher solid percentage and volume is critical. It is recommended in general that the solid content of the wax must be above 18% and higher application rates could be applied. The fungicide, TBZ, is known to decrease the development of chilling injury, however the prevention is much more effective when the fungicide is applied to the fruit via drench before waxing as the fungicide has shown to precipitate and not offer uniform coverage when applied with the wax (Kellerman, 2014; Ehlers, 2016). Thus, when evaluating pre-harvest factors, it might be possible to predict the outcome of the fruit during postharvest stress and thus counter the development of physiological disorders to ensure that all the fruit including the tolerant and susceptible fruit are exported under cold storage regulations without any disorders. The goal is to treat sensitive fruit to reduce their sensitivity to allow delivery in the market without any symptom development.

Technology transfer

- Pre-season Packhouse workshops: 2018 and 2019.
- CRI Citrus Research Symposium, 2018 August.

5.2.3 PROGRESS REPORT: Postharvest fruit quality in mandarin, lemons and navels oranges under shade netting

Project 1207 (2018/19-2019/20) by Paul Cronje, Sean Moore, Wayne Kirkman, Jade North (CRI), Johane Botes (SRCC/CRI), Bahlebi Kibreab Eiasu and Phillipine Moabelo (University of Fort Hare, Alice Campus) [*project to be changed in 2019 season to PHI-4-12*]

Summary

Fruit quality of navel, lemons and mandarin fruit during commercial production under shade netting was qualified to determine if the shade had a negative impact on any quality aspect such as colour, internal quality or chilling susceptibility. The preliminary data from the first season did not indicate a problematic influence of shade netting under these conditions. The rind colour and internal quality was not considerably affected. Furthermore, the incidence of chilling injury was also not influenced in the orchards during the first season. However, these preliminary results will be further expanded during the second season before any detailed conclusions can be made.

Opsomming

Die vrugkwaliteit van nawels, suurlemoene mandaryne was geëvalueer vir enige invloed agv produksie onder saknette. Die voorlopige data beskikbaar na een seisoene dui op geen waarneembare tendens en negatiewe impak nie. Die skil kleur en interne kwaliteit was nie geaffekteer nie. Verder meer kon daar nie 'n verbad getrek word tussen die voorkoms van koueskade en produksie onder skadunet nie. Daar moet egter op gelet word dat hierdie data slegs die eerste jaar se resultate bevat en na die tweede seisoen sal afleidings gemaak kan word.

5.2.4 **PROGRESS REPORT: Ambient loading and forced air cooling of citrus for cold sterilisation markets. Including: Validation of ambient loading in the False codling moth Management System (FMS) for citrus export to the EU (PHI 3 extension)**

Project number: 1125 (PHI 3-01/2018 and CRI 1219) (2014/15 – 2018/19) by Paul Cronje (CRI-SU) and Tarl Berry (SU)

Summary

Citrus production is expected to increase significantly over the next few years and will place considerable stress on fruit precooling facilities to achieve the required throughput. Additionally, international phytosanitary requirements are expected to become progressively more stringent in the coming years, further increasing the demand for cooling at precooling facilities. Ambient loading is a cold chain approach whereby palletised fruit by-pass a precooling facility and are loaded warm into the reefer container, after which the fruit are cooled within the container. This approach is a promising solution towards reducing stress on facilities, costs and waiting times. However, cooling rate and uniformity is often inadequate. This project thus aims to develop and enhance cold chain technologies and techniques to reduce the strain on cold store facilities at ports. This includes the identification of improved container loading approaches to improve cooling performance in containers. The study further explores the use of rail networks that allow citrus fruit to bypass precooling facilities. Finally, the project will explore the integration of conventional vessels into the FMS program, which may also be an aid in reducing pressure at ports. To date the project has met all its expected milestones. The rail related research has been completed, work relating to container loading approaches is currently underway and conventional loading approaches will be performed in 2020.

Opsomming

Die verwagting is dat sitrusproduksie oor die volgende paar jaar aansienlik sal toeneem en druk op vrugvoorkoelingsgeriewe sal plaas om die vereiste deurset te bereik. Boonop word verwag dat internasionale fitosanitêre vereistes in die komende jare geleidelik strenger sal word, wat die aanvraag op voorverkoelingsfasiliteite verder sal verhoog. "Warm laai" is 'n progressiewe koueketting opsie waartydens gepalletiseerde vrugte nie verkoel word in 'n voorverkoelingsfasiliteit nie en direk na pak die houer geplaas word, waarna die vrugte binne die houer afgekoel word. Hierdie benadering is 'n belowende oplossing om die

druk van verkoelingsfasiliteite te haal asook om koste en tyd te bespaar. Die tempo van verkoeling asook die en homogeniteit in alles posisies in die houer is egter dikwels onvoldoende. Verder verbetering in die proses sluit in identifisering van problematiese aspekte in laai van houers wat direk verkoeling kan verbeter. Hierdie projek poog om dus om die kouekettingtegnologieë en -tegnieke verder te ontwikkel en verbeter om die druk op koelkamers in hawens te verminder. In die program was die gebruik van die spoornetwerke ondersoek as deel van die sitruskoueketting. In die toekoms sal die program die integrasie van konvensionele verskeping in die FMS-program insluit, wat sodoende ook die druk in die hawens kan verminder. Die projek het tot dusver aan al die verwagte mylpale behaal. Die spoorverwante navorsing is afgehandel en navorsing met betrekking tot die laai van houers is tans aan die gang en die in die volgende seisoen sal konvensionele verskeping aandag geneit.

5.3 PROGRAMME: PRODUCTION AND QUALITY

Programme coordinator: Pieter Raath (CRI)

5.3.1 Programme summary

During the 2018/2019 research period, one new project was initiated in the Production and Quality Research Programme, one was running and two completed. This document therefore contains two final reports as well as a termination report summary for the running project that is finalised in 2019.

In Project 1113 (*Nitrogen and potassium release from organic soil amendments over time*) an attempt was made to elucidate concerns that citrus fruit colour and quality could be affected by delayed nitrogen (N) release from applied organic materials. In both an incubation study and field trial it was shown that almost all potassium (K) in all types of compost used (vermicompost, cattle manure, citrus waste compost, wood based compost) is readily released after application. High initial availability of nitrate (NO_3^-) in the composts, with citrus waste compost releasing the most, was also detected in the incubation studies. The citrus waste compost, however, showed an increased rate of NO_3^- a delayed release from 28 days of incubation up to 90 days. In accordance with the incubation studies, soil mineral N and K showed fluctuations that were dictated by application times of the composts, e.g. increased concentrations following applications, with subsequent decreases. It was concluded that an application up to 16.5 m³ compost/ha (32 L per tree) can improve yield with no adverse consequences to fruit quality. The mineral N and K released must however be taken into account in the fertilisation programme.

In a comprehensive project (Project 1123 – *The benefits of shade netting for citrus fruit quality*) the use of shade netting to change light quality and quantity, with the primary focus to increase return on investment by reducing damage to fruit, was investigated. Examining the impact of this altered ambient climate on tree and fruit physiology, water use efficiency and susceptibility of fruit/trees to damage by insects and infection by fruit pathogens, indicated that 20% white shade netting affected the microclimate of a 'Nadorcott' mandarin orchard in Citrusdal positively. Productivity and profitability of the 'Nadorcott' mandarin orchard was increased. The use of this technology can therefore be recommended in areas that experience extensive yield losses due to adverse climatic conditions. Furthermore, after also doing a comparative analysis of drape netting vs. permanent shade netting, it was shown that 'Nadorcott' mandarin growers that are considering using non-permanent netting would most probably have to upwardly adjust foliar spray applications of pesticides and fungicides, and possibly also plant growth regulators (PGRs) and mineral nutrient foliar sprays to ensure efficacy thereof.

Interim results of a one-year investigative project (Project 1234 - *The use of novel soil conditioners to improve citrus phosphorus (P) nutrition and tree performance*) confirmed that for soil with high P concentrations, application of P is not required since available soil P is then at sufficient levels even in high pH (e.g. pH_{KCl} 7.2-7.8) soils. Furthermore, promising results that show significantly increased microbial enzyme activities by various elemental S (phosphatase), *Bacillus/Pseudomonas* spp. (B-glucosidase & urease) or *Bacillus/Azospirillum* (B-glucosidase, phosphatase & urease) inoculants to potentially enhance both P and N availability were obtained. The results will be confirmed by an additional set of sampling data.

From the research addressed in this Programme Report, nine presentations were made at the CRI Citrus Research Symposium (2018); eight at industry extension meetings; three papers were presented at international symposia; one scientific article was published and three MSc Students graduated.

The following remaining knowledge gaps might be considered in future research:

- Elucidation of the value of so-called humic and fulvic acids compared to the use of organic material.
- The use of shade poses unanswered questions, e.g. how factors such as various cultivars and geographic location influences the effect shade nets have on fruit quality during both pre- and post-harvest conditions.

Programopsomming

Gedurende die 2018/19 navorsingsperiode is een nuwe projek in die Produksie en Kwaliteit Navorsingsprogram geïnisieer, terwyl twee afgehandel is en een steeds lopend was. In hierdie dokument bevat dus twee finale verslae asook 'n beëindigingsverslag vir die lopende projek wat in 2019 afgehandel sal word.

In Projek 1113 (*Stikstof en kalium vrystelling van organiese grond verbeteraars oor tyd*) is besorgdheid dat sitrus vrugkleur en gehalte benadeel kan word deur vertraagde vrystelling van stikstof (N) uit toegediende organiese materiaal aangespreek. In beide 'n inkubasiestudie en veldproef is getoon dat omtrent al die kalium (K) in alle vorme van kompos (erdwurmkompos, beesmiskompos, kompos van sitrusafval, houtgebaseerde kompos) geredelik vrygestel word. Daar is ook in die inkubasiestudie gevind dat heelwat nitraat (NO_3^-) ook inisieël uit al die kompostipes vrygestel word, met die sitrusafvalkompos wat die meeste vrystel. Laasgenoemde het boonop 'n toename in NO_3^- vrystellingstempo vanaf 28 dae tot 90 dae inkubasie getoon. Ooreenstemmend met die inkubasie studie, het die grond se minerale N en K konsentrasies gewissel in tesame met die kompos se toedieningstye, nl. 'n toename in konsentrasies is waargeneem na toedienings, met 'n afname wat mettertyd voorkom. 'n Gevolgtrekking dat toedienings van tot $16,5 \text{ m}^3$ kompos per hektaar (32 L per boom) wel opbrengs kan verhoog sonder 'n nadelige effek op vruggehalte. Die minerale N en K wat vrygestel word moet egter in ag geneem word in die bemestingsprogram.

In 'n omvattende projek (Projek 1123 – *Die voordele van skadunet vir sitrus vruggehalte*) is die gebruik van skadunet ondersoek om liggehalte en hoeveelheid te verander, met 'n hoofokus om 'n opbrengs op belegging te verkry deur skade aan die vrugte te verminder. Die impak van die veranderde klimaatstoestande op boom- en vrugfisiologie, watergebruikseffektiwiteit en voorkoms van insek en siekte-infestatsie van die bome/vrugte is bepaal. Daar is gevind dat 20% wit skadunet die klimaat van 'n "Nadrocott" mandarin boord in Citrusdal sodanig positief verander dat produktiwiteit en winsgewendheid van die boord verhoog het. Die gebruik van hierdie tegnologie kan dus aanbeveel word in areas waar groot verliese in opbrengs ervaar word as gevolg van ongunstige klimaatstoestande. Verder, tydens 'n vergelykende studie waar die gebruik van drapeurnette teenoor permanente skadunet vergelyk is, is gevind dat "Nadrocott" mandarin produsente wat dit oorweeg om nie=permanente nette te gebruik waarskynlik hul spuit-toedienings van plaagbeheer- en siektebeheerprodukte, asook groeireguleerders en moontlik blaarvoedings sal moet verhoog om die effektiwiteit daarvan te verseker.

Voorlopige resultate van 'n eenjarige ondersoekende projek (Projek 1234 – *Die gebruik van grondkondisioneerders om sitrus P-voeding en boomprestasie te bevorder*) het bevestig dat onderhoudsbemesting op gronde met hoë pH's nie nodig is as die grond se P-vlakke hoog is nie. Verder is belowende resultate verkry waar mikrobiologiese ensiemaktiwiteite deur o.a. elementêre swawel, *Bacillus/Pseudomonas* spp. of *Bacillus/Azospirillum* innokulante verhoog is, met die moontlikheid dat beide N en P beskikbaarheid bevorder kan word. Hierdie resultate sal bevestig word deur 'n verdere stel monsternemingsdata.

Voortvloeiend uit die navorsing wat in hierdie Navorsingsprogram Verslag vervat is, is nege aanbiedings tydens die CRI Simposium (2018) gedoen; agt voorligtingslesing by Industrievergaderings; drie wetenskaplike lesings by internasionale simposiums; een wetenskaplike artikel en drie MSc student het afstudeer.

Die volgende bestaande kennisgapings mag dalk oorweeg word in formulering van toekomstige navorsingsvoorstelle:

- Uitklaring van die waarde van sogenaamde humien- en fulviensure as grondtoedienings in vergelyke met die gebruik van organiese materiaal.
- Onbeantwoorde kwessies rondom die gebruik van skadunette, naamlik hoe faktore soos kultivar en geografiese ligging die effek van skadunette beïnvloed, met spesiale verwysing na vruggehalte in beide die vooroes en na-oes periodes.

5.3.2. **FINAL REPORT: Nitrogen and potassium release from organic soil amendments over time** Project 1113 by JT Vahrmeijer (CRI), CM van Heerden (UP), E Tesfamariam (UP)

Summary

In citrus production the use of compost to improve the nutrient content and physical properties of soil is increasing. The concern exists, however, that citrus fruit colour and quality could be affected by delayed nitrogen (N) release from the soil organic matter. Incubation studies and field trials were conducted to determine the release rates of specific nutrients from different composts (vermicompost, cattle manure, citrus waste compost, wood based compost). Results from a 240-day incubation study indicated that potassium (K) is readily released from the different composts, with vermicompost (VC) having the most K released (2.67% decrease in VC K content). This suggests that application of organic soil ameliorants/composts that contain significant amounts of K can lead to an excessive supply of K in the first season of application and thereafter, if application is repeated.

Compost analyses indicated that the N concentration of cattle manure (CM) increased from 2.19% to 2.50% and for wood based compost (WBC) from 0.25 – 0.41%, over a period of 240 days. Vermicompost and citrus waste compost (CWC) had no significant change in its N concentration during the 240-day incubation study. In the incubation studies a high initial availability of NO_3^- in the composts, with CWC releasing the most, was also detected. In contrast to the other materials, CWC also showed an increased rate of NO_3^- release from 28 days of incubation up to 90 days.

Field trials were conducted with different compost treatments applied in a randomised block design, during September of 2015 and 2016. At regular intervals leaf, soil and compost samples, as well as leaching water from wetting front detectors, were collected from the different treatment plots and analysed. In accordance with the release of mineral N and K from the composts in the incubation studies, soil mineral N and K showed fluctuations that were dictated by application times of the composts, e.g. increased concentrations following applications that decreased thereafter.

Increased yield was obtained where compost was used compared to the control (conventional fertilisation). This is ascribed to the beneficial effect of an organic mulch, other than the nutritional value, which improves tree performance, e.g. improved water infiltration, less rapid fluctuation in soil water content and temperature, improved soil aeration, structure, etc.

Opsomming

Die gebruik van kompos in sitrusproduksiesisteme is besig om toe te neem. Daar is egter kommer oor die effek wat vertraagde stikstof (N) vrystelling uit organiese materiaal op vrugkleur en kwaliteit mag hê. Inkubasiestudies en veldproewe is gedoen om die tempo van vrystelling van spesifieke voedingselemente uit verskillende tipes kompos (erdwurmkompos, beesmis, kompos gemaak van sitrusafval, hout gebaseerde kompos) te bepaal. Resultate verkry na 'n 240 dae inkubasie proef het aangetoon dat kalium (K) vinnig vrygestel word uit die verskillende komposte met erdwurmkompos (EWK) wat die meeste K vrygestel het ('n 2.67% afname van die K-inhoud vir EWK). Dit impliseer dat die gebruik van organiese materiaal/kompos met hoë K

konsentrasies daartoe aanleiding kan gee dat 'n oormaat hoeveelheid K in die eerste seisoen van toediening vrygestel kan word, en ook daarna met elke herhaalde toediening.

Komposontledings toon dat 'n toename in die N-konsentrasie van die beesmiskompos (BMK) voorgekom het, te wete van 2.19% tot 2.50%. In die geval van houtgebaseerde kompos (HGK) was daar ook 'n toename van 0.25 tot 0.41%. In die geval van EWK en kompos gemaak van sitrusafval was daar geen verandering in N-konsentrasie oor die 240 dae inkubasie periode nie. In die inkubasiestudies is 'n hoë inisiële beskikbaarheid van NO_3^- waargeneem, met kompos gemaak van sitrusafval wat die meeste vrygestel het. In kontras met die ander tipes kompos, het die sitrusafvalkompos ook 'n toename in NO_3^- vrystelling getoon vanaf 28 dae tot 90 dae inkubasie.

Veldproewe is gedoen waar verskillende kompos behandelings gedurende September 2015 en 2016 toegepas is in 'n gerandomiseerde blok ontwerp. Blaar, grond en komposmonsters is gereeld geneem, asook logingswater wat in "wetting front detectors" opgevang is. In ooreenstemming met die vrystellingspatroon van N en K uit die verskillende tipes kompos in die inkubasiestudie, het die grond se minerale N en K fluktuasies getoon wat ooreenstem met toedieningstye van die kompos, naamlik dat konsentrasies in die grond gestyg het kort na toediening, gevolg deur 'n afname oor tyd.

Oesdata is in die veldproef geneem gedurende September 2017, waartydens elke behandeling apart gemonster is en die totale opbrengs (kg boom^{-1}) asook vruggrootteverspreiding bepaal is. Vergeleke met die kontrole, het *al* die behandelings tot 'n toename in opbrengs aanleiding gegee.

Introduction

With the increase of fertiliser prices in South Africa, farmers are investigating alternatives to inorganic fertilisers. It is a well-known fact that soil organic matter is a major source of plant nutrients and improves the physical properties of soil; such as the water holding capacity, aggregate stability and soil porosity (Ouédraogo *et al.*, 2007). However, there is a decrease in the organic matter content of agricultural soils worldwide, due to natural mineralisation processes and changes in agricultural practices (Barrel *et al.*, 2011). To counter this decrease in soil organic matter, farmers increasingly use organic fertilisers, manures, cover crops and compost as organic soil amendments to improve soil structure and the nutrient content of soils (Barrel *et al.*, 2011). Timely availability of N, P and K from organic soil amendments however is important for optimum crop production. Nitrogen released from composts or any organic material in soils is influenced by the microbial decomposition of the compost, which is driven by the carbon (C) requirements of microorganisms and the amount of N assimilated by microbes that feed on organic matter (Hadas *et al.*, 1997). Decomposition rate and subsequent nutrient release are influenced by soil temperature, soil moisture and composition of the decomposer communities and have an important influence on the organic matter and nutrient budget of soils (Bayala *et al.*, 2005). Organic matter added to the soil is considered to comprise of three pools *viz.* the carbohydrate-like, cellulose-like and the lignin-like fractions of the residues. Each of these pools has its own rate of decomposition (influenced by soil temperature and water content) and the release of C and N is determined by the mineralisation and immobilisation processes that occur (Probert *et al.*, 2005). Mineralisation data for cattle manures consistently shows an initial immobilisation or delay in mineralisation that lasts for several weeks, while plant materials with low C:N ratios typically have positive net mineralisation from the commencement of the incubation period (Constantinides & Fownes, 1994).

If mineralisation of organic soil amendments (pelleted chicken manure, compost from manure, etc.) in citrus orchards is delayed, N-release may occur too late in the season and will have a negative effect on fruit quality, such as a delay of colouring, thicker peels, shortened shelf life and reduced fruit size (Coetzee, 2007). Therefore, it is important to determine N mineralisation for different organic materials at different temperatures and water contents for the use in N release models.

Stated objectives

1. To determine the different fractions of N associated with the organic material to be used as a soil amendment.

2. To determine N-release curves at different temperatures and water content for different organic material to be used as soil amendments.
3. Determining the nitrate, ammonium and total N-content over time in soil cores at different temperatures, soil water content and type of organic soil amendment.

Materials and methods

Incubation Study: The incubation study was initiated on 2 December 2016 and ended 30 July 2017, a period of 240 days. A volume of 100 ml of each organic soil amendment (OSA), e.g. type of compost, was incubated in a temperature controlled room at 25°C and a fairly constant moisture content was maintained by applying 25 ml of water to the samples every 7 to 14 days. The weight was recorded for each sample, which enabled the calculation of each sample's specific gravity (SG). In total 112 samples were incubated, namely four replicate samples of each of the four OSA's (vermicompost, cattle manure compost, wood based compost and citrus waste compost) to be analysed after 1, 7, 14, 28, 60, 120 and 240 days of incubation.

The total N and K concentration of each OSA was determined prior to incubation. After the consecutive periods of incubation, a 1:1.5 water extract for NH_4^+ , NO_3^- and K^+ analysis was done on the samples, as well as the total N%, K% and the moisture %. It was assumed that the total N, as determined prior to incubation, is equal to the OSA N% analyses on day 1 plus 1:1.5 extract analyses of NH_4^+ and NO_3^- , plus the amount of N loss into the atmosphere. Last mentioned could therefore be determined by deduction (e.g. after one day of incubation the OSA total N% analyses + 1:1.5 extract analyses + X = original OSA N% of day 0 with X = the amount of N loss into the atmosphere). This applied for all the removed samples until day 240.

Field study: The field study was conducted at Letaba Estates in the Letsitele area in an eight-year-old "Valentia Late" block, grafted on Swingle. A randomized block design was used, consisting of five applied treatments (the four OSA's applied at a rate of 16.5 m³/ha (32 L per tree) and a control that was the standard fertilizer program followed by the farm) replicated four times. Each experimental plot consisted of 15 trees, e.g. 3 rows of 5 trees per row. Samples were taken from the three central trees in each row.

Wetting front detectors were also installed to collect drainage water for N analyses. Representative compost, soil, soil water and leaf samples were collected at intervals of roughly one month and respectively analysed for total N & K (composts); NH_4^+ , NO_3^- & K (soil) and all nutrients (leaves).

At the end of the 2015/2016 season, the fruit was harvested by labourers without prior arrangement with the research team. During the 2016/2017 season (1 and 2 September 2017) harvest data was however successfully collected from each block's central data tree. The total fruit weight per tree was established, thereafter a culling factor was determined as well as the fruit size distribution.

Statistical Analysis: Yield data in weight per bin (kg) was analysed with analysis of variance (ANOVA) to test for differences between the five treatment (compost) effects (Shapiro & Wilk, 1965; O'Neill & Mathews, 2002). The counts per fruit size were non-Normal and the generalized linear models (GLM) procedure with the Poisson distribution and logarithmic link was used to test for differences between treatment effects. The water extract and soil analysis data were non-Normal (skew distributed) and factorial GLM analysis with the Gamma distribution and logarithmic link was used to test for differences between treatment, day and the treatment by day interaction effects.

The OSA, soil and leaf analysis were analysed with the REML, or Linear mixed model, procedure to test for differences between treatment, day and the treatment by day interaction effects. As the residuals after analysis were Normal, but with heterogeneous treatment variances, comparison of means were done at the 1% level (Glass, Peckham & Sanders, 1972). In all analyses, means were separated using Tukey's test at either the 5% or 1% level of significance (Freund, Mohr & Wilson, 2010). All data were analysed using the statistical program GenStat® (VSN International, 2017).

Results and discussion

In Table 5.3.2.1 analysis of the composted materials that were used in this trial as OSA's are presented. In the field trial, where each OSA was applied at a rate of 16.5 m³ ha⁻¹ to the ridges, this resulted in an additional application of N that ranged between 162.4 and 223.0 kg ha⁻¹ (Table 5.3.2.2).

Table 5.3.2.1. Analysis of initial organic soil amendments* that were used in both the incubation and field trials.

Type of compost	N	Moisture	Ash	pH	C	C:N	Density
	%				%		kg l ⁻¹
Vermicompost	1.93	14.7	43.0	7.6	33.2	17:1	0.51
Cattle manure	2.18	30.8	32.0	7.6	39.5	18:1	0.62
Citrus-waste compost	1.33	26.8	65.1	9.1	20.3	15:1	0.88

*The analyses result for the wood based compost was not reported by the research team.

Table 5.3.2.2. Kilogram of nitrogen and carbon applied per hectare for each OSA applied at a rate of 16.5 m³ ha⁻¹.

Type of compost	N	C	Theoretic increase in soil C for the top 15cm layer
	kg ha ⁻¹		%
Vermicompost	162	2 790	0.14
Cattle manure	223	4 044	0.20
Citrus-waste compost	193	2 950	0.15

Incubation Study: The incubation studies showed a high initial availability of NO₃⁻. In contrast to the other materials, CWC showed an increased rate of NO₃⁻ release from 28 days of incubation up to 90 days. From the onset of incubation, the total NO₃⁻ that was released throughout the incubation period, was also the highest for CWC (Figure 5.3.2.1). This might be due to a higher activity of N-mineralising bacteria resulting from the N-source being more available for utilisation, as well as the lower C:N ratio compared to the other materials.

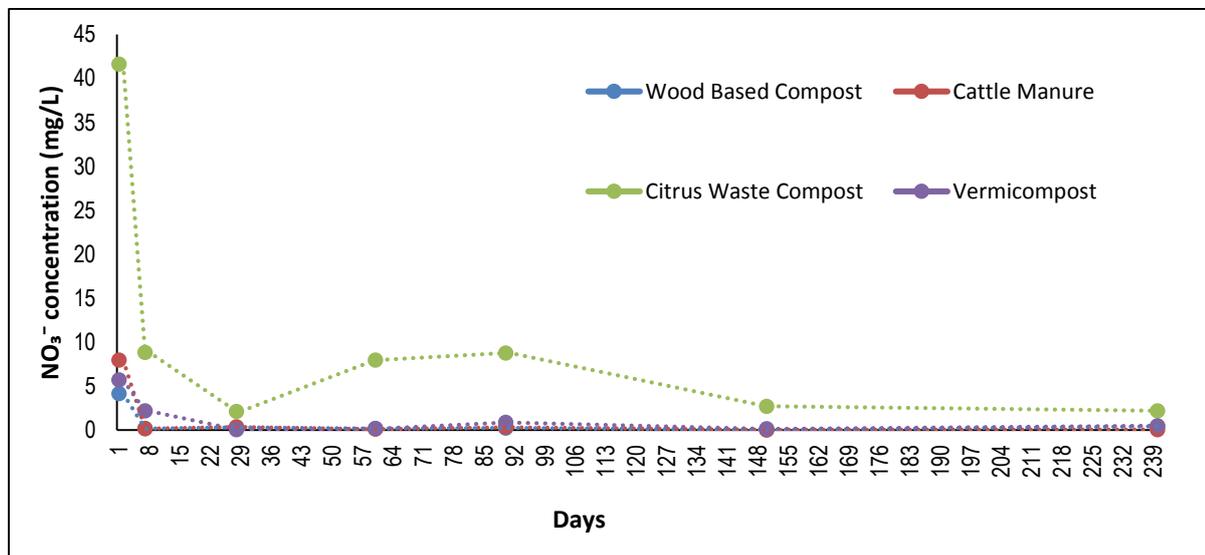


Figure 5.3.2.1. Nitrate (NO₃⁻) concentration in a 1:1.5 water extract of organic soil amendments during incubation.

Compared to NO₃⁻, much higher rates of NH₄⁺ was released during incubation. Similar to NO₃⁻, NH₄⁺ also showed a significant availability during the first 28 days of incubation. However, thereafter no significant rise in NH₄⁺ release was obtained (as for NO₃⁻) indicating that delayed release of NH₄⁺ does not occur after 28 days. At the onset of incubation, both CM and VC released a much higher amount of NH₄⁺ (Figure 5.3.2.2). This is ascribed to the higher total N concentration of both materials compared to WBC and CWC (Figure 5.3.2.3).

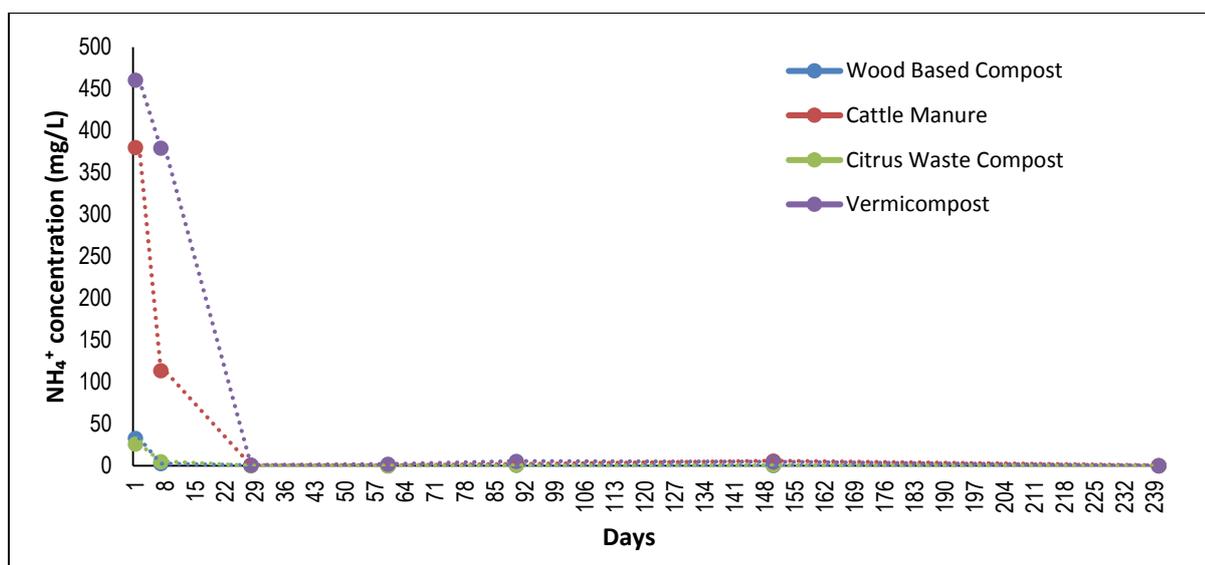


Figure 5.3.2.2. Ammonium (NH₄⁺) concentration in a 1:1.5 water extract of organic soil amendments during incubation.

Using the 1:1.5 water extraction values the total available mineral N supplied by the different OSA's directly after application could be calculated (Table 5.3.2.3). None of the OSA's will result in an initial excessive application of N.

Table 3. Mineral N available in the different OSA's at the time of application.

Organic soil amendment	Mineral N per m ³ (kg)			Total mineral N applied per ha at an OSA application rate of 16.5 m ³ /ha (kg/ha)
	NO ₃ ⁻	NH ₄ ⁺	Total mineral N	
Vermicompost	0.055	0.24	0.29	4.79
Cattle manure	0.007	0.23	0.24	4.01
Citrus-waste compost	0.003	0.015	0.02	0.29
Wood based compost	0.004	0.023	0.03	0.45

Throughout the period of incubation, total N content (N %) of the CM and VC was higher than that of the CWC and WBC. The N concentration of the materials remained fairly stable throughout the period of incubation, although a slight increase from 2.19% to 2.50% was observed for CM and from 0.25% to 0.41% for the WBC (Figure 5.3.2.3). Last mentioned is ascribed to loss of carbon (C) from the material during incubation due to microbial respiration or oxidation.

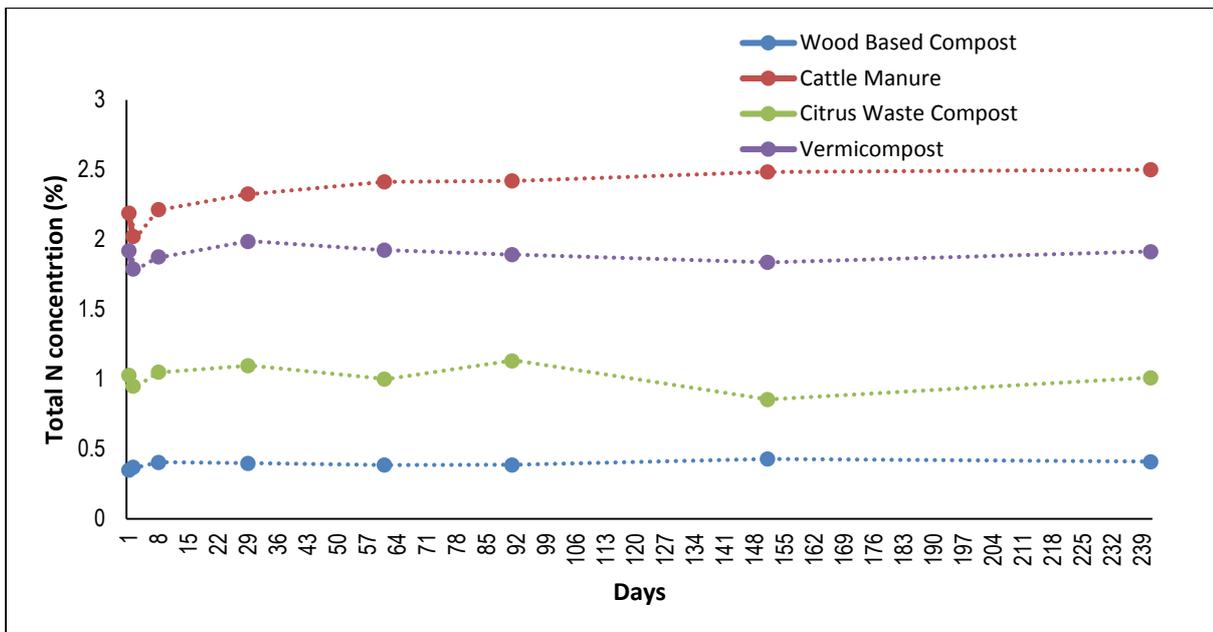


Figure 5.3.2.3. Nitrogen (N) concentration of the organic soil amendments during incubation.

From the onset of incubation, as well as throughout the whole period of incubation, both CM and VC released much higher amounts of K^+ , than the WBC and CWC (Figure 5.3.2.4). This corresponds to the higher total K concentration in the CM and VC (Figure 5.3.2.5). The total available mineral K supplied by the different OSA's directly after application are indicated in Table 5.3.2.4. A significant initial application of available K was made for both CM and VC. Given the significant amounts of K extracted up to 28 days of incubation, there is a significant risk of over-application of K when using CM or VC. Annual applications of the volumes used in the field trials should therefore be avoided.

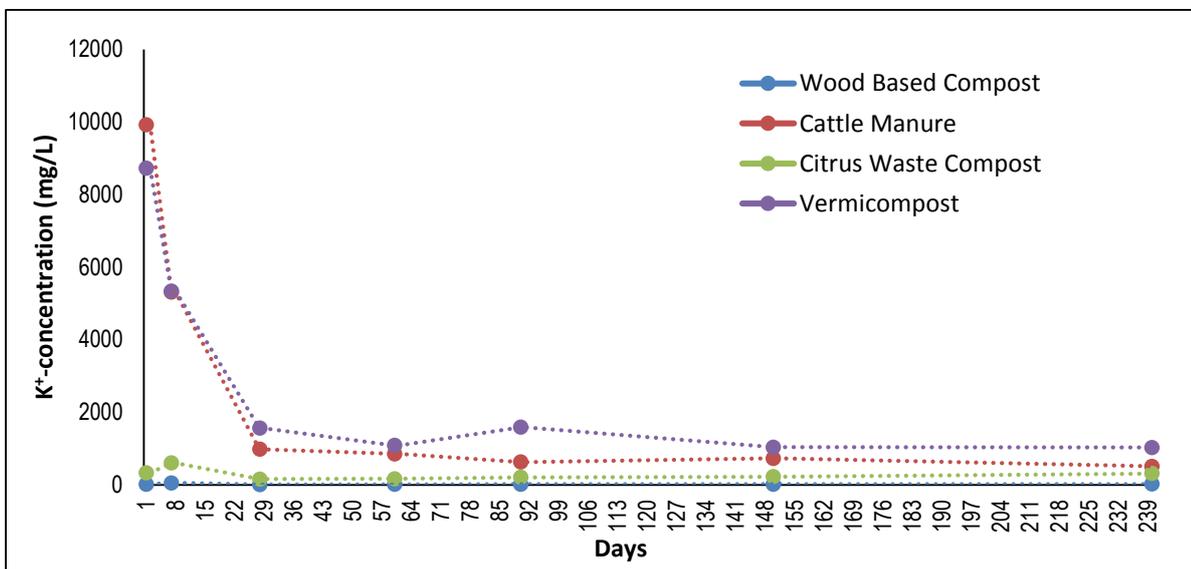


Figure 5.3.2.4. Potassium (K^+) concentration in a 1:1.5 water extract of organic soil amendments during incubation.

The rapid initial decrease in total K concentration of CM and VC (Figure 5.3.2.5) is more than the decrease in K^+ extracted (i.e. 1:1.5 water extract), which seems to indicate that K^+ might have been lost from the materials through leaching when the samples were wetted. The general trend was that the K concentration of the OSA's continued to decrease over the total 240-day period of incubation. This is in accordance with K^+ being extracted over the total period.

Table 5.3.2.4. Mineral K available in the different OSA's at the time of application.

Organic soil amendment	Mineral K per m ³ of OSA (kg)	Total mineral K applied per ha at an OSA application rate of 16.5 m ³ /ha (kg/ha)
Vermicompost	6.7	110.1
Cattle manure	9.2	152.2
Citrus-waste compost	0.4	7.2
Wood based compost	0.01	0.2

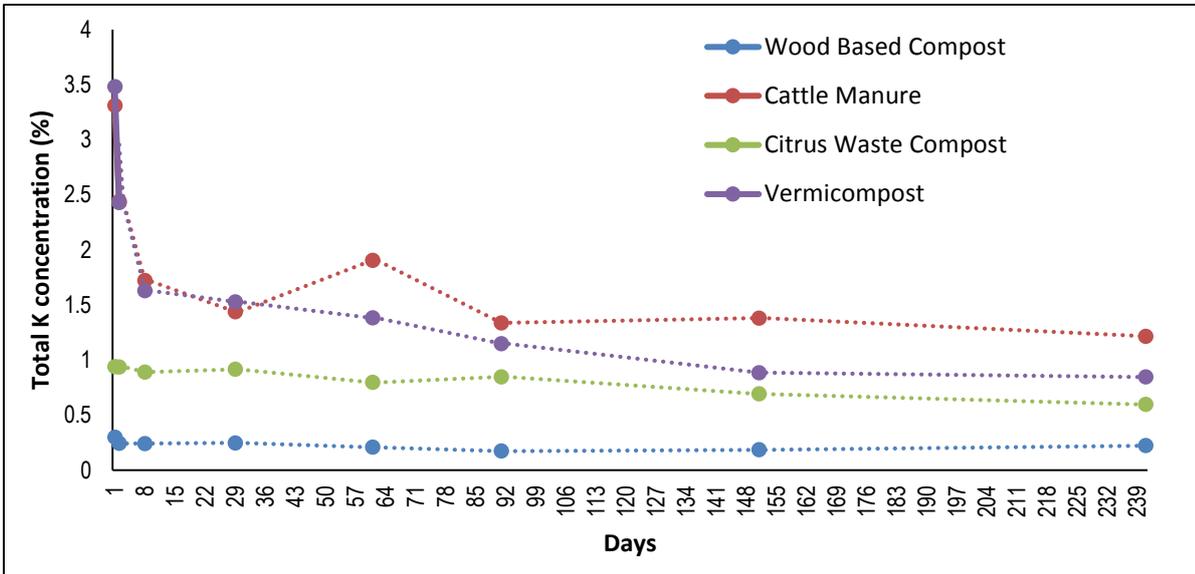


Figure 5.3.2.5. Change in total potassium concentration of the organic soil amendments during incubation.

Field Study: Application of all types of OSA, as well as fertiliser (control), resulted in an immediate increase in soil K concentration for both the years that the trial was conducted (e.g. from 04/09/2015 to 04/11/2015 and February 2016 to October 2016). The rapid decrease in soil K concentration subsequent to the two applications, is ascribed to utilisation and leaching loss of K⁺. This indicates to the K being readily available (in ionic form) in the OSA's, as also suggested by the incubation study data. In accordance with the K concentration of the materials, CM and VC showed the highest increase in soil K (Figure 5.3.2.6).

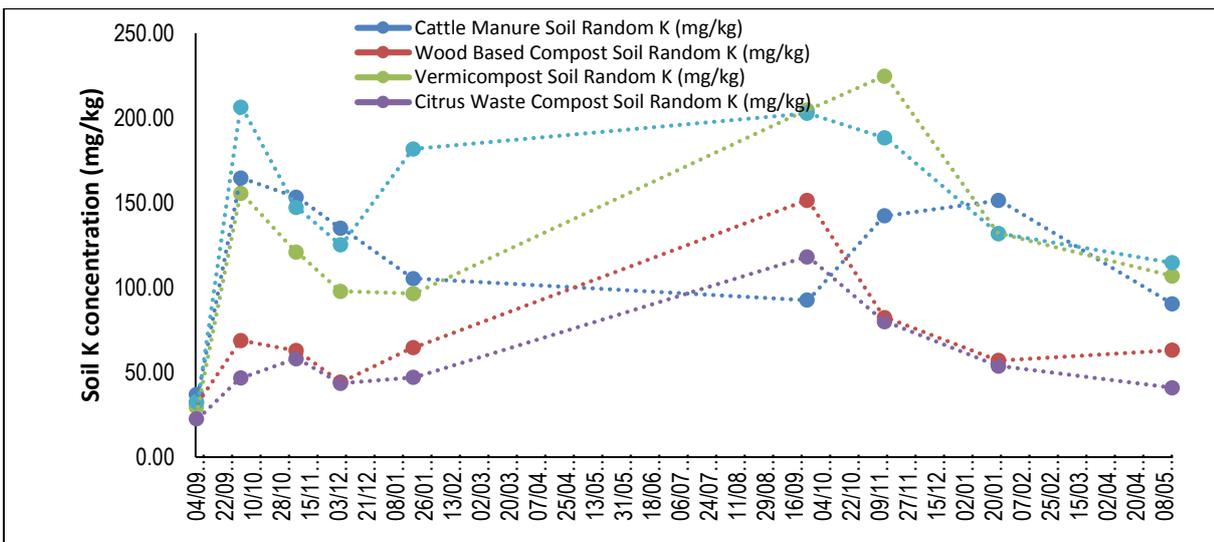


Figure 5.3.2.6. Potassium (K) concentration of soil as affected by different organic soil amendments applied to citrus soil during field trials.

Soil NH₄⁺ concentration decreased sharply after the first application of the OSA's. The decrease is ascribed to nitrification of the NH₄⁺, uptake by the trees and possible volatilisation. The second application was followed

by a slower rate of decline in NH_4^+ concentration (Figure 5.3.2.7). This could be due to different soil conditions (e.g. temperature, irrigation) than in the first year. Except for differences in the initial samples, taken shortly after application of the OSA's, no statistical differences were obtained between the NH_4^+ concentrations in the soil for the different OSA treatments, nor with the control.

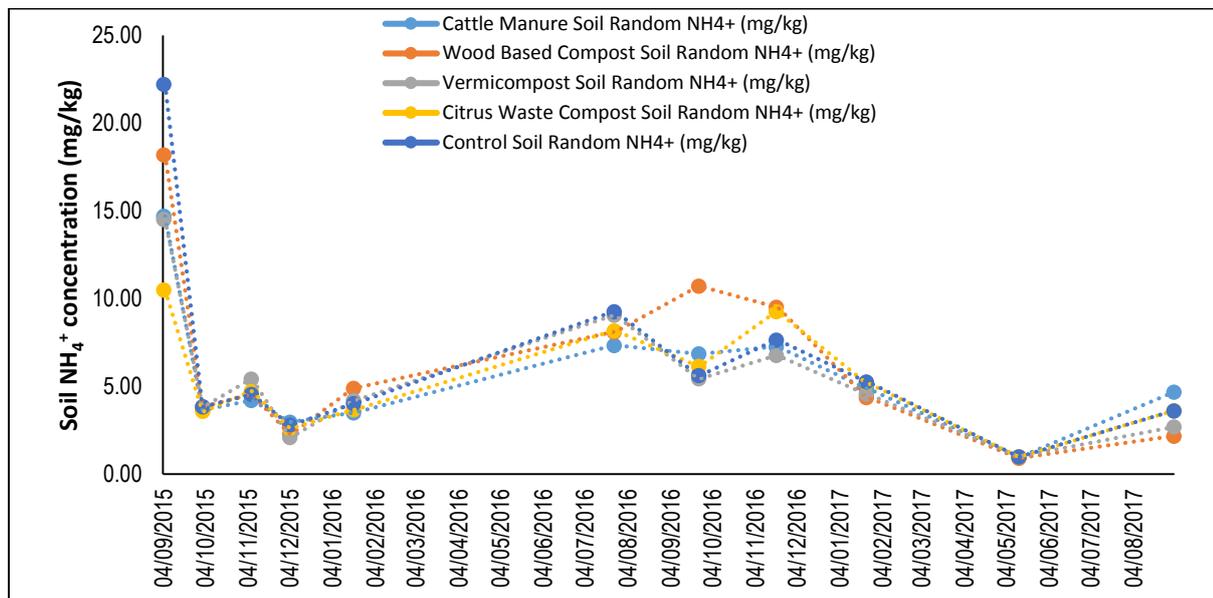


Figure 5.3.2.7. Ammonium (NH_4^+) concentration of soil as affected by different organic soil amendments applied to citrus soil during field trials.

In accordance with the OSA containing some NO_3^- , and similar to the NH_4^+ trend, the field trial's soil NO_3^- concentration increased directly after the two annual applications of OSA's (Figure 5.3.2.8). As for NH_4^+ , no significant differences in soil NO_3^- concentration was obtained between the treatments and the control. This, as for the NH_4^+ , indicates that the OSA's resulted in similar mineral N concentrations in the soil than when fertiliser is applied.

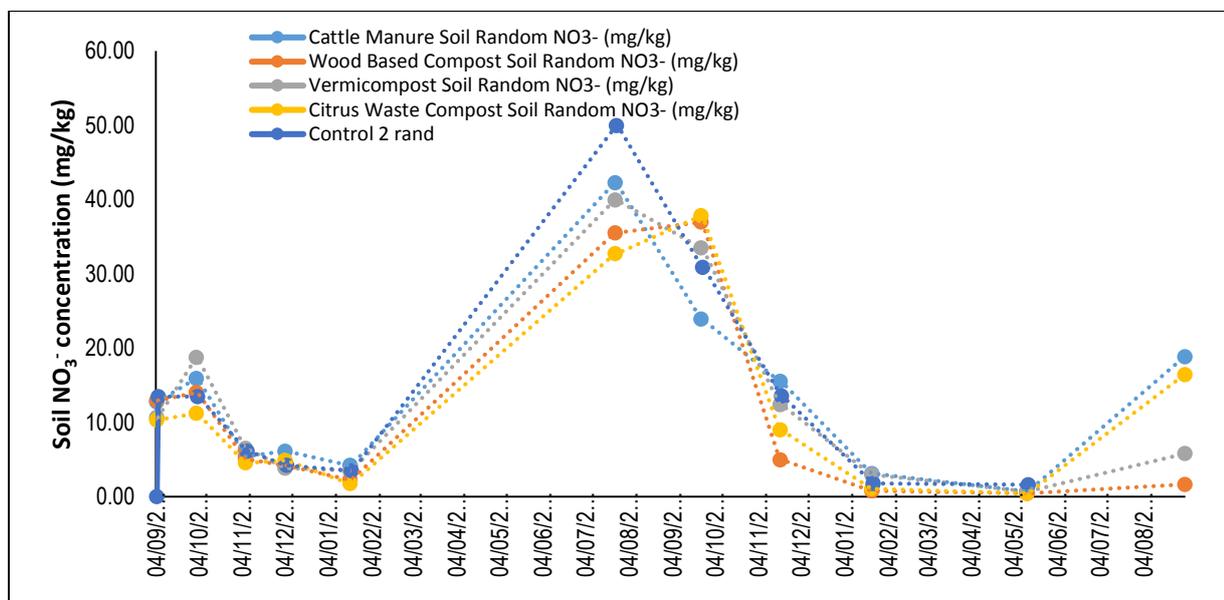


Figure 5.3.2.8. Nitrate (NO_3^-) concentration of soil as affected by different organic soil amendments applied to citrus soil during field trials.

In accordance with the lack of statistical differences between the treatments for mineral N and K concentrations of the soil, also no differences in leaf N and K concentrations were obtained (data not shown).

Soil C content showed significant variance between sampling times over the period 04/09/2015 to 04/08/2017. This is ascribed to sampling methodology that might have differed at the different times. For all sampling times, however, no significant difference in soil C content was obtained between the treatments (data not shown). This indicate to the application rate of the OSA's (e.g. 16.5 m³/ha) not being enough to have an impact on the soil's organic matter content (as indicated in Table 5.3.2.2).

Compared to the control, yield was increased by all the OSA treatments (Figure 5.3.2.9). This indicate to some benefit to tree performance obtained from the application of organic material. There however does not seem to be a correlation between yield response and nutrient content or release of mineral nutrients from the different OSA's. The benefit of the OSA's to increase yield is therefore probably due to other reasons, e.g. typical benefits obtained by an organic mulch like improved soil water regimes and temperature, improved soil structure, soil aeration, etc.

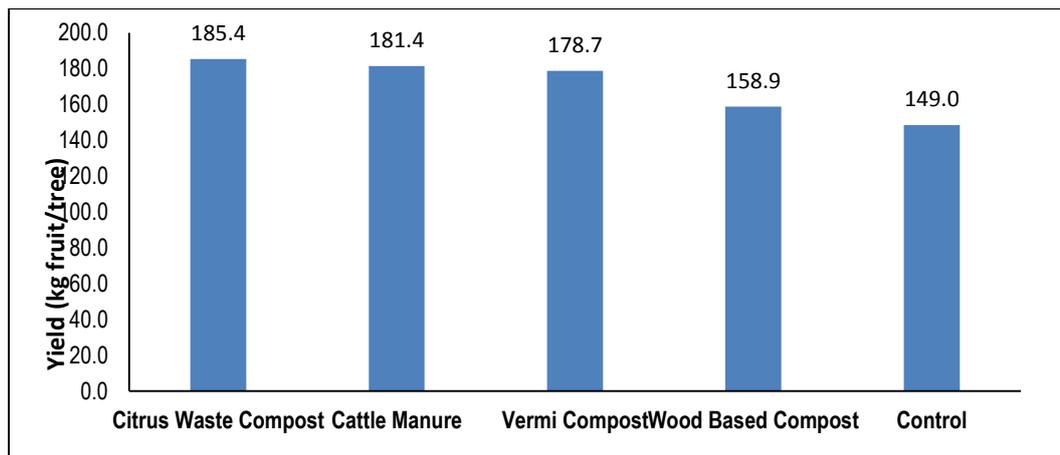


Figure 5.3.2.9. Average yield obtained as affected by different organic soil amendments applied to citrus soil in the field trial.

Conclusion

The incubation studies showed a high initial availability of NO₃⁻ in the OSA's, with CWC releasing the most. In contrast to the other materials, CWC also showed an increased rate of NO₃⁻ release from 28 days of incubation up to 90 days. The incubation study furthermore indicated that K⁺ is readily released from the different composts. This suggests that application of OSA's that contain significant amounts of K can lead to an excessive supply of K in the first season of application and thereafter, if application is repeated.

In accordance with the release of mineral N and K from the OSA's in the incubation studies, soil mineral N and K showed fluctuations that were dictated by application times of the OSA's, e.g. increased concentrations following applications, that decreases thereafter.

Increased yield was obtained where OSA's were used compared to the control (conventional fertilisation). This is ascribed to the beneficial effect of an organic mulch, other than the nutritional value, which improves tree performance, e.g. improved water infiltration, less rapid fluctuation in soil water content and temperature, improved soil aeration, structure, etc.

Future research

Elucidation of the value of so-called humic and fulvic acids compared to the use of organic material is still required.

Technology transfer

Some of the data from this project was incorporated in the following presentation:

Raath, P.J. 2018. Citrus tree nutrition: Paradoxes to be managed and unravelled. CRI Citrus Research Symposium, Champagne Castle, KZN, South Africa, 19-22 August 2018.

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5.3.3. FINAL REPORT: The benefits of shade netting for citrus fruit quality

Project 1123 (2015/6 – 2017/8) by Paul Cronje, Jakkie Stander, Jade North, Martin Gilbert, Claire Love, Jan van Niekerk (CRI), Graham Barry (XCInt Citrus), Robert Brown, Johane Botes and Du Toit Prins (SU)

Summary

The use of shade netting to change light quality and quantity is primarily focused on increasing return on investment by reducing the occurrence of damage to fruit. After installing permanent shade netting over a 'Nadorcott' mandarin orchard planted in 2012 in Citrusdal, Western Cape Province, South Africa, the expected change to the microclimate, which in turn could affect a tree's physiology, was recorded in both netted and open situations. Wind speed was reduced. Likewise, solar radiation was reduced by ~17% under shade netting, and mean and maximum temperatures were reduced on top of the canopy, whereas minimum temperature was higher. However, the temperature within the canopy (1.5 m from soil level) was higher under shade netting, and this led to a significant increase in effective heat units as well as relative humidity which in turn lowered the VPD. Average soil temperature was higher under shade netting resulting from less diurnal fluctuations between the minimum and maximum temperatures. Soil water content was increased by ~17% over the two years. Cumulatively, the shade netting had a positive effect on carbon assimilation during the summer months, coinciding with phase II of fruit growth. In order to evaluate the impact of netting on external and internal quality of citrus fruit, monthly evaluations of fruit size, rind colour, internal quality parameters (°Brix/citric acid ratio) and the incidence of sunburn were performed. Fruit diameter was not influenced by

shade net in 2016. However, in 2017 a larger fruit diameter was measured for shade net fruit. Rind colour development and internal quality were not influenced negatively by the treatment. Shade net was effective in reducing the incidence of sunburn. Moreover, postharvest fruit quality was not negatively affected by the shade net treatments. In general, flowering was not affected by the shade nets, but during the second season, flowering intensity on summer vegetative shoots was higher under shade net. Fruit set, fruit yield, and fruit internal quality were not affected by the shade net treatment. Shade netting did not influence the ability of uniconazole soil-drench treatment to reduce vegetative growth or the efficacy of synthetic auxin fruit thinning agents. Apart from fruit size, a combination of shade netting and chemical fruit thinning treatments had no effects on other important fruit quality attributes. The use of drape-nets in citrus, a popular practice in certain countries to protect crops against hail damage, and/or to prevent cross-pollination and seed development in mandarins was compared to the permanently netted and open area in the orchard. Three drape-net treatments were applied from two months before full bloom until directly after physiological fruit drop; from pre-bloom until before colour break; and from pre-bloom until commercial harvest. The drape-net and permanent shade net treatments significantly reduced the percentage of sunburnt fruit. The inside-canopy deposition of low volume foliar sprays was significantly lower for drape-net treatments. Wild insect pest populations were very low in the area, with nets having varying effects on pest populations, as well as sterile released FCM. From the budget model generated in this study, it can be concluded that 20% of white permanent shade netting resulted in increased orchard profitability, despite a high establishment cost and increase in production costs. To conclude, 20% white shade netting affected the microclimate of a 'Nadorcott' mandarin orchard in Citrusdal and thereby positively affected the trees' physiology to increase productivity and profitability of a 'Nadorcott' mandarin orchard. The use of this technology can be recommended in areas that experience extensive yield losses due to adverse climatic conditions.

Opsomming

Die gebruik van skadunette om die ligkwaliteit en -hoeveelheid te verander, is hoofsaaklik daarop gemik om die opbrengs te verhoog deur die voorkoms van skade aan vrugte te verminder. Na die installing van permanente skadunet oor 'n vier jaar oue 'Nadorcott' mandaryn boord wat aangeplant was in 2012 in Citrusdal, in die Wes-Kaap Provinsie, Suid-Afrika, was dit te verwagte dat die verandering van die mikroklimate 'n boom se fisiologie sal beïnvloed. Daar is gevind dat die windsnelheid verminder was, sowel as 'n 17% afname in straling. Verder was gemiddelde en maksimum temperature bo die blaardak verminder, terwyl die minimum temperatuur hoër was. Die temperatuur in die blaardak (1,5 m vanaf grondvlak) was egter hoër onder die skadunet en dit het gelei tot 'n beduidende toename in effektiewe hitte-eenhede asook RH. Die gevolg was dat die VPD verlaag het. Gemiddelde grondtemperatuur was hoër onder skadunet as gevolg van minder daaglikse fluktuasies en grondwaterinhoud was oor die twee jaar ~ 17% hoër. Kumulatief het die skadunet 'n positiewe uitwerking gehad op koolstofassimilasie gedurende fase II van vruggroei. Die impak van skadunette op die eksterne en interne kwaliteit was gevalueer deur maandelikse meetings van vruggrootte, skilkleur, interne gehalte parameters ($^{\circ}$ Brix/sitroensuurverhouding) asook sonbrand. In die tweede seisoen was vrugdeursnee vergroot terwyl skilkleur en interne kwaliteit ontwikkeling nie negatief beïnvloed is nie en sonbrand minder was. Daarbenewens is die na-oeskwaliteit nie negatief beïnvloed deur die skadunet behandelings nie. Oor die algemeen is blom nie geraak deur die skadunette nie, maar gedurende die tweede seisoen was blom intensiteit op die somer vegetatiewe lote hoër onder skadunet. Vrugset, opbrengs en interne kwaliteit is nie geraak deur die skadunetbehandeling nie, maar die deursnee van die vrugte het in die tweede seisoen toegeneem. Skadunet het geen invloed gehad op die vermoë van onikonazol-grondbehandeling om vegetatiewe groei te beheer nie en die doeltreffendheid van sintetiese oksien uitdingsmiddels was nie verminder nie. Afgesien van vruggrootte het 'n kombinasie van skadunet en chemiese uitdunning geen invloed gehad op ander belangrike vrugtekwaliteitskenmerke nie. Die gebruik van "drape-net" in sitrus, 'n gewilde praktyk in sekere lande om gewasse teen haelskade te beskerm en / of kruisbestuwing en saadontwikkeling in mandaryne te voorkom, was vergelyk met die permanente en oop areas in die boord. Drie "drape-net" toemaak tye was aangewend, naamlik vanaf twee maande voor volblom tot direk na fisiologiese vrugval; van volblom tot voor kleurbreek; en van volblom tot kommersiële oes. Die "drape-net" en permanente skadunetbehandelings het die % sonbrande vrugte verminder. Die hoeveelheid spuit middel aan die binnekant van die boom was aansienlik laer vir "drape-net" behandelings. Uit die begrotingsmodel wat in hierdie studie gegenereer word, kan die gevolgtrekking gemaak word dat skadunet tot verhoogde vrugbaarheid van die boorde lei, ten spyte van 'n hoër inset en toename in produksiekoste. Ter afsluiting: 20% wit skadunet het die

mikroklimaat van 'n 'Nadorcott' mandaryn boord in Citrusdal beïnvloed en sodoende die bome se fisiologie positief beïnvloed. Die produktiwiteit en winsgewendheid van 'n 'Nadorcott'-mandaryn boord het verhoog en die gebruik van die tegnologie kan aanbeveel word in gebiede wat, weens klimaatsomstandighede, opbrengsverliese ervaar.

Introduction

The sustainable economic success of the South African citrus export industry depends heavily on increased production and export of high-quality citrus fruit to the high-value, but discerning, Northern Hemisphere markets. It is evident from literature and extrapolating from other horticulture crops in different countries that various advantages could be gained by using shade netting in citrus orchards. This technology has received inadequate research focus in both the worldwide citricultural industry as well as in South Africa. The high cost of the implementation of this technology demands clear advantages in fruit quality that would translate into a significant increase in export quality fruit and return on investment. Central to the use of shade netting stands the aspect of carbon fixation and allocation under the reduced photosynthetic active radiation (PAR) conditions. Photosynthesis in sun-acclimated citrus trees reaches a saturation point after about one-third of full sunlight (Syvertsen, 1984). In high light environments, this excess PAR can increase leaf and fruit temperature by as much as 9°C above air temperature (Syvertsen and Albrigo, 1980), resulting in higher water loss (higher VPD) and reduced stomatal control. These physiological responses to high light and heat conditions cascade into a reduction of photosynthesis, as the closed stomata result in a drop in growth and fruit yield (Goldschmidt, 1999) due to lower carbohydrate accumulation and water use efficiency.

Various fruit quality aspects could benefit from the reduction of heat in citrus orchards covered by shade netting, i.e. fruit set and rind condition (colour and lack of blemishes). The reduced heat load under the shade netting could increase fruit set due to lower ambient temperature at this physiologically sensitive period, especially with the valuable seedless cultivars. The impact of shade netting on the fruit, foliar and soil borne pests and diseases is also unknown.

Research was undertaken over three seasons using an orchard with and without shade netting to study the responses of both trees and fruit to the altered environment and determine whether this technology makes financial sense for harsh, marginal climates. The primary aim of this project was to improve yield as well as internal and external fruit quality of citrus in order to realise higher export volumes.

Stated objectives

To determine the impact of an altered ambient climate on:

1. Key tree and fruit physiological responses that determines fruit quality due to microclimatic changes.
2. Water use efficiency.
3. Susceptibility of fruit/tree to damage by insects and infection by fruit pathogens.
4. Comparative analysis of drape netting vs. permanent shade netting (**RCE-4 extension**).

Reporting was done in separate sections for the different focus areas within the larger project and contains only key results from the study. The three complete MSc theses, from which the results were selected, are available from the researchers on request. A copy of each is available in the CRI Nelspruit library.

- Du Toit, P. M., 2018. The impact of shade netting on the microclimate of a citrus orchard and the tree's physiology. MSc Thesis. Dept. Horticultural Science University of Stellenbosch, South Africa.
- Botes, J., 2018. Impact of shade netting on internal and external quality of 'Nadorcott' mandarin fruit. MSc Thesis. Dept. Horticultural Science University of Stellenbosch, South Africa.
- Brown, R., 2018. Effect of permanent shade netting on 'Nadorcott' mandarin tree phenology and productivity. MSc Thesis Dept. Horticultural Science University of Stellenbosch, South Africa.

1. **Key tree and fruit physiological responses that determine fruit quality due to microclimatic changes.**
- 1.1 **Microclimate and tree physiology of 'Nadorcott' mandarin are affected by shade netting**

Introduction

Covering of complete citrus orchards with shade netting and producing citrus under “protected cultivation” is a relatively new and developing cultural practice in South Africa, and elsewhere in the world. This research was initiated due to a lack of comprehensive data on the changes to the microclimate of a citrus orchard under shade netting which is receiving increased interest in the South African citrus industry. The high capital cost to install permanent shade netting structures combined with relatively little reliable data being available to facilitate investment decisions guided the objectives of this research namely: to quantify the effects of 20% permanent white shade netting in a Mediterranean-type climate, viz. Citrusdal, Western Cape Province, South Africa, over two seasons on 1) canopy microclimate, 2) tree physiology and 3) CHO accumulation.

This study formed part of a larger citrus industry and Department of Trade and Industry funded project in which the impact of shade netting on the phenology of citrus trees, the efficacy of plant growth regulators (Brown, 2018) and fruit quality (Botes, 2018) were quantified and documented.

Materials and methods

Site, plant material and shade net properties

This experiment was conducted in Citrusdal (32°35'22"S, 19°0'53"E), Western Cape Province, South Africa, in a commercial 'Nadorcott' mandarin (*Citrus reticulata* Blanco) orchard with 'Carrizo' citrange as rootstock, planted in 2012 at a spacing of 5.5 x 2.5 m. A permanent structure covered with “20%” white shade netting at 5.5 m height was constructed over the trees in four randomly allocated blocks (75 x 25 m) a month before full bloom in September 2015.

Above-canopy microclimatic evaluation

Microclimate parameters (solar radiation, wind speed, air and soil temperatures, relative humidity, and soil water content) were measured hourly using two Campbell Scientific weather stations at ± 4 m above the soil and ± 1 m below the netting. One weather station was placed under one of the shade netted areas and the another in an open block above the tree canopy. The data were used to calculate and compare temperature ranges relevant to citrus physiology and phenology, and VPD was calculated to compare the impact of changes in all climatic parameters on the tree's physiology.

Within tree canopy climatic measurements

Additional air temperature data were gathered in each replicate (n=4) using TinyTag Plus 2 TGP-4510 data loggers, placed within the tree at 1 - 1.5 m above ground level and fixed to the main branch out of direct sunlight.

Physiological measurements

Ambient air temperature and relative humidity within the canopy were measured hourly using TinyTag Plus 2 TGP-4510 data loggers one hour (0800_{HR}) before physiological measurements started until the end of measurements (1200_{HR}). One TinyTag data logger was used per replicate, and the values were used to calculate the vapour pressure deficit (VPD).

Physiological measurements of CO₂ assimilation, stomatal conductance, and leaf transpiration were done using two uniform sun-exposed leaves in the middle of an eastern facing canopy position for each replicate (n = 4). Measurements were done between 0900_{HR} and 1200_{HR} on cloudless days on a monthly basis (30-day interval) from the start of the season (July 2016) until the end of the season before harvest (June 2017). The CO₂ assimilation rate (A_c), leaf stomatal conductance (g_s) and leaf transpiration (E) data were measured with a closed chamber infra-red gas analyser (IRGA). The flow rate of air was set at 200 $\mu\text{mol}\cdot\text{s}^{-1}$, air CO₂ concentration was set at 400 ppm, photosynthetic photon flux density (PPFD) at 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and leaf temperature maintained at 25 °C, with the RH% controlled manually to ensure a VPD to be maintained below

2 kPa. From the assimilation and transpiration values, the photosynthetic water use efficiency (WUE) was calculated.

Statistical analysis for within-canopy temperatures

Analysis of variance (ANOVA) was used, or repeated-measures ANOVA was performed when responses were repeated on the same respondent. Mean separations were carried out using Fisher's least significant difference test, where applicable, at $P \leq 0.05$ and 0.1 . The experimental design was a randomised complete block design (RCBD) with four blocks per treatment ($n = 4$), i.e. control and shade netting.

Results

Solar radiation

Average daily radiation was reduced under the shade netting by 18% (1.00 to 0.82 MJ·m⁻²) in the first season and 16% (0.68 to 0.57 MJ·m⁻²) in the second season. Maximum radiation levels followed the same trend as the average levels under the shade netting with a 17% reduction in the first season and a 12.5% reduction in the second season from 2.4 to 2.0 MJ·m⁻² and 2.4 to 2.1 MJ·m⁻², respectively. Seasonal differences were also recorded; throughout the summer the shade netting reduced radiation levels by 12% (1.6 to 1.4 MJ·m⁻²) and 9% (1.1 to 1.0 MJ·m⁻²) in the first and second seasons, respectively. During the cooler part of the year, i.e. autumn to spring, the shade netting had a greater effect with a 17 to 19% reduction in light levels.

Ambient air temperature

Average air temperature above the canopy was only slightly lower under the shade netting, e.g. by 0.7 °C and 0.1 °C in the two respective seasons. Small differences in minimum and maximum daily temperature were recorded. Despite minimal differences in the lower temperature ranges under the shade netting the total hours below 0 °C in July 2017 was decreased by 22 hours. Shade netting reduced the maximum temperature above the canopy by 0.7 °C (3.3% reduction) in the first season and 1.0 °C (3.6% reduction) in the second season. However, although the average temperature did not differ to a large extent, the shade netting reduced the temperatures classified as extreme, i.e. >35 °C, by 18% (12 hours) in the first season and by 20% (54 hours) in the second season.

Within the tree canopy, the average air temperature throughout the year showed significant differences between the treatments within months and between months. From Nov. 2016 until Mar. 2017 there was a significant increase under shade netting of 0.6 °C in average air temperature within the tree canopy. The same pattern occurred for the average monthly maximum temperatures with a significant difference between the month and treatments within a month ($P = 0.003$). From Nov. 2016 until Feb. 2017 monthly maximum temperatures within the canopy under shade netting was significantly higher than the open. However, the total hours above 35 °C and between 26 and 30 °C throughout the year showed no significant increase. In the spring, summer, autumn, and winter no significant differences were observed between treatments. Minimum air temperatures did not differ between the treatments throughout the season. However, a trend of minimum increase values is evident, especially during the coldest period (June to July).

Relative humidity (RH)

Average daily RH in the first season was reduced by 0.5% under the netting; however, in the second season, the shade netting had a 6% higher RH. The minimum RH in 2016 was only 1% higher in the open, and for the second season, there was an increase of 3.4% under shade netting. These elevated RHs were more evident during the summer months when temperatures were higher.

Vapour pressure deficit (VPD)

The average maximum daily VPD during the summer months was reduced by 0.024 kPa in 2016 (Feb. – Mar.), however in 2017 (Jan. – Mar.) a larger reduction in VPD was recorded (0.212 kPa) resulting in a 38% difference between treatments. In the first season, the shade netting reduced the average daily VPD by 2.3% (0.0941 kPa), and in the second season by 15% (0.189 kPa).

Soil temperature

Mean daily soil temperature under shade netting during the two seasons was increased by 0.5 °C and 2 °C respectively. Although the averages were higher, the maximum daily soil temperature was reduced by 4% (1 °C) in the first and by 23% (8 °C) in the second season, respectively. A higher average minimum soil temperature was recorded under shade net resulting in a 1.3 °C (15%) and 4.1 °C (44%) increase compared to the control in the two seasons. Soil temperatures under the shade netting, therefore, had less daily fluctuation between the maximum and minimum temperatures compared to the open orchard's soil temperature.

The volumetric soil water content

The volumetric soil water content and the average daily water availability under the shade netting were increased by 10% and 12% in the two respective seasons. The maximum daily water content, in general, was also higher under shade netting for both years by 17% and 7% for 2016 and 2017 respectively.

Wind speed

The shade net treatment reduced the total wind hours during the first season, with the average maximum daily wind speed being reduced by 21% from 7.4 to 4.8 km·h⁻¹. Furthermore, from Sept. until Oct., which coincides with the period when major wind damage to fruitlets occurs, a reduction in wind speed of 40% (3.4 km·h⁻¹) occurred. During 2017, the same trend occurred with the maximum wind speeds being reduced up to 80% from 7.3 to 1.8 km·h⁻¹.

Plant physiological responses

Air temperature (°C) between 0800-1200_{HR} did not differ significantly between treatments on the day of physiological measurements. Relative humidity (RH) was 1.6% higher under the shade netting, but was not significantly different for the whole season. However, in Jan. 2017 the shade netting environment had a significantly higher RH of 48.3% compared to the control of 44.4%. In Feb. 2017, RH was at the lowest for both the shade netting and control, before increasing towards June. Although there was no difference in air temperature between treatments, the slightly higher RH values under shade netting, especially during the summer months, affected the VPD during this period.

Differences between treatments during the summer months, especially in Jan. and Feb. 2017, occurred with the shade netting significantly lowering VPD in Jan. 2017 from 2.4 to 2.1 kPa (13% reduction) and in Feb. 2017 by 10% (from 3.9 to 3.5 kPa). In Mar. 2017 a smaller reduction of 8% (from 2.5 to 2.3 kPa) occurred ($P = 0.0669$). From Apr. 2017 until June 2017 no differences between treatments were observed and VPD was lower due to low temperatures and high RH during winter.

Throughout the season the physiological response of the 'Nadorcott' mandarin trees were only moderately affected by the shade netting with the A_c ($P = 0.1069$) and g_s ($P = 0.0819$) being higher under the shade netting over the season; although not significant at 90% confidence levels. A_c was 9% higher under the shade netting, raising assimilation rates from 5.23 to 5.75 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Stomatal conductance was also higher throughout the season, but E and WUE remained unaffected over the whole season. Transpiration rates followed the same pattern as stomatal conductance (g_s) throughout the season. Photosynthetic WUE expressed as ratio $A_c:E$ was not influenced by the shade netting treatment (Fig. 5.3.3.1).

From Nov. 2016 under the shade netting a higher trend in A_c , g_s , and E started to occur and persisted until Apr. 2017. From May 2017 onwards this trend became less apparent as the weather changed after summer. There was no distinct trend of a higher or lower WUE between the shade netting and control during the season. In Jan. 2017 the trees under shade netting had a higher A_c rate ($P = 0.0465$) compared to the control (7.08 vs. 8.35 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Similar differences occurred in Feb. 2017 and Mar. 2017 with higher A_c rates by 1.16 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ($P = 0.07$) and 1.12 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ($P = 0.081$), respectively. At the beginning of autumn (Apr. 2017), no differences occurred between the shade netting and control (Fig. 5.3.3.2A). Stomatal conductance followed the same trend as A_c with higher conductance during these months, with Jan. 2017 being 21% ($P = 0.0038$) higher under the shade netting (0.11 vs. 0.14 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Fig. 5.3.3.2B). In mid-summer (Feb. 2017) g_s was the lowest for all measurements. However, the shade netting treatment had higher g_s by 0.02 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ compared to the control ($P = 0.0922$). Thereafter, no further differences were observed between treatments for g_s . During Jan. 2017, E rates were 0.3 $\text{mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ higher under the shade netting ($P = 0.0791$) compared to the control trees (Fig. 5.3.3.2C); thereafter, however, only numerical increases

occurred. With differences in A_c rates and minimal differences in E experienced in these months, WUE remained high but unaffected by the shade netting for Jan. 2017 to Mar. 17. In Apr. 2017 WUE was 0.6 $\mu\text{mol}\text{-CO}_2 \text{ mmol H}_2\text{O}^{-1}$ higher (from 3.9 to 4.5 $\mu\text{mol}\text{-CO}_2 \text{ mmol H}_2\text{O}^{-1}$) under the shade net ($P = 0.0488$) compared to the control (Fig. 5.3.3.2D). This difference was due to higher A_c and not a reduction in E for the specific month.

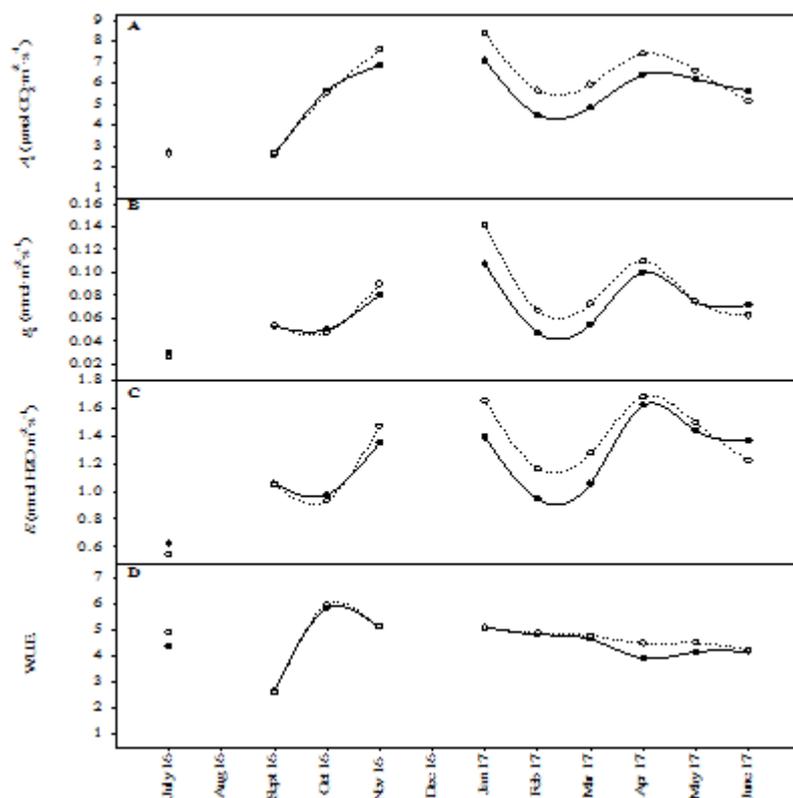


Figure 5.3.3.1. Leaf physiological parameters A_c (A), g_s (B), E (C) and WUE (D) trend of a ‘Nadorcott’ mandarin under 20% white shade netting (\circ) compared to the control (\bullet) on a monthly basis in Citrusdal ($n = 4$).

Discussion

Permanent shade netting is becoming a popular technology in citrus production, not only to produce high-value seedless fruit but also to manipulate the orchard microclimate by reducing the amount of solar radiation entering an orchard. However, it was unknown to what extent the reduction in solar radiation caused by 20% shade netting would influence the microclimate of an orchard in a Mediterranean-type climate and how that change would affect the physiology of ‘Nadorcott’ mandarin trees.

The permanent 20% shade netting reduced solar radiation by 17% and acted as a synthetic windbreak and as a result changed the orchard microclimate and affected leaf photosynthesis and stomatal conductance during the summer months. During summer (Jan. to Feb.) when heat stress typically occurs but also coincides with stage II of citrus fruit growth, the shade netting increased stomatal conductance due to a lower VPD, and in return resulted in a higher photosynthetic rate compared to the open, unnetted trees. Similar reduction in irradiation was recorded by several authors using netting of different shade percentages and colours (Jifon and Syvertsen, 2003, Shahak *et al.*, 2004a; Shahak *et al.*, 2004b; Stamps, 2009). Although irradiation levels were reduced, only small differences occurred in mean daily temperatures above the canopy throughout the two years, with the largest reduction of 1 °C measured under shade netting in the second season; although there were differences in maximum temperature on hot, summer days. These results concur with previous studies (Nicolás *et al.*, 2008; Kittas *et al.*, 2012). Average wind speed was reduced by 40% and maximum windspeed was reduced by up to 80%. This reduction in wind speed is of particular importance from bloom until 8 weeks after full bloom when citrus fruitlets are most sensitive to wind blemishes (Albrigo, 1976).

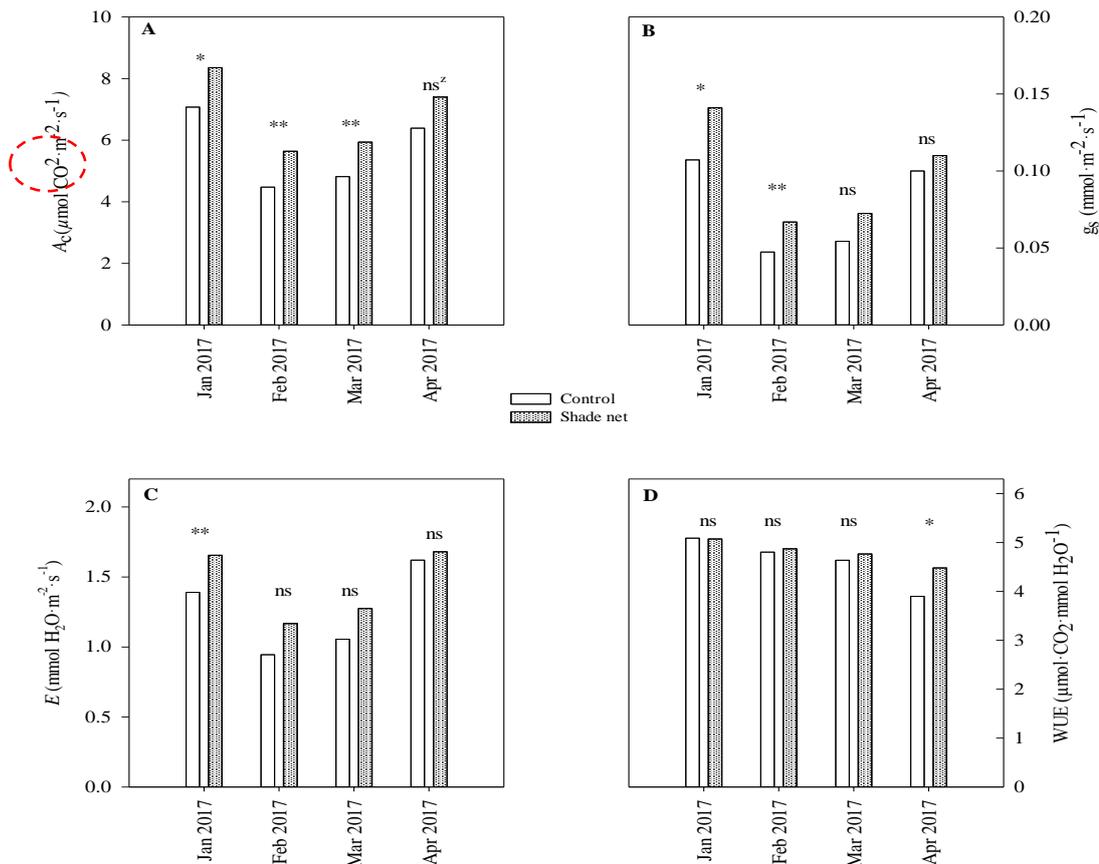


Figure 5.3.3.2. The effect of 20% white shade netting during the summer months on physiological parameters of a 'Nadorcott' mandarin tree physiology, i.e., A_c (A), g_s (B), E (C) and WUE (D); ($n = 4$). * and ** indicate significant differences between treatments within a month as tested by Fisher's LSD test ($P \leq 0.05$ and $P \leq 0.10$); ^z indicates no significant differences between treatments within a month.

Not only was VPD altered by the shade netting, but also an increase was recorded in average soil temperature and water content over the two seasons. The higher, less negative, tree water potential under the shade netting indicates the trees experienced less water stress (data not shown). Therefore, the trees under shade netting could use the available water more efficiently during the day due to a reduction in evapotranspiration from the soil and plant tissue. This increased tree water potential could also be associated with the increased stomatal conductance observed during the summer months which led to increased carbon assimilation. Therefore, an orchard under shade netting could potentially receive less water without negatively impacting on the yield due to the increase in the water use efficiency. These results indicate a potential to reduce irrigation in a 'Nadorcott' mandarin orchard due to the possibility of using water more efficiently under shade netting. This concurred with previous research linking the positive correlation between soil water matrix potential and tree water potential of citrus (Levy *et al.*, 1978; Ginestar and Castle, 1996).

Therefore, 20% permanent white shade netting over a 'Nadorcott' mandarin orchard caused a decrease in water stress experienced under the shade netting and could lead to improved stomatal conductance for parts of the day, thereby, increasing the potential to assimilate CO_2 and improve carbohydrate status of 'Nadorcott' mandarin trees. These positive impacts on the tree physiology under the shade netting could lead to increased fruit growth and increased the production of larger fruit. It can be hypothesized that the trees under shade netting could use the available water more efficiently during the day due to a reduction in evapotranspiration from the soil and plant tissues.

To conclude, shade netting altered the microclimate of a 'Nadorcott' mandarin orchard in Citrusdal, Western Province, South Africa, as well as plant physiology associated with photosynthesis especially during the

summer when heat stress typically occurs. The increased soil water content and lower solar radiation experienced under the shade netting increased the tree water potential. Due to reduced evaporative demand in summer, the shade netting could offer the potential to reduce irrigation volumes without compromising on tree water requirements.

During this study, the complexity of using available natural resources to realise a commercial aim was made clear. By altering only one major environmental factor, i.e., use of shade netting, the reduction in solar radiation resulted in a cascade of changes in the tree physiological response. Shade netting technology offers advantages to citrus producers but will add complexity to tree management.

1.2 The influence of 20% white shade nets on fruit quality of 'Nadorcott' mandarin

Introduction

In citrus cultivation, fruit size and external appearance, including rind coloration and the absence of blemishes are important to determine market value. Additionally, the sugar to acid ratio is the critical internal quality parameter influencing continued purchasing (Ting and Attaway, 1971). In citriculture environmental factors such as temperature and light intensity influence fruit size (Moon *et al.*, 2011), acidity, a soluble solid content (SSC) (Verreynne *et al.*, 2004; Reitz and Sites, 1949) and rind colour (Cronje *et al.*, 2013). The influence of canopy position is mainly due to the change in light spectral quality as it penetrates the canopy, resulting in lower photosynthetic active radiation (PAR) levels inside the canopy (Cronje *et al.*, 2013). However, microclimatic factors such as air temperature and relative humidity also differ between canopy positions and will affect fruit quality. The SSC in citrus fruit is the result of the translocation and accumulation of leaf photosynthates in the juice sacs (Yakushiji *et al.*, 1998). Therefore, factors such as light and other environmental factors that influence photosynthesis play an important role in the fruit's SSC. Sites and Reitz (1949) established a correlation with SSC in the fruit pulp and the amount of light received, and this is also evident from studies which reported a higher SSC for outside canopy fruit (Fallahi and Moon, 1988; Verreynne *et al.*, 2004).

Light levels and low temperature are known to influence the chloro-chromoplast conversion associated with colour development (Goldschmidt, 1988; Thomson *et al.*, 1967). The characteristic green colour of immature fruit is due to the abundant chlorophyll pigment located in the chloroplast, while carotenoids which provide the yellow-orange colouration of mature citrus fruit is present in the chromoplast (Gross, 1987). The rate of chlorophyll production is reduced at a low light intensity, with enhanced chlorophyll degradation under cool temperatures (Meredith and Young, 1969), whilst for carotenoids, light promotes the carotenoid levels and result in improved rind coloration (Cronje *et al.*, 2013). In contrast to the low light levels, excessive solar radiation levels alone or in combination with high air temperatures have been shown to cause sunburn (Ketchie and Ballard, 1968), which result in the yellow rind discoloration or in some cases necrotic tissue, thereby affecting the rind appearance and reducing the fruit value.

Extreme environmental stress conditions are difficult to predict and control. However, shade netting, a relatively new introduction in citriculture, is being implemented in orchards as protection against such climatic events (Rajapakse and Shahak, 2007). However, the impact of netting on citrus internal and external fruit quality aspects has not been as clear as its documented success in reducing sunburn (Syvertsen *et al.*, 2003; Wachsmann, 2014). The aim of this study was to determine the influence of 20% white permanent shade netting on the quality aspects critical for fruit export of 'Nadorcott' mandarin (*C. reticulata* Blanco) at harvest and during long-term cold storage at -0.6 and 4 °C.

Material and methods

The experiment was carried out over two seasons (2016 and 2017) in a commercial orchard of 'Nadorcott' mandarin trees on *Carizzo citrange* (*Poncirus trifoliata* x *C. sinensis*) in Citrusdal (-32.542140, 19.011877), Western Cape Province, South Africa. The orchard was planted in 2012, with a tree spacing of 5.5 m between and 2.5 m within rows, in a north to south row orientation. The orchard management followed standard citriculture practices for both treatments. The experimental layout was a randomised complete block design

(RCBD), where each experimental block was divided into two plots of 75 m x 25 m (n = 4). The treatments consisted of either the shade netting or the control (no netting), where the shade plots were covered by permanent 20% white shade netting (Plusnet, 13 Bussing Road, Aureus, Randfontein, 1759, Gauteng, South Africa) prior to the first season. In order to compensate for a border effect, trees in the first 10 m at either side of the rows and the first two rows from the sides of each plot were excluded from data collection. Two weeks prior to commercial harvest 40 fruit were sampled in each replicate block from two adjacent trees of uniform size, health, and crop load. The fruit were selected mid-way of the tree canopy height, \pm 1-1.5 m from ground level, within the first 20 cm of the outer canopy boundary. The day after harvesting the fruit the rind colour and thereafter the internal quality parameters were determined.

Fruit rind colour was measured with a chroma meter (CR-400, Konica Minolta Sensing, Inc., Japan) on the sun-exposed side of each fruit and expressed as Hunter *a/b* ratio. Fruit diameter was determined with an Electronic Fruit Size Measure and Data Logger (GÜSS Manufacturing (Pty) Ltd., Strand, South Africa). The juice of ten fruit of each replicate was extracted (8-SA10, Sunkist®, Chicago, USA), strained through a muslin cloth, whereafter the soluble solid content (SSC), measured as °Brix, was determined with a digital refractometer (PR-32 Palette, ATAGO CO, Tokyo, Japan). From the extracted juice, a 50 mL sample was used to determine the citric acid content, with a potentiometric titrator (888 Titrand, Metrohm, Switzerland), using Tiamo™ software. Due to citric acid being the major component of organic acids within *Citrus*, the percentage of citric acid was also used in calculating the sugar to the acid ratio (°Brix/citric acid percentage). The remaining 30 fruit per replicate were allocated to lots of 15 per replicate prior to cold storage at either 4°C or -0.6°C for 32 days plus seven days at shelf life before evaluation for non-chilling rind disorders (staining) and chilling injury (% incidence). The sunburn incidence was graded based on a visual scoring scale, after colour break, according to severity, ranging from 0 = no sunburn incidence, 1 = moderate to light yellow coloration to 2 = severe, bright yellow coloration, leathery and necrotic and used to calculate percentage incidence. Mixed model repeated measures ANOVA was performed using Statistica 13's VEPAC module (TIBCO Software Inc., 2017). For post hoc testing, the Fisher least significant difference (LSD) post hoc test was used. Sunburn incidence was analysed by means of a one-way ANOVA.

Results

No significant difference in the fruit diameter between the shade net and control fruit was recorded during the first season. However, in the second season, the average fruit diameter for the shaded fruit was significantly larger at 68.8 mm compared to 66.2 mm respectively. The yield (fruit per tree and kg per tree) were not affected by the treatment (Table 5.3.3.1). Shade net did not influence rind colour development as rind colour development patterns were similar between control and shaded fruit, with no significant difference detected between the treatments, over both seasons (Table 5.3.3.2). However, it was visually noted that the colour development in the second season was not as progressed at harvest compared to the first season which is reflected in the colorimeter values. The internal quality parameters measured showed no significant difference between treatments at harvest (Table 5.3.3.2) as well as after cold storage (data not shown). Both treatments impacted on SSC (°Brix) and citric acid content in the juice, showing a similar trend over the developmental period (data not shown) but resulted in no significant difference at harvest. Furthermore, the ratio of the SSC to % citric acid ratio was not differently influenced for fruit grown under shade netting, compared to that of fruit grown without shading, over the fruit development period as well as at harvest (as no treatment differences occurred). The presence of shade netting effectively reduced the percentage sunburn incidence, in both seasons (Table 5.3.3.3). In both seasons the 20% white shade netting significantly reduced the percentage of sunburned with 18% and 16% in the respective seasons. No significant difference in the incidence of chilling and non-chilling postharvest disorders was observed after cold storage (Table 5.3.3.4).

Discussion

The shade net treatment did not influence the fruit development pattern, regardless of the season, with no fruit size difference reported in the first season. However, during the second season, the average fruit size for shaded-fruit was increased by 3.9%, resulting in these fruits being 2.6 mm larger at the end of the season. Results from the 2016 season concur with Wachsmann *et al.* (2014) where no influence of shade netting on 'Orri' mandarin fruit size was reported for 18% white, 25% red, 24% yellow or 13% transparent netting. The

lack in yield (fruit and kg per tree) difference between treatments could be explained by the relatively young orchard status (4 to 5th year of production) and it is possible that due to the increase vegetative growth under the shade net (Brown, 2018) an increase in yield per tree would become evident in subsequent season. However, the increased fruit size under the shade net had a positive commercial impact to increase export volume as the shifted the fruit size distribution towards the larger counts, and had a direct impact on an improved return on investment even at this early stage of the orchard's production (Brown, 2018).

Shade nets or screens are known to reduce solar radiation (Jifon and Syvertsen, 2001; Lee *et al.*, 2015) and light intensity that can also differ amongst canopy positions (Cronje *et al.*, 2013). Verreynne *et al.* (2004) reported that light intensity influences the fruit size of mandarin types, with greater size for outside canopy, positioned fruit. However, in this study, a reduction of light intensity (17-20%) (Prins, 2018) by the shade net did not affect the fruit growth and size negatively.

The general reduction in photosynthetic photon flux (PPF) caused by shade nets, may result in higher photosynthesis due to a decrease in photo-inhibition which is associated with high light and leaf temperatures (Prins, 2018; Syvertsen *et al.*, 2003). The inconsistent results obtained in our study regarding fruit size between seasons can therefore not directly be ascribed to the role of photosynthesis in providing carbohydrates to drive fruit development. Syvertsen *et al.* (2003) reported a higher photosynthesis rate under the 50% shade cloth, yet fruit size of 'Spring Navel' orange was not affected. Moon *et al.* (2011) suggest that fruit growth is not only dependent on light intensity but aspects such as sink strength, hormone balance, fruit load and leaf to fruit ratio all being influential. Possible climatic differences between the two seasons might be responsible for producing fruit size differences since high heat and humidity is known to lead to large fruit (Prins, 2018). However, Reuther (1988) proposed air temperature to have a broad influence on fruit growth and that heat accumulation could be used as a better indicator to relate to fruit growth.

Rind colour was not negatively affected by the shade net treatment in either season. Colour development generally occurs during the end of the cell enlargement phase (Rodrigo *et al.*, 2004). In this study, however, in both seasons, colour change from green to yellow-orange only commenced between May and June. These results, however, do not concur with other studies where shade net treatment affected the rind coloration, either positively or negatively. Syvertsen *et al.* (2003) and Otero *et al.* (2011) reported that the rind coloration of 'Spring' navel orange was improved by a 50% shade cloth. In contrast, Jifon and Syvertsen (2001) found that rind coloration was delayed for 'Ruby Red' grapefruit and 'Hamlin' orange when exposed to a 50% reflective shade screen. It is thought that the shade percentage factor and type of netting product, along with the time of application and the tree age are critical to affecting rind colour. Light intensity is important for rind coloration, in and a reduction in light intensity reduce carotenoid levels (Cronje *et al.*, 2013). However, in this study, the reduced light levels and the expected climatic alterations associated with shade nets was not detrimental to the rind coloration (Prins, 2018). The shade netting was effective in reducing the percentage of sunburn in both seasons and sunburn is strongly linked to high sunlight exposure of fruit (Ketchie and Ballard, 1968) and concurs with results of Lee *et al.* (2015) on 'Ponkan' mandarin.

In terms of export value, in addition to the external appearance, the taste of the fruit as expressed by the SSC, acids and the sugar to acid ratio, is also of the utmost importance. The internal quality development of shaded fruit was not affected, which is in agreement with Cohen *et al.* (2000), where the sugar and acids in 'Marsh' grapefruit juice were not influenced by the application of either 30 or 60% shade. However, both concurring and contradicting findings to the current study were reported by Jifon and Syvertsen (2001). In their study, shade screens (50%) applied either early (after the bloom-peak 'May-June drop') or continued (after bloom to harvest) or late (only in August to harvest) did not affect the Brix of 'Hamlin' sweet orange, whereas continued shade application decreased the °Brix/acid ratio. Otero *et al.* (2011) reported that the use of 50% shading cloth on 'Spring' navel oranges, reduced the total soluble solids, but without affecting the ratio. In addition, Wachsmann *et al.* (2014) documented that 'Orri' mandarin under 18% white shade net to have had significantly higher sugar to acid ratio, therefore reaching commercial maturity earlier, due to the desired reduced acid levels. A lack of postharvest physiological disorders is, in addition to good rind colouration, key to determine the market value of fruit. An undocumented postharvest disorder for this cultivar did occur, i.e. rind staining at both cold storage temperatures, but was not affected by shade netting. The absence of any negative impact

of the shade netting with regard to inducing postharvest disorders in 'Nadorcott' mandarin is of great commercial significance and is considered a key result from this study.

To conclude, the 20% white shade netting had a positive influence on the fruit size in the second season that could indicate a possible advantage for increases in export volumes. The shade net was effective in reducing the percentage of sunburn, without affecting the rind coloration. Furthermore, the maturity of the fruit grown under the shade net was not influenced. Therefore the internal quality was not affected.

Table 5.3.3.1 The influence of 20% white shade netting on 'Nadorcott' mandarin fruit diameter and yield (fruit and kg per tree), during two consecutive seasons, 2016 and 2017 in Citrusdal, South Africa.

	2016			2017		
	Fruit diameter (mm)	Fruit·Tree ⁻¹	Kg·tree	Fruit diameter (mm)	Fruit·Tree ⁻¹	Kg·tree
Control	48.1 ^{NS}	367 ^{NS}	31.89 ^{NS}	51.6 ^b	550 ^{NS}	55.31 ^{NS}
Shade net	48.8	432	40.19	53.7 ^a	526	58.69
<i>P-value</i>	0.648	0.384	0.218	0.034	0.790	0.712

^{NS} Denotes non-significant difference at 5% significant level.

^a Different letters denotes significant difference between means within a column for at $P \leq 0.05$ according to Fisher's LSD test (n=4).

Table 5.3.3.2 The influence on rind colour and internal quality at harvest, of 20% shade netting on 'Nadorcott' mandarin fruit compared during two consecutive seasons, 2016 and 2017 in Citrusdal, South Africa

	2016				2017			
	Rind colour (a/b)	TSS (°Brix)	Citric Acid (%)	Ratio (Brix/C-acid)	Rind colour (a/b)	TSS (°Brix)	Citric Acid (%)	Ratio (Brix/C-acid)
Control	-0.35 ^{NS}	14 ^{NS}	1.3 ^{NS}	10.5 ^{NS}	-0.59 ^{NS}	12.2 ^{NS}	1.1 ^{NS}	10.8 ^{NS}
Shade net	-0.34	13.6	1.3	10.8	-0.59	11.9	1.1	11.1
<i>P-value</i>	0.697	0.436	0.415	0.481	0.980	0.591	0.451	0.401

^{NS} Denotes non-significant difference at 5% significant level (n=4).

Table 5.3.3.3 The influence on sunburn incidence of 20% white shade netting on 'Nadorcott' mandarin fruit during two consecutive seasons, 2016 and 2017 in Citrusdal, South Africa

	Treatment	Percentage (%)
2016	Control	23.7 a
	Shade net	5.75 b
	<i>P</i> -value	0.002
2017	Control	20.8 a
	Shade net	5.00 b
	<i>P</i> -value	0.031

* Different letter denotes significant difference between means within a column for each season at $P \leq 0.05$ according to Fisher's LSD test ($n=4$).

Table 5.3.3.4 The influence on the incidence of postharvest disorders of 20% white shade netting on 'Nadorcott' mandarin fruit during two consecutive seasons, 2016 and 2017 in Citrusdal, South Africa. The fruit were stored for 32 days at 4 or -0.6°C plus at 7 days at ambient conditions before evaluations.

Storage temperature	4 °C	-0.6 °C
	Staining (%)	Chilling injury (%)
Control	26.9 ^{ns}	3.25 ^{ns}
Shade net	26.9	4.75
<i>P</i> -value	1.000	0.680

^{ns} Denotes non-significant difference between means within a column according to Fisher's LSD test at $P \leq 0.05$ ($n=4$).

2. Tree water use and reaction on a change in soil water

The use of shade netting in agriculture in South Africa is rapidly increasing, primarily for the protection of crops from hail and wind damage. It is well documented that shade nets modify the microclimate of the orchard of which they cover by reducing incoming solar radiation, reducing the daytime temperature, decreasing vapour pressure deficit (VPD), and decreasing the wind speed. The objectives of the study are to measure and compare water use and water use productivity of mandarins under shade netting and in the open while also gaining an understanding of the reasons for any of the observed effects of the shade netting.

Materials and methods

The study in Citrusdal on an orchard of 'Nadorcott' mandarin on Carrizo rootstock planted in 2013 and with a spacing of 2.5 x 5.5m is being used in the trial. The trial is set up with shade net treatments arranged in a randomized complete block design. Soil moisture content at varying distances from irrigation drippers is measured by capacitance probes. Irrigation frequency and application amount are also measured using flow meters installed on dripper lines. Leaf and stem water potential, stomatal conductance, leaf area index and canopy dimensions of all the trees used in the trial are periodically measured.

To evaluate the effect of different irrigation treatments on tree water status as influenced by shade netting during fruit growth, the application of irrigation volumes was changed a month before full bloom on 3 Sept. 2016. Water was supplied through double row drip irrigation with each tree having four drippers of supplying a high (3.2L/h/dripper), low (0.8L/h/dripper) and control (1.6L/h/dripper) volume of water and designated 2X, 0.5X and X. The frequency of irrigation was not changed. This technique is commercially used to monitor the impact of irrigation scheduling on fruit size in orchards and would create contrasting effects of under- and over-irrigation to be compared with standard irrigation volumes applied.

Results and discussion

The 20% white netting created a more shaded environment, and as a result altered the soil and tree water status of the 'Nadorcott' mandarin orchard. A higher Ψ_{tree} under the shade netting indicates that the trees were in a positively modified microclimate resulting in reduced water demand. In addition, the shade netted trees receiving the control irrigation volume had an 11% higher tree water potential (less negative) compared to the open trees during the measurement period, indicating a less water stressed environment. Furthermore, those trees under the shade netting that received half the normal irrigation, had a similar Ψ_{tree} compared to the open trees receiving the control irrigation volume.

In general, it is known that the driving force for plant water uptake and transpiration is the difference between the soil and leaf water potential, and a reduction in transpiration normally coincides with reduced water uptake (Taiz *et al.*, 2015). This occurs in citrus trees during periods of high temperatures and low humidity as part of a seasonal cycle as well as within a day. The result was a decreased stomatal conductance and transpiration due to an increased water vapour deficit (VPD) and eventual reduction in water uptake (Cohen *et al.*, 1985a; Brakke and Allen, 1995). Therefore, a change in the microclimate as seen under the shade netted orchard in this study can potentially have an effect on the trees' response in terms of water uptake due to changes above and below ground as illustrated by the higher Ψ_{tree} .

It was established that under the shade netting the soil water content ($\text{m}^3 \cdot \text{m}^{-3}$) was increased by 17% compared to the open treatment (Fig. 5.3.3.1). Furthermore in this part of the study the tree water potential, measured at pre-dawn, provides a suitable reflection of soil water status (Germanà and Sardo, 1988) and tree water uptake, was higher resulting in a possible reduced water stressed condition during the day. This concurred with previous research linking the positive correlation between soil water matrix potential and tree water potential of citrus (Levy *et al.*, 1978; Ginestar and Castle, 1996). Soil water availability has a direct effect on hydraulic resistance between the roots and soil (Jones *et al.*, 1985) and as a result during deficit irrigation, water uptake by roots can be reduced by up to 50% due to the higher hydraulic resistance (Cohen *et al.*, 1983b). In the shade netting treatment more soil water was available and could potentially reduce the hydraulic resistance between the soil and root interface and thereby increased water uptake and increased the tree water potential (Fig. 5.3.3.2).

An additional positive effect of the shade netting was the increase in optimal hours for root growth and function. This was not quantified in the study, but it could be construed that these more favourable conditions could lead to more roots being initiated under the shade netting. Therefore, in addition to more available water, it is possible that a greater rooting density could lead to a reduction in hydraulic resistance and have a positive effect on tree water balance. Furthermore the shade netting treatment resulted in the minimum daily soil temperature being higher and the maximum soil temperature lower compared to the control, which could have a positive effect on root permeability (Ramos and Kaufmann, 1978) and thereby increasing water uptake, especially at night. This aspect of improved root conditions should be further evaluated in shade netting studies to quantify to what extent changes in soil temperature influence root metabolism and not only water uptake but also nutrient uptake and, therefore, potentially influence fertigation regimes.

Less solar radiation on shaded leaves reduces the leaf temperature and vapour pressure (Syvertsen and Albrigo, 1980; Cohen *et al.*, 1997, Cohen and Naor, 2002), which lowers the evaporative demand and in return can reduce transpiration and increase water potential in a citrus leaf (Jones *et al.*, 1985). In this study, shade netting reduced both solar radiation ($\text{MJ} \cdot \text{m}^{-2}$) as well as VPD, however, without affecting the transpiration rate in the mornings from 0900_{HR} to 1200_{HR}. However, during the warmest part of the day, i.e., 1200 to 1500_{HR}, the reduction in radiation and VPD under the shade netting could result in increased stomatal conductance for this part of the day (Jifon and Syvertsen, 2003). If water loss through stomatal openings exceeds that of the water uptake by roots, due to a high evaporative demand during midday, stem water potential can become more negative (Dzikiti *et al.*, 2007). The lower VPD during the midday period could decrease transpiration demand of a tree and prevent further moisture loss (Syvertsen, 1982), as reported in apricot and apple orchards (Nicolás *et al.*, 2005; Smit, 2007). It could, therefore, be possible that the shade netting treatment decreased leaf transpiration demand during the period known as midday depression, in order for roots to maintain water uptake for a longer period. This could have prevented moisture loss from the tree and increased the pre-dawn tree water potential as measured.

In citriculture a high pre-dawn water potential is important as it effects stomatal conductance throughout the day (Fererer *et al.*, 1979) thereby directly impacting on carbon assimilation. This study has indicted that under the shade netting there exists the possibility that adequate stomatal conductance can be maintained for longer periods during the day, resulting in an increase in gas exchange and carbon assimilation.

In addition to a citrus's tree water status being critical to enable carbon assimilation, it is important to allow all the physiological processes critical to realise a commercially valuable crop such as flowering, fruit set, as well as directly influencing the final fruit size, quality, and yield (Ginestar and Castle, 1996). In the current study the increased water potential under the shade netting did not increase the number of fruit per tree, which concurs with results obtained under 50% continuous shade with no differences between the two treatments (Jifon and Syvertsen, 2001). In addition the control irrigation volume (X) had the highest fruit number per tree, with 21% more fruit compared to the 0.5X and 12% to the 2X irrigation treatments. This could be indicative of a possible under- and over-irrigation scenario.

Even though fruit number (set) was not increased by the shade netting, the evident shift towards larger fruit size could be due to more water availability during the second phase of fruit growth, responsible for cell enlargement (Ginestar and Castle, 1996; Gonzà Lez-Altozano and Castle, 1999). In addition, the increased photosynthesis measured during the cell enlargement stage under the shade netting treatment could also have positively impacted on the fruit size distribution (Goldschmidt and Koch, 1996). The 2X irrigation treatment indicates how more water available could increase fruit size by shifting the fruit size to larger fruit.

Tree canopy volume was not affected by irrigation treatments, however, canopy volume growth tended to be smaller with less water (0.5X). It is, therefore, possible that by continuing these treatments over two or more seasons, a cumulative effect of reduced irrigation would negatively affect canopy volume due to decreased shoot elongation of vegetative flushes (Gonzà Lez-Altozano and Castle, 2000).

Internal and external fruit quality was not negatively affected by shade netting which is critical from a commercial point-of-view. The irrigation treatments did affect the internal quality to some extent with differences seen in citric acid (%), °Brix and the ratio thereof. However, the irrigation did not affect the eating quality of the fruit in terms of commercial guidelines as indicated by Botes (2018). Core gumming is a new undocumented internal physiological fruit disorder of 'Nadorcott' mandarin. It was found that the shade netting resulted in less gumming incidence, which is the first indication that a possible reduced lowered heat and water stress could reduce the incidence thereof and should be further investigated.

The mineral nutrient content of the fruit at harvest was analysed to document any possible changes due to the treatments. The mineral uptake of the fruit did not change significantly under the shade netting, however, the irrigation had an effect on Ca, Mg, Mn and B content and all of these nutrients were increased by the half irrigation volume treatment. As nutrient studies in fruit trees are a complex topic and with different nutrients impacting the tree physiology and phenology differently throughout a growing season, more in-depth research for citrus trees under shade netting is suggested.

To conclude, 20% permanent white shade netting over a 'Nadorcott' mandarin orchard increased the soil water content and in return improved tree water potential. This decrease in water stress experienced under the shade netting could lead to improved stomatal conductance for parts of the day, thereby, increasing the potential to assimilate CO₂ and improve carbohydrate status of 'Nadorcott' mandarin trees. Under the shade netting the higher soil water availability, as well as tree water potential could have led to the increased fruit growth and increased the production of larger fruit. It can be hypothesised that the trees under shade netting could use the available water more efficiently during the day due to a reduction in evapotranspiration from the soil and plant tissues. On a practical level, the data indicate the possibility to reduce irrigation volumes under shade netting in citrus orchards without reducing yield or fruit quality. Therefore, during a restrictive water period, in a season or between seasons, shade netting of trees could receive less water and still maintain a commercially valuable crop.

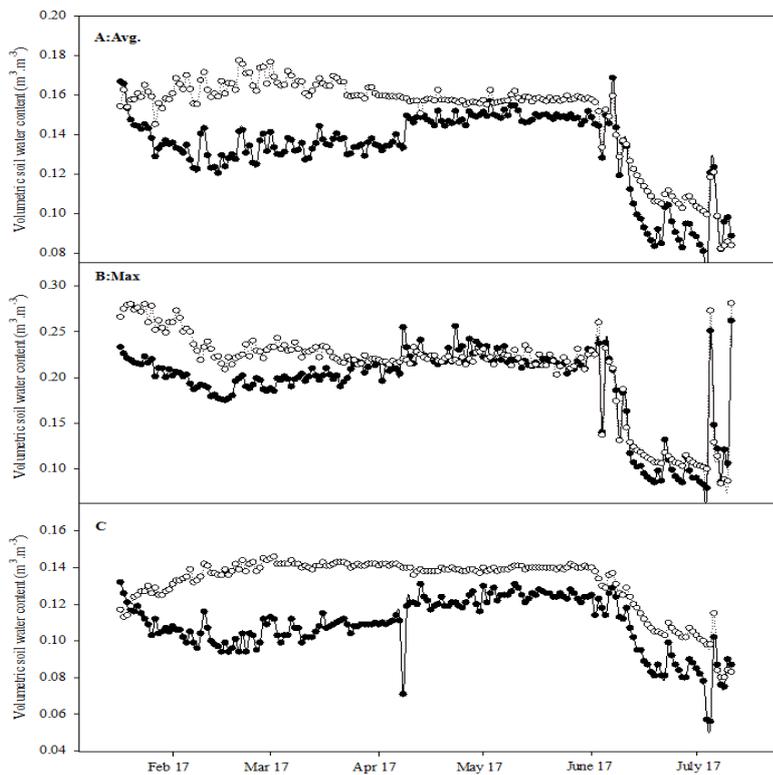


Fig. 5.3.3.1. Monthly daily mean (A), maximum (B) and minimum (C) volumetric soil water content ($\text{m}^3\cdot\text{m}^{-3}$) of the second season and how 20% white shade net affect the water availability in a uniform clay loam soil of a 'Nadorcott' mandarin orchard in Citrusdal. (\circ = Shade net; \bullet = Control).

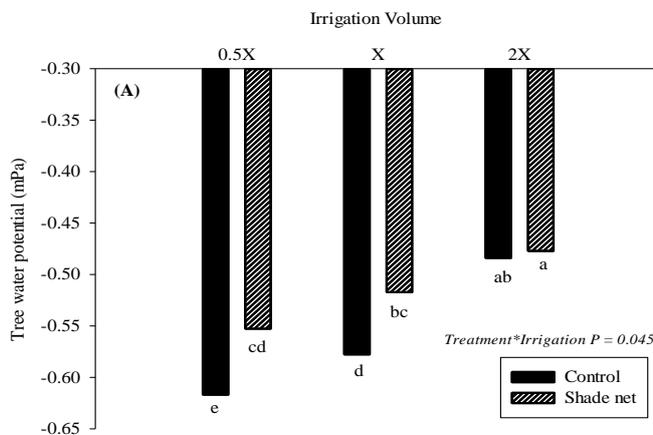


Fig. 5.3.3.2 The effect of 20% white shade netting combined with different irrigation treatments on the pre-dawn (after 0300_{HR}) tree water potential (mPa) 'Nadorcott' mandarin from physiological fruit drop (Nov. 2016) throughout fruit enlargement phase II (Jan. to Mar. 2017) in Citrusdal, South Africa. Different letters within and between irrigation treatments (0.5X, X, 2X) differ significantly at 95% confidence level as tested by Fisher's least significant difference (LSD) test; (n=4).

3. Susceptibility of fruit/tree to damage by insects and infection by fruit pathogens.

Shade nets have become a common feature of the South African citrus industry, but with the increase in netting options available comes a greater need for understanding the potential impact on insect pest populations. Pest populations were monitored weekly and fruit damage evaluated for 20% white, permanent, partially enclosed nets. Three open control blocks and three netted replicates were monitored. False codling moth (FCM), *Thaumatotibia leucotreta*, (Lepidoptera: Tortricidae) was monitored using yellow Delta traps and FCM pheromone (Chempac, Simondium, South Africa), while Mediterranean fruit fly, *Ceratitidis capitata* numbers were monitored with yellow bucket traps and Biolure (Chempac, Simondium, South Africa) attractants. Data

stations consisting of five trees were marked out in each of the replicates and any fallen fruit or fruit showing signs of possible infestation were collected weekly. Fruit evaluations were also conducted on 10 fruit on 10 trees in each replicate shortly prior to harvest to determine any pest damage. The experimental area was within the XSite ((Pty.) (Ltd.) Citrusdal, South Africa) sterile FCM release control programme, therefore differentiation was made between sterile and wild moths. Wild moth numbers were very low and no significant difference was found between nets and open controls. Sterile moth catches were higher on average for the open control areas than permanently netted areas. Overall, fruit fly catches were low, but were higher in the open areas. Fruit collected weekly from data stations showed no indications of FCM or fruit fly damage. Similarly, no FCM or fruit fly damage was found during fruit evaluations done just prior to harvest. Citrus thrips, *Scirtothrips auranti* (Thysanoptera: Thripidae), and bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae), damage was recorded, but there was no significant difference in damage between netted and open areas. This research is ongoing and very relevant due to the continual increase in citrus orchards being netted

4. Comparative analysis of drape netting vs. permanent shade netting (RCE-4 extension).

Introduction

Various different types of permanent protective netting structures and/or different colours of shade netting have been tested for their effects on tree physiology and fruit development and quality (Manja and Aoun, 2019; Mupambi *et al.*, 2018; Stamps, 2009; Wachsmann *et al.*, 2014; Zhou *et al.*, 2018). In *Citrus* spp., non-permanent netting (NPN) is used in certain production regions during a particular stage in the season to protect trees and fruit from damage that could be caused by hail (Wachsmann *et al.*, 2014). It is also used in some citrus cultivars during flowering, to exclude bees thereby preventing cross-pollination and unwanted seed development (Gambetta *et al.*, 2013; Gravina *et al.*, 2016; Otero and Rivas, 2017). In commercial 'Nadorcott' mandarin production, where the potential for cross-pollination is high and seed development likely, some growers cover trees entirely with NPN during flowering in order to exclude pollinators such as honey bees (Gravina *et al.*, 2016; Otero and Rivas, 2017). This method is successful and environmentally safe. However, it is unclear how the covering of trees with NPN before, during and after flowering for an extended period, encompassing tree growth and fruit development, impacts on the deposition of foliar sprays that are applied, insect pest prevalence and fruit production. The monitoring of two important insect pest species was included in the experiment in order to determine the effect of NPN on their numbers. False codling moth (FCM), *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae) and Mediterranean fruit fly, *Ceratitidis capitata* Weidemann (Diptera: Tephritidae) are classified as a phytosanitary pest by a number of export markets (Grout and Moore, 2015). Therefore, monitoring and control of this pest is important for the industry.

Objectives of this study were to evaluate the effects of NPN on foliar spray deposition, insect pest prevalence, leaf mineral nutrient concentration, fruit yield, and quality and rind surface damage of 'Nadorcott' mandarin fruit.

Materials and methods

Plant material and experimental site

The study was conducted in a commercial orchard of 6-year old 'Nadorcott' mandarin trees budded onto 'Carrizo' citrange (*C. sinensis* × *Poncirus trifoliata*) rootstock at Citrusdal in the Western Cape province of South Africa. The region is semi-arid and experiences typical Mediterranean-type climatic conditions. The summer occurs from December to February, autumn from March to May, winter from June to August and spring from September to November. The region receives an annual rainfall of between 400 and 650 mm, of which the majority occurs from the end of autumn to the end of winter (May to August). Trees were planted in a sandy soil at a spacing of 5.5 × 2.5 m, amounting to 727 trees per hectare, and the orchard is orientated in a North-South row direction. The selected orchard was bordered on the north and south sides by 'Nadorcott' mandarin trees, and on the east and west, by 'Navel' sweet orange trees at distances of approximately 200 and 400 m, respectively. The nearest potential cross-pollinator was 'Nules Clementine' mandarin trees located approximately 900 m to the south.

Trees were irrigated using a drip system with four emitters per tree, and total water supply was approximately 8000 L per tree per annum. All trees received consistent and standard fertilizer applications, with the rate of application ($\text{kg}\cdot\text{ha}^{-1}$) based on annual leaf mineral nutrient analysis and a target fruit yield of 60 to 70 ton per ha. Micronutrients were applied mostly in the form of low-volume foliar spray applications from early spring to late summer, and macronutrients *via* the soil (fertigation). A foliar application of gibberellic acid (GA_3) [Progibb® 40% soluble granule (SG) formulation containing 400 $\text{g}\cdot\text{kg}^{-1}$ active ingredient of GA_3 , Valent BioSciences Corporation, Libertyville, IL] was applied to all treatments at 80% petal drop in October 2017.

Experimental design and treatments

The experiment was set up in a completely randomized design and consisted of an untreated control, i.e. open trees without NPN, and three different NPN treatments. The following NPN treatments were applied prior to flowering in Aug. 2017 at 51 state of the *Citrus* BBCH phenological scale (Agustí *et al.*, 1997): 1) trees covered with NPN from Aug. 2017 to Nov. 2017 (NPN Aug. to Nov.), 2) trees covered with NPN from Aug. 2017 to Mar. 2018 (NPN Aug. to Mar.), and 3) trees covered with NPN from Aug. 2017 until harvest in Jul. 2018 (NPN Aug. to Jul.). Each treatment consisted of four replicates ($n=4$) that were applied to entire tree rows. Trees were covered to the ground with a white (18% shade) AHN-55 ($55 \text{ g}\cdot\text{m}^{-2}$) mesh type NPN (Drape Net SA, Johannesburg, South Africa). In each treatment replicate, two data trees were used in the determination of the treatments' effects on fruit yield, fruit quality, leaf mineral nutrient concentration and fruit surface damage ($n=8$). For the determination of the treatments' effects on foliar spray deposition, three data trees were used within each treatment replicate.

Data collection

Foliar spray deposition

To evaluate the effects of NPN on the deposition of foliar sprays in the tree canopy, the control and NPN treatments were sprayed in January 2018 (summer) and in June 2018 (winter) with different water volumes of commercial foliar sprays with a 2 000 L oscillating spray machine (Nieuwoudt Spray, Citrusdal, South Africa). The January foliar sprays were applied at water volumes of 3 500 and 15 000 $\text{L}\cdot\text{ha}^{-1}$. The June sprays were applied at water volumes of 3 500, 7 000 and 15 000 $\text{L}\cdot\text{ha}^{-1}$. Each foliar spray contained fluorescent pigment [South Australian Research and Development Institute (SARDI) Yellow Fluorescent Pigment, 40% EC (SARDI, Loxton, South Australia)] allowing for visualization of the spray deposition on the leaf and fruit surfaces subsequent to spray application. The pigment dosage was adjusted according to the respective spray volumes, viz. 1, 2 and 3 $\text{mL}\cdot\text{L}^{-1}$ pigment was added to the 15 000, 7 000 and 3 500 $\text{L}\cdot\text{ha}^{-1}$ foliar sprays. Foliar sprays were applied to both sides of ten adjacent trees in single rows of NPN and control treatment replicates. Leaves and fruit were sampled from three randomly selected uniform trees within the respective treatment replicates. For the January evaluation, only leaves were sampled; whereas fruit and leaves were sampled for the June evaluation. Samples were collected subsequent to application of the foliar sprays from different tree canopy positions: three vertical positions, viz. top, middle and bottom; and two horizontal positions, viz. inner canopy (leaves 30 to 50 cm inside the tree canopy and in the top, middle or bottom of the tree canopy) and outer canopy (leaves or fruit on the outside of the top, middle or bottom of the tree canopy). From each position, 12 leaves (amounting to a total of 72 leaves per data tree) were collected and transported to the laboratory in labelled plastic bags. Fruit were sampled from the same positions as for leaves. Five fruit were sampled from each position, amounting to a total of 30 fruit per data tree. The side of the fruit that faced the spray machine was marked with a permanent marker pen before the fruit were sampled and placed in labelled carton containers and transported to the laboratory. The leaves and fruit were stored at 4 °C until deposition analysis was conducted as described by Van Zyl *et al.* (2013). Images of leaves and fruit were captured in a dark room with an illuminated ultra-violet light source (UV-A, $\approx 365 \text{ nm}$, Labino Mid Light; www.labino.com). A red Perspex box (300 × 210 × 110 mm) was used to reduce any shadowing and to enhance edges of leaves. A glass pane (200 × 200 × 2 mm) was used to cover leaves, and fruit were placed on a small plastic stand. A camera (Canon EOS 40D, Tokyo, Japan) equipped with a 60 mm macro lens was used to capture digital images in RAW file format (.CR2 $\approx 10 \text{ MB}$) of the upper and lower leaf and fruit surfaces. Digital images were captured of the fruit surface that faced the spray machine and of the fruit surface that faced away from the spray machine. The camera was mounted on a tripod in a fixed position above the Perspex box. RAW images were converted to 8-bit Exif-TIFF (.TIF $\approx 30 \text{ MB}$) format using Digital Photo Professional software version 3.1.0.0 (CANON INC.; www.canon.com) to determine the deposition parameters in a digital analysis (Van Zyl

et al., 2013). For leaves, a camera aperture setting of F10, and an ISO setting of 100 were used. Whereas, an aperture setting of F14 and an ISO setting of 160 were used for fruit.

Deposition quantity was measured as the percentage of leaf or fruit area that was covered by pigment particles, i.e. the percentage fluorescent particle coverage (FPC%). For the deposition quality assessment, the leaf or fruit areas were divided into equally-sized squares of 100 × 100 pixels (10000 pixels). The area size of the leaf or fruit amounted to between 20 and 250 individual squares per leaf. The squares could therefore be used to calculate the percentage area of the fruit or leaf surface that was covered by fluorescent pigment particles, independent of the size of the leaf or fruit. The percentage of the Interquartile Coefficient of Dispersion (ICD%) for each leaf or fruit was used as a measurement of deposition quality, i.e. the uniformity of deposition on the leaf or fruit surface. Low ICD% values were indicative of better deposition quality. Deposition uniformity between leaves or fruit was calculated as the uniformity in pigment deposition in a batch of 12 leaves or 5 fruit (standard deviation × 100/mean). Deposition efficiency was expressed as deposition quantity normalised to FPC% per 1000 L water per hectare.

Insect pest prevalence

False codling moth (FCM) was monitored using a yellow Delta trap which contained a sticky floor and a pheromone lure (Chempac, Simondium, South Africa) to attract males. Mediterranean fruit fly, *C. capitata* were monitored using a Sensus trap (River Bioscience, Port Elizabeth, South Africa) which contained a Capilure (trimedlure) capsule and a small dichlorvos-impregnated block to attract and kill male fruit flies. One trap of each type was placed in the tree canopy in each control, NPN Aug. to Mar., and NPN Aug. to July replicate. The insect pest prevalence in the NPN Aug. to Nov. treatment was not monitored due to the insect lures only being available shortly before the end of November. However, fruit from this treatment were nevertheless evaluated for insect damage and/or insect infestation at time of commercial harvest. Trap examinations were carried out at two-week intervals. False codling moth lures in traps were replaced every 3 months, and fruit fly lures were replaced every 6 weeks. The farm was included in the commercial area-wide Sterile Insect Release programme [Xsit (Pty.) Ltd, Citrusdal, South Africa] in which sterile, male FCM releases are conducted aurally on a weekly basis as part of an Integrated Pest Management (IPM) program. Both wild and sterile FCM males were therefore monitored by traps.

Leaf mineral nutrient concentration

Leaves were sampled according to the South African citrus industry standard, specifically from four- to six-month-old fruiting shoots in March (Du Plessis *et al.*, 1992; Du Plessis and Koen, 1992). One leaf sample consisted of eight leaves that were collected from four shoots between 0800 and 1000 HR on 01 Mar. 2018, from the same treatment replicates that were used to determine effects on fruit yield and quality. After sampling, the leaves were kept cool and washed with distilled water, before being frozen at –80 °C and freeze-dried (Christ Beta 1–8 LD Freeze Dryer, Martin Christ Gefrier Trocknungsanlagen GmbH, Osterode am Harz, Germany). The leaves were ground to a fine powder with an analytical grinder (Yellow line, A10, IKA-Werke, Staufen, Germany) and stored at –80 °C until analysis. Mineral nutrient analyses in leaf samples were conducted by an accredited commercial chemical and microbiology analytical laboratory [Bemlab (Pty) Ltd., Strand, South Africa] according to published protocols (Hou and Jones, 2000). Briefly, 1 g of dried leaf tissue was ashed at 800 °C and made up to a volume of 50 mL with a 50:50 hydrochloric acid (50%) solution for extraction through filter paper. The mineral nutrient concentrations were analysed using inductively coupled plasma-emission spectroscopy (Varian PRX–OEX, Varian Inc., Palo Alto, CA) against suitable standards and subsequent to a nitric-hydrochloric total acid digestion step. For analysis of total nitrogen (N), 0.15 g of each sample was combusted at 850 °C and analysed using a LECO N analyzer (LECO FP528 Nitrogen analyzer, LECO cooperation, St. Joseph, MI) by thermal conductivity. The concentrations of the mineral nutrients in the leaf were expressed as mg·g⁻¹ leaf dry weight (DW) for macronutrients, or mg·kg⁻¹ leaf DW for micronutrients.

Fruit yield

Commercial fruit harvest commenced at the end of July 2018 when fruit quality indices complied with specifications established by fruit export markets as set out by the minimum export standards and requirements for soft citrus (DAFF, 2015). Harvesting was completed by the end of August. To determine the total fruit yield of the respective treatments, fruit from two individual trees within each treatment replicate row were harvested separately on the same day, prior to the start of commercial harvest and the total fruit yield

was determined for each replicate in kg fruit per tree. A sample of 50 fruit was randomly collected from each replicate and the transverse diameter (mm) of each fruit was measured using an electronic fruit size measuring caliper (CD-6" C; Mitutoyo Corp, Tokyo, Japan). Each fruit was assigned to a fruit size category of which the average fruit weight was determined and fruit size distribution from each treatment replicate was extrapolated for the determination total fruit yield per tree in number of fruit per tree.

Fruit quality

To determine the effect of treatments on fruit quality attributes, i.e. fruit size (diameter), total soluble solids (Brix°) of the juice, titratable acidity (TA) of the juice, and fruit juice content (%), a sample of 12 randomly selected fruit was collected from the two individual trees within each treatment replicate row that were used for fruit yield determination. Fruit diameter was measured using an electronic caliper. The fruit were cut longitudinally to evaluate treatment effects on internal quality and any presence of seeds. A citrus fruit juicer (Sunkist®, Chicago, IL) was used to extract the juice from each fruit, whereupon juice percentage (%) could be calculated by dividing the weight of the juice by the total fruit weight. A refractometer (PR-32 Palette, Atago Co., Tokyo, Japan) was used to measure the total soluble solids content as Brix°, and TA was measured (888 Titrando, Metrohm AG, Herisua, Switzerland) and expressed as the citric acid percent. The sugar to acid ratio was calculated by dividing the Brix° by the citric acid (TA) content (Brix°: TA).

Fruit surface damage

Fruit surface damage evaluations were done at time of commercial harvest in Jul. 2018 on the same fruit that were used for fruit quality evaluations. Fruit were examined for sunburn damage, light or severe wind damage and any evidence of pest damage or chemical burn. Wind scarring on citrus is caused by young fruit coming into prolonged repetitive contact with parts of the tree vegetative structure e.g. leaves and twigs. Wind scarring can be a major source of downgrading of citrus fruit and varies depending upon the climate of the area in which the crop is grown (Bedford 1998). Light wind damage was defined as surface scarring equivalent to plates 1 and 2 of the Capespan citrus culling standards (still suitable for export), while severe wind damage was defined as equivalent to plate 3-6 (unsuitable for export). The wind damage categories may have included damage that was caused by the abrasion between the fruit and the NPN, since it was impossible to distinguish between damage caused by wind and damage caused by NPN scarring.

Statistical analysis

STATISTICA data analysis software version 14 (Dell Inc. 2017, Round Rock, TX) was used to analyse the fruit yield, fruit quality and leaf mineral nutrient concentration data. Analyses of variance (ANOVA) were performed and mean separations were carried out using Fisher's least significant difference test where applicable, at $P \leq 0.05$. Deposition quantity (FPC%), quality (ICD%) and uniformity (CV%) data for the different spray volume and NPN (covered or open) treatment combinations were subjected to ANOVA using SAS version 8.2 statistical software (SAS institute Inc., 1999). The Student's *t*-test for least significant difference ($P \leq 0.05$) was used. The skewing effect of outliers was negated by using median FPC% values of the 12 leaves or 5 fruit for deposition analysis. Data from upper and lower leaf surfaces were analyzed separately but were combined when describing the results.

Results

Foliar spray deposition

The January foliar spray deposition analysis on leaves showed a significant interaction between foliar spray volume and horizontal canopy position of leaves for deposition quantity (FPC%) ($P < 0.0001$), deposition uniformity (CV%) ($P = 0.0004$) and deposition quality (ICD%) ($P = 0.0028$) (not shown). There was also a significant NPN treatment (covered vs. open) effect for FPC% ($P = 0.0055$) and CV% ($P = 0.0062$). For ICD%, this effect was non-significant ($P = 0.1207$) (not shown).

The interaction between foliar spray volume and horizontal canopy position showed that the 3 500 L·ha⁻¹ foliar spray volume resulted in a FPC% of 9.6 on leaves on the outside of the tree canopy. This was similar to the FPC% of the 15 000 L·ha⁻¹ foliar spray volume, irrespective of horizontal canopy position of leaves (FPC% inside = 8.5 and FPC% outside = 8.8). The 3 500 L·ha⁻¹ foliar spray volume resulted in an FPC% of 2.8 on

leaves on the inside of the tree canopy. This was significantly lower than the FPC% of the 15 000 L·ha⁻¹ foliar spray volume at the different horizontal canopy positions.

The CV% results showed that the 15 000 L·ha⁻¹ foliar spray volume resulted in the best application uniformity on leaves on the outside of the tree canopy (CV% of 45.8), which was similar to the same application for leaves on the inside of the canopy (CV% of 50.2). These values were both significantly better (lower CV%) than the CV% in the 3 500 L·ha⁻¹ foliar spray volume. For the 3 500 L·ha⁻¹ foliar spray volume, the CV% on leaves at the inside of the canopy was 108.1 and at the outside of the canopy it was 76.3 CV%, with these two values being significantly different.

The best deposition quality (lowest ICD%) was observed on leaves on the inside of the tree canopy, with the 15 000 L·ha⁻¹ foliar spray volume (ICD% of 59.5). For leaves on the outside of the tree canopy the 15 000 L·ha⁻¹ foliar spray volume resulted in an ICD% of 66.1, which was significantly poorer compared to the ICD% for leaves on the inside of the tree canopy. Whereas, the 3 500 L·ha⁻¹ foliar spray volume resulted in similar ICD% on leaves on the inside and outside of the tree canopy (64.4 vs. 61.7 ICD%, respectively).

For the January foliar spray deposition analysis, the greatest FPC% was observed on control leaves of NPN treatments (8.8 and 6.1 FPC%, respectively), regardless of foliar spray volume (Table 5.3.3.5). Similarly, foliar spray volume had no influence on CV%, although it was significantly better on leaves in control compared to NPN treatments (64.9 and 75.2 CV%, respectively) (Table 5.3.3.5). There was no significant difference in the ICD% on leaves between NPN and control treatments (64.4 and 61.5 ICD%, respectively) (Table 5.3.3.5).

Table 5.3.3.5 Effects of non-permanent netting (NPN) on deposition quantity (FPC%), deposition uniformity (CV%) and deposition quality (ICD%) of foliar spray on leaves of 'Nadorcott' mandarin trees at either 15 000 or 3 500 L·ha⁻¹ in January 2018

Treatments	FPC%	CV%	ICD%
Control	8.8a ^z	64.9b	61.5
NPN	6.1b	75.2a	64.4
<i>P</i> value	0.0055	0.0062	0.1207

^z Different letters in the same column denote significant differences between values at the 95% confidence level.

In the June leaf deposition analysis, there was a significant interaction between foliar spray volume and vertical and horizontal canopy positions for FPC% ($P = 0.0062$). The reason for this interaction was most likely due to the 3 500 L·ha⁻¹ spray volume having significantly lower FPC% values at the different horizontal and vertical canopy positions compared to the other two spray volumes. However, bearing in mind the aim of the study, more important was the significant NPN treatment effect (covered vs. open) ($P = 0.0035$). For CV%, the analysis showed a significant interaction between foliar spray volume and vertical tree canopy position of leaves ($P = 0.0043$), as well as a significant relationship between foliar spray volume and horizontal canopy position of leaves ($P = 0.0002$). The different spray volumes resulted in mean CV% values at the different vertical canopy positions that were between 54.4 CV% and 99.8 CV%. Similarly, the mean CV% resulting from the different spray volumes at the different horizontal canopy positions ranged from 48.3 CV% and 86.6 CV%. For CV%, an important effect was again the significant NPN treatment (covered vs. open) ($P = 0.0014$) effect. Results for ICD% showed a significant horizontal canopy position effect ($P = 0.0021$), foliar spray volume effect ($P < 0.0001$) and also a NPN treatment (covered vs. open) ($P = 0.0393$) effect.

The NPN treatment effect results on leaves in June showed that, irrespective of the foliar spray volume, significantly more fluorescent pigment was deposited on leaves of control trees compared to leaves of NPN trees (4.8 and 3.1 FPC%, irrespectively) (Table 5.3.3.6). Comparison of the CV% in trees from the different

NPN (covered vs. open) treatments showed that CV% on leaves of control trees was better compared to leaves of NPN trees (59.6 vs. 80.5 CV%), irrespective of foliar spray volume (Table 5.3.3.6).

Table 5.3.3.6 Effects of non-permanent netting (NPN) on deposition quantity (FPC%), deposition uniformity (CV%) and deposition quality (ICD%) of foliar spray on leaves of 'Nadorcott' mandarin trees at either 15 000, 7 500 or 3 500 L·ha⁻¹ in June 2018

Treatments	FPC%	CV%	ICD%
Control	4.8a [‡]	59.6a	79.4b
NPN	3.1b	80.5b	84.2a
<i>P</i> value	0.0035	0.0014	0.0393

[‡]Different letters in the same column denote significant differences between values at the 95% confidence level.

For ICD%, irrespective of NPN treatments (covered vs. open) or canopy position, the best ICD% was obtained at the 7500 L·ha⁻¹ foliar spray volume, which was similar to the 15 000 L·ha⁻¹ foliar spray volume (73.2 vs. 77.7 ICD%), but significantly better than the 3 500 L·ha⁻¹ (94.4 ICD%). Regardless of application volume or NPN treatments (covered vs. open), the leaf deposition analysis showed that the ICD% on leaves on the outside of the tree canopy was significantly better compared to leaves on the inside of the tree canopy (79.8 and 83.8 ICD%, respectively). However, as for FPC% and CV%, the ICD% in control trees was significantly better compared to NPN trees (79.4 and 84.2 ICD%, respectively) (Table 5.3.3.6).

The ANOVA of the fruit deposition analysis done in June indicated that there was a significant interaction between foliar spray volume, NPN treatments (NPN vs. covered) and vertical canopy position of leaves for FPC% ($P = 0.0342$). The results from this interaction indicated that the FPC% resulting from the different spray volumes at the different vertical canopy positions ranged between 5.5 FPC% and 22.6 FPC%. The results also showed a significant interaction between foliar spray volume and horizontal canopy position of leaves ($P = 0.0018$). For this interaction it was seen that, irrespective of NPN treatment, the 3 500 L·ha⁻¹ application resulted in statistical similar FPC% values on the inside (9.2 FPC%) and outside (6.9 FPC%) of tree canopies. The inside value was similar to that of the 7 500 L·ha⁻¹ application on the inside (10.1 FPC%) on the inside of the canopy. On the outside of the canopy, this volume resulted in a FPC% of 15.3. This was statistically similar to those obtained by the 15 000 L·ha⁻¹ application on the outside (17.9 FPC%) and inside (16.9 FPC%) of the tree canopy. Most importantly for this study was the observed significant interaction between foliar spray volume and NPN treatments (covered vs. open) for FPC% ($P = 0.0262$).

The 15 000 L·ha⁻¹ foliar spray volume resulted in the best FPC% on fruit at both horizontal canopy positions (inside = 16.9 FPC% vs. outside = 17.9 FPC%), irrespective of NPN treatment (covered or open) (Table 5.3.3.7). The 7 500 L·ha⁻¹ foliar spray volume resulted in a FPC% of 15.26 on fruit on the outside of the tree canopy, which was similar to the FPC% on fruit for the 15 000 L·ha⁻¹ foliar spray volume. However, on fruit on the inside of the tree canopy, the 7 500 L·ha⁻¹ foliar spray volume resulted in significantly poorer FPC% (10.14). The poorest FPC% on fruit resulted from the 3 500 L·ha⁻¹ foliar spray volume (inside = 9.3 FPC% and outside = 6.9 FPC%) (Table 5.3.3.7). The FPC% in the 3 500 L·ha⁻¹ foliar spray volume was similar on fruit in the control (8.8 FPC%) and NPN treatments (7.4 FPC%) (Table 3). For the 7 500 L·ha⁻¹ foliar spray volume, the FPC% values on fruit differed significantly between the control and NPN treatments. On the fruit from control trees, the FPC% was 19.3 and 6.1 on fruit from NPN treatments (Table 5.3.3.7). At the 15 000 L·ha⁻¹ foliar spray volume the FPC% on fruit was similar for NPN and control trees (15.3 vs. 19.5 FPC%).

The results on fruit for deposition uniformity (CV%) showed a significant interaction between foliar spray volume, NPN treatments and vertical canopy position of fruit ($P = 0.0344$). This interaction was most likely again due to the different spray volumes resulting at wide ranging CV% values at the different vertical positions. These values ranged from 26.7 CV% and 80.8 CV%. A significant interaction between foliar spray volume and horizontal canopy position ($P = 0.0104$) was furthermore observed for CV%, along with a significant interaction

between foliar spray volume and NPN treatments ($P = 0.0484$). At the different horizontal canopy positions, varied results were observed in terms of the mean CV%. On the inside of the tree canopies the 3 500 L·ha⁻¹ application had a 40.0 CV% that was statistically comparable to that of the 15 000 L·ha⁻¹ application at the inside (40.3 CV%) and outside (47.1 CV%). On the outside of canopies the 3 500 L·ha⁻¹ resulted in a CV% of 48.6. This was again similar to the 7 500 L·ha⁻¹ at the outside (55.8 CV%) of the canopies. This volume performed the poorest at the inside of the tree canopies (65.3 CV%).

Results from the spray volume and NPN treatment interaction showed that the 15 000 L·ha⁻¹ foliar spray volume resulted in similar CV% on fruit from NPN and control trees (43.1 and 44.3 CV%) (Table 5.3.3.7). On fruit from control trees, the 7 500 L·ha⁻¹ foliar spray volume resulted in similar CV % compared to that obtained for the 15 000 L·ha⁻¹ foliar spray volume. However, in the NPN treatment, the 7 500 L·ha⁻¹ foliar spray volume resulted in the poorest (70.3 CV%) ICD% (Table 5.3.3.7). The 3500 L·ha⁻¹ foliar spray volume performed similar to the 15 000 L·ha⁻¹ foliar spray volume in terms of CV%, for both NPN and control treatments (47.8 and 40.9 CV%) (Table 5.3.3.7).

The results for deposition quality (ICD%) analysis showed a significant vertical canopy position effect on fruit ($P = 0.0386$), along with a significant interaction between foliar spray volume and horizontal canopy position of fruit ($P = 0.0205$). Furthermore, a significant interaction occurred between foliar spray volume and NPN treatment ($P = 0.0157$). For the vertical canopy position effect it was seen that at the bottom (65.9 ICD%) and top (66.2 ICD%) the ICD% was statistically similar but better than at the middle of the tree canopies (70.4 ICD%). In terms of the volume and horizontal canopy position interaction the 15 000 L·ha⁻¹ application had the best ICD% at the inside (57.7 ICD%) and outside (58.2 ICD%) of canopies. This was followed by the 7 500 L·ha⁻¹ on the outside (65.0 ICD%) that was poorer than observed for the 15 000 L·ha⁻¹. The poorest ICD% was achieved by the 3 500 L·ha⁻¹ application. This resulted in an ICD% of 74.1 on the inside of the canopy and 77.8 ICD% on the outside of the canopy.

Table 5.3.3.7 Mean deposition quantity (FPC%), deposition uniformity (CV%) and deposition quality (ICD%) values for the significant foliar spray water volume x treatment interaction on the fruit from non-permanent netting (NPN) or control trees sprayed at either 15 000, 7 500 or 3 500 L·ha⁻¹ in June 2018

Foliar spray water volume (L·ha ⁻¹)	Treatments	FPC%	CV%	ICD%
15 000	Control	19.5a ²	44.3b	54.9b
	NPN	15.3a	43.1b	60.9b
7 500	Control	19.3a	50.9b	57.1b
	NPN	6.1b	70.3a	79.9a
3 500	Control	8.8b	40.9b	74.1a
	NPN	7.4b	47.8b	77.8a
<i>P</i> value		0.0262	0.0484	0.0157

² Different letters in the same column denote significant differences between values at the 95% confidence level.

For the volume and NPN treatment interaction the poorest ICD % values were recorded on fruit from trees sprayed with 3 500 L·ha⁻¹ foliar spray volume, for both the NPN and control trees. These values were similar to that of fruit from NPN trees that were sprayed with 7 500 L·ha⁻¹. On fruit from control trees, the ICD% was significantly better compared to fruit from the NPN trees (57.1 and 79.9 ICD %, respectively) (Table 5.3.3.7). As for the CV%, the 15 000 L·ha⁻¹ foliar spray volume resulted in the best ICD%, which was similar on fruit from control and NPN treatments (54.9 and 60.9 ICD%, respectively) (Table 5.3.3.7).

Insect pest prevalence

Overall, the experimental area had a low pest prevalence (Fig. 5.3.3.3). Nevertheless, meaningful results were obtained. The average number of weekly sterile male FCM catches in the control traps was considerably higher most weeks when compared to both NPN treatments between January and June (Fig. 5.3.3.3A). However, a few sterile moths were caught in traps that were under the NPN (Fig. 5.3.3.3A). An increase in sterile male FCM activity was observed for the Aug. to Mar. NPN treatment once the nets had been removed in March, leading to a peak in activity in July. Trap catches of wild FCM were very low, with four wild males trapped in the control and one wild male in the NPN August to March treatment for the duration of the experiment. No wild FCM males were caught in the NPN treatments during the times when the netting was in place (Fig 5.3.3.3B). Wild FCM males were only caught in the control treatment and in one of the NPN treatments after the netting had been removed (Fig. 5.3.3.3B). Regarding fruit flies, the same pattern was repeated as with FCM catches greatly reduced beneath the NPN (Fig. 5.3.3.3C).

Leaf mineral nutrient concentrations

There were no significant differences between the concentrations of any of the macronutrients nitrogen (N), phosphorous (P), potassium (K), calcium (Ca) and magnesium (Mg) in leaves of the control and the NPN treatment in March (Table 5.3.3.8). For micronutrients, the concentrations of zinc (Zn) ($P = 0.0317$) and iron (Fe) ($P = 0.0041$) were significantly higher (by 81 and 78%, respectively) in leaves of the control treatment compared with leaves of the NPN treatment (Table 5.3.3.8). There were no differences in the concentrations of sodium (Na), manganese (Mn), copper (Cu) and boron (B) between leaves of the control treatment and leaves of the NPN treatment in March (Table 5.3.3.8).

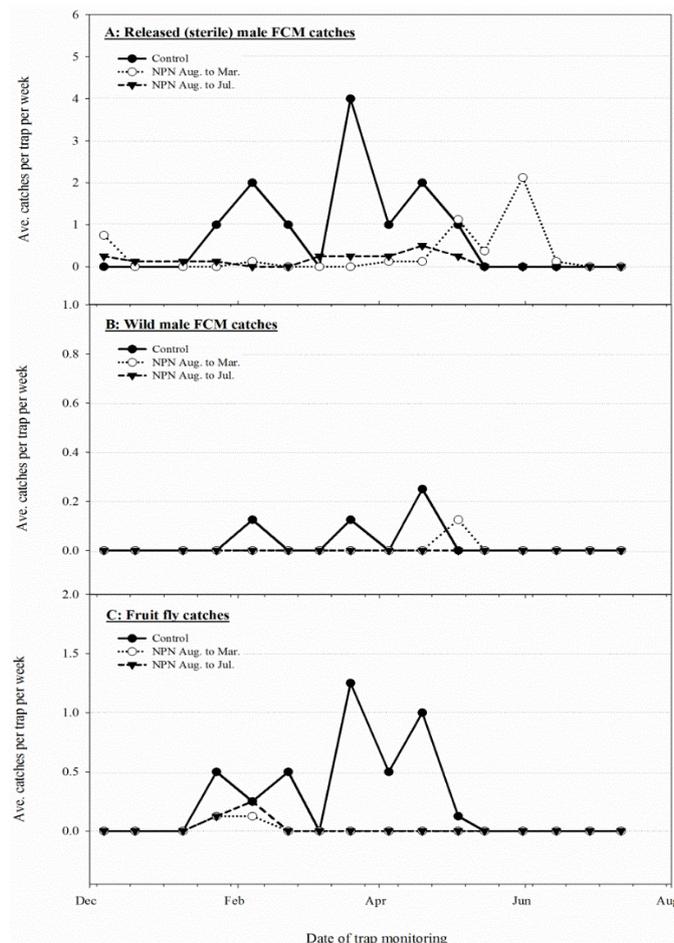


Figure 5.3.3.3. The average number of A) released (sterile), male false codling moth (FCM) (*Thaumatotibia leucotreta*), B) wild, male FCM, and C) fruit flies (*Ceratitis capitata*) catches per trap per week for control and non-permanent netting (NPN) treatments from Dec. 2017 to Jul. 2018.

Table 5.3.3.8 Effects of non-permanent netting (NPN) on leaf mineral nutrient concentration in March 2018

Treatments (n=8)	Leaf macronutrients concentration (mg·g ⁻¹ leaf DW)					
	<u>N</u>	<u>P</u>	<u>K</u>	<u>Ca</u>	<u>Mg</u>	
Control	31.1	2.0	12.3	37.1	3.1	
NPN	32.9	1.9	12.9	38.1	3.3	
<i>P</i> value	0.0516	0.7873	0.6653	0.7413	0.6545	
	Leaf micronutrients concentration (mg·kg ⁻¹ leaf DW)					
	<u>Na</u>	<u>Mn</u>	<u>Zn</u>	<u>Fe</u>	<u>Cu</u>	<u>B</u>
Control	387	132	40a [‡]	277a	3.5	60
NPN	401	107	22b	156b	3.5	73
<i>P</i> value	0.7884	0.3623	0.0317	0.0041	1.0000	0.2109

[‡]Different letters in the same column denote significant differences between values at the 95% confidence level.

Fruit yield

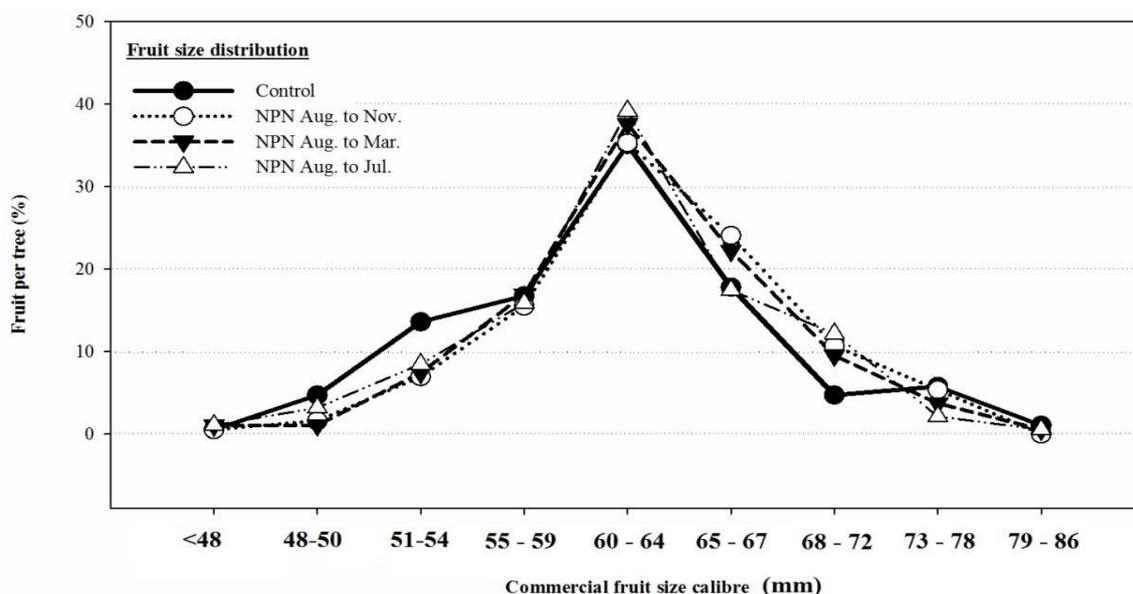
In the NPN August to November and NPN August to March treatments, fruit yield (kg fruit per tree, $P = 0.0008$; no. fruit per tree, $P = 0.0002$) was significantly lower compared to the control and the NPN August to July treatments (Table 5.3.3.9). The NPN August to March treatment resulted in the lowest fruit yield (69 kg and 765 fruit per tree), but it did not differ significantly from fruit yield of the NPN August to November treatment (72 kg and 782 fruit per tree) (Table 5.3.3.9). The NPN August to July treatment resulted in the highest fruit yield (102 kg and 1157 fruit per tree), but it did not differ significantly from fruit yield of the control (95 kg and 1112 fruit per tree) (Table 5.3.3.9). Fruit yield of all the NPN treatments consisted of a higher amount of larger sized fruit (SC1 to SC1XX) (not significantly), whereas fruit yield in the control consisted of a greater amount of smaller sized fruit (SC4 to SC 5) (Fig. 5.3.3.4).

Table 5.3.3.9 Effects of different non-permanent netting (NPN) treatments on fruit yield of 'Nadorcott' mandarin

Treatments (n=8)	Fruit yield	
	<u>kg per tree</u>	<u>No per tree</u>
Control	95a [‡]	1112a
NPN Aug. to Nov.	72b	782b
NPN Aug. to Mar.	69b	765b
NPN Aug. to Jul.	102a	1157a
<i>P</i> value	0.0008	0.0002

[‡] Different letters in the same column denote significant differences between values at the 95% confidence level.

Figure 5.3.3.4 Effects of different non-permanent netting (NPN) treatments on the distribution of different commercial fruit size calibres (SC) in the yield of 'Nadorcott' mandarin.



Fruit quality

The NPN treatments had no significant effects on average fruit diameter, fruit rind colour, juice content and juice Brix° compared to the control (Table 5.3.3.10). Fruit from the NPN August to November treatment had a significantly lower juice TA content compared to the control and the NPN August to March and NPN August to July treatments (Table 5.3.3.10). The NPN August to November and the NPN August to July treatments had similar Brix°: TA ratios, but significantly higher Brix°: TA ratios compared to the control and the NPN August to March treatments (Table 5.3.3.10). No seeds were found in any of the sampled fruit, in any treatments

Table 5.3.3.10 Effects of different non-permanent netting (NPN) treatments on fruit quality of 'Nadorcott' mandarin

Treatments (n=8)	Diameter	Colour	Brix°	TA	Brix°:	
					TA	Juice%
Control	62	1.2	12.3	1.10a ²	13.4b	61
NPN Aug. to Nov.	63	1.3	11.9	0.99b	15.1a	62
NPN Aug. to Mar.	63	1.3	11.8	1.09a	13.4b	63
NPN Aug. to Jul.	62	1.3	11.8	1.09a	15.1a	62
P value	0.8754	0.8520	0.1884	0.0387	0.0358	0.8741

²Different letters in the same column denote significant differences between values at the 95% confidence level.

Fruit surface damage

Overall, light wind damage was most common (42%), followed by undamaged or clean fruit (36%), and fruit showing severe wind damage (19%) (Fig. 5.3.3.5). Other types of damage noted were very low, with 1.4% of fruit showing indications of pest damage and 1% of fruit showing chemical burn. The pest damage was highest for leafhopper (*Empoasca distinguenda* Paoli) at 0.8%, followed by bollworm (*Helicoverpa armigera* Hübner) at 0.3% and citrus thrips (*Scirtothrips aurantii* Faure) at 0.3%. When comparing the wind damage on fruit from inside the canopy, the percentage of light wind damage was higher for fruit from the control treatment compared to NPN treatments (Fig. 5.3.3.5). Inner canopy fruit in the NPN August to November treatment had

the highest percentage of clean fruit and the lowest percentage of light wind damage, but also had the greatest percentage of severe wind damaged fruit (Fig. 5.3.3.5A). For the outer canopy fruit, the amount of fruit suffering wind damage, particularly in the severe wind damage category, was significantly greater than that of the inner canopy. The NPN August to July treatment showed an equal percentage of clean fruit to the NPN August to March treatment, but the percentage of severe wind damage was lower (Fig. 5.3.3.5B).

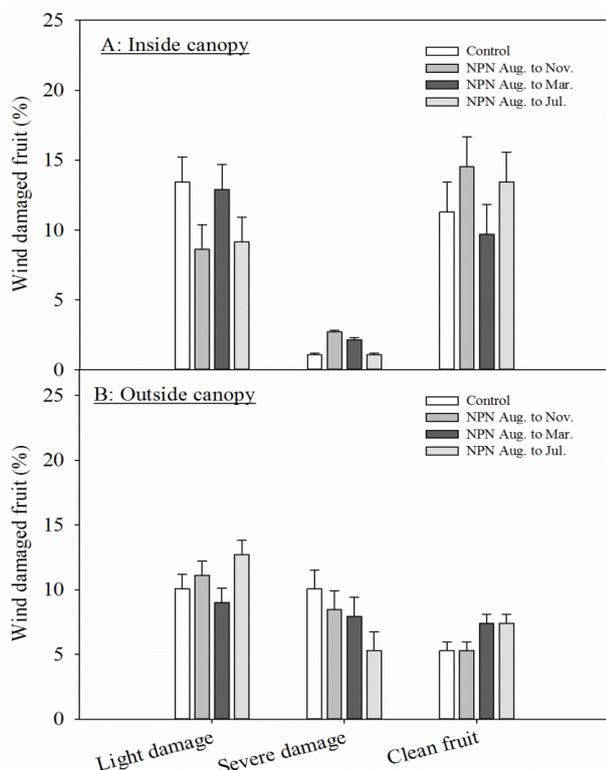


Figure 5.3.3.5 Wind damage of fruit from A) inside and B) outside tree canopies of control and non-permanent netting (NPN) treatments at time of commercial harvest in Jul. 2018.

The NPN August to November (22%) and the NPN August to March (21%) treatments resulted in similar fruit sunburn damage compared to the control (27%) (Fig. 5.3.3.6). The longer NPN treatment (NPN August to July) resulted in significantly lower percentage (9%) of sunburned fruit compared to the control, but similar percentage of sunburned fruit compared to the NPN August to March treatment (Fig. 5.3.3.6).

Discussion

The use of NPN reduced the efficacy of foliar spray applications to 'Nadorcott' mandarin trees by reducing the deposition quantity of foliar sprays in the tree canopy by up to 44%, the deposition uniformity by up to 35% and deposition quality by up to 40%, compared to open trees. These effects were obtained at low and high foliar spray volumes in summer, and on leaves from different canopy positions. In winter, NPN reduced foliar spray deposition of a medium volume foliar spray on leaves and fruit at different canopy positions. The practice of applying NPN in orchards is popular for short periods in citrus producing countries such as Australia, Chile and the USA (personal communication with Etienne Rabe, Wonderful Citrus, California, and Tim Grout, CRI), because of the efficacy of the method in excluding bees and to produce seedless fruit (Gambetta *et al.*, 2013; Otero and Rivas, 2017). In the main, producers would not be able to produce seedless fruit without NPN, and the method is safe to the environment. However, this is the first study to quantify the effects of NPN on foliar spray deposition on leaves and fruit in citrus tree canopies. Our results show that 'Nadorcott' mandarin growers that are considering using NPN would most probably have to adjust foliar spray applications of pesticides and fungicides, and possibly also plant growth regulator (PGR) and mineral nutrient foliar sprays to increase efficacy of targeted foliar sprays.

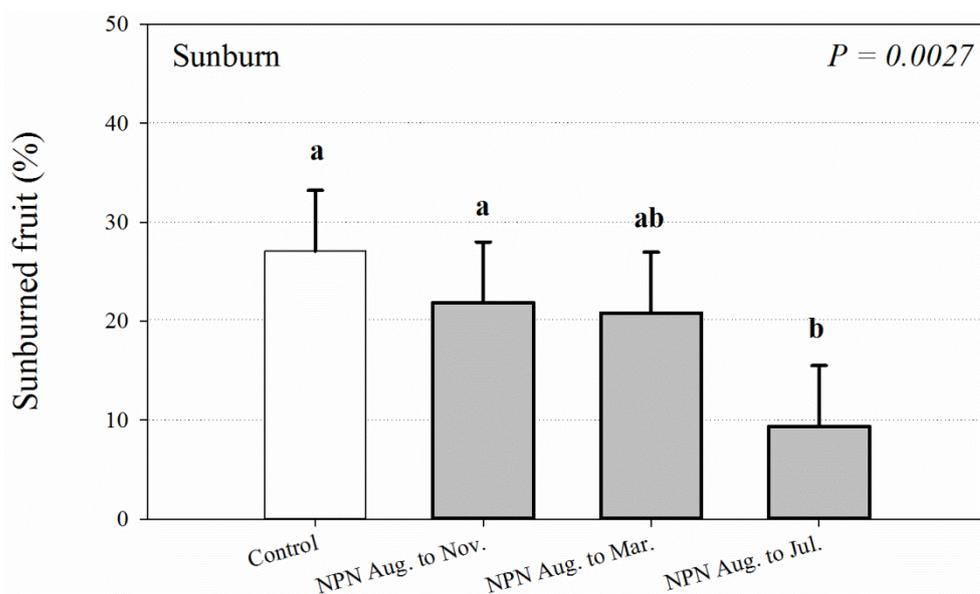


Figure 5.3.3.6. Effects of different non-permanent netting (NPN) treatments on sunburn of 'Nadorcott' mandarin fruit.

The trees in the experimental orchard were planted in a sandy soil, where it is standard practice to supply a proportion of certain mineral nutrients by low-volume foliar spray applications in spring and early summer (Lovatt, 2013), the period when coverage of trees by NPN is recommended (Gambetta *et al.*, 2013; Otero and Rivas, 2017). There were no differences in the concentrations of macronutrients in leaves between control and NPN treatments. The concentrations of the micronutrients Mn (<23%, $P = 0.3623$), Zn (<81%, $P = 0.0317$) and Fe (<78%, $P = 0.0041$), however, were lower in leaves of NPN treatments compared to the control. Considering that NPN treatments reduced spray deposition quantity by 44%, it could have been possible that a large portion of the foliar-applied micronutrients did not reach the trees. Furthermore, the deposition uniformity of foliar sprays in NPN was reduced by 35%, and the translocation of Zn and Mn from sprayed to unsprayed leaves is usually minimal (Embleton *et al.*, 1988; Swietlik, 1996). Micronutrients such as Mn and Zn play important roles in citrus (Embleton *et al.*, 1988), particularly during spring and summer, and can be limiting to fruit quality, especially in sandy soils (Smith, 1966). For example, Swietlik (1996) reported an increase in fruit yield of grapefruit (*C. paradisi*) trees following a Zn foliar spray when 20% or more leaves showed Zn deficiency symptoms; and Embleton *et al.* (1988) showed that a Mn deficiency in citrus leaves correlates with reduced fruit size and a high Brix°: TA ratio in fruit juice at time of harvest.

The long NPN treatment (August to July) reduced fruit sunburn damage by 17%, but outer canopy fruit suffered increased wind damage or scarring compared to the control. Except for a lower TA in the shortest NPN treatment (August to November) and a higher Brix°: TA ratio in two NPN treatments, the lower micronutrient concentrations in leaves did not impact on other fruit quality attributes.

Short NPN treatments (August to March) reduced fruit yield by 33 and 37% compared to the control, most likely through a reduction in fruit set, and/or an increase in fruit drop (Gambetta *et al.*, 2013; Gravina *et al.*, 2016; Otero and Rivas *et al.*, 2017). In 'Nadorcott' mandarin trees that were completely covered by NPN, Gambetta *et al.* (2013) reported that NPN reduced fruit set, and Otero and Rivas (2017) showed that entire coverage of 'Nadorcott' mandarin tree canopies by NPN reduced fruit yield by 66%. In this study, late removal of NPN treatments had no negative effects on fruit yield compared to the control, and confirm that 'Nadorcott' mandarin is an obligate parthenocarp (Gambetta *et al.*, 2013). It indicates that, although NPN reduced the deposition quantity of foliar sprays, lower fruit yields in shorter NPN treatments were probably not related to the effects of NPN treatments on fruit set and foliar spray deposition, especially of GA₃ sprays at petal drop to increase fruit set (El-Otmani *et al.*, 2000; Otero and Rivas *et al.*, 2017). Trees covered by NPN for a period longer than March received the same foliar sprays in spring compared to trees covered by NPN for a shorter period. If the reduction in fruit yield was related to the lower foliar spray deposition, especially of GA₃, the coverage of trees by NPN until fruit harvest would have had the same impact on fruit yield than shorter NPN

treatments. In fact, a longer NPN treatment would probably have excluded other important foliar sprays too. However, the longer NPN treatment had up to 38% more fruit per tree than shorter NPN treatments (1157 vs. 782 and 765, $P = 0.0002$), and similar fruit yield compared to the control (1157 vs. 1112, $P = 0.0002$).

In other studies, on the use of NPN in 'Nadorcott' mandarin, an application of GA₃ to trees under NPN was able to compensate for the negative effects of NPN on fruit set (Otero and Rivas, 2017). In this study, however, the lower fruit yield in shorter NPN treatments did not appear to be related to any negative effects of NPN on fruit set or GA₃ sprays. The fruit yield reduction was most likely caused by fruit drop that was exacerbated by the physical act of NPN removal. Shorter NPN treatments were removed at the end of November, close to the end of physiological fruit drop period when fruitlets are particularly sensitive to abscission. In order to totally exclude bees, the NPN are draped over entire tree canopies and covered by sand or rocks at the base of the tree row. The weight of the NPN and the dragging action by which it is removed from tree rows put pressure on the tree and causes a shaking, and, in some cases, breaking of tree limbs, fruiting branches and twigs, which could exacerbate fruit drop. Of particular interest in this study, is that 'Nadorcott' mandarin fruitlets were sensitive to drop until as late as March and only became insensitive to fruit drop when fruit were fully mature by July.

Although it did not appear that the lower micronutrient concentrations in leaves were related to the lower fruit yield or fruit quality, a lower leaf micronutrient concentration could affect fruit production in the long term, or in other orchards, especially if nutritional sprays or application methods are not adjusted. The differences in the leaf micronutrient concentrations between NPN and control treatments were significant. However, the rate of nutrient applications in the experimental orchard was high and the concentrations of various mineral nutrients in leaves of both the control and the NPN treatments remained within the biological norm for citrus (Du Plessis *et al.*, 1992; Du Plessis and Koen, 1992). In commercial orchards where the rate of mineral nutrient applications is insufficient, the effects of NPN on leaf micronutrient concentrations through reduction in foliar spray deposition could impact negatively on fruit production.

This experimental site did not have a high level of citrus pest infestation and the disease pressure in the production region is known to be low due to the semi-arid and typical Mediterranean type climate. It would be of interest to test NPN in other areas where pest populations are high, or a particular species is known to be a problem. Nevertheless, the use of NPN in this study resulted in a clear reduction in moth trap catches in the case of both sterile and wild FCM when compared to the open controls and showed that NPN could help to exclude larger citrus pests. This is unlikely to be total exclusion, as it was still possible to capture released, sterile FCM males and fruit flies under NPN which may be due to gaps forming in the nets as a result of wear and tear.

Conclusion

In general, the use of NPN in the production of 'Nadorcott' mandarin reduced foliar spray deposition on leaves and fruit, and resulted in lower concentrations of certain micronutrients in leaves. In trees where NPN was removed before July, fruit yield was reduced by up to 37%, but a longer NPN treatment resulted in similar fruit yield compared to the control. The reduction in fruit yield by the shorter coverage periods of the trees was not related to the effects of NPN on foliar spray deposition in spring and early summer, or to lower leaf micronutrient concentration. The lower fruit yield in short NPN treatments was most likely caused by fruit drop that was exacerbated by the physical removal of NPN. In orchards where the rate of mineral nutrient applications is insufficient, the effects of NPN on leaf micronutrient concentrations through reduction in foliar spray deposition could impact negatively on fruit production in the long term. The use of NPN had no effects on commercial fruit quality attributes and in the NPN treatment applied until fruit harvest, reduced sunburn damage of fruit by 17%. However, outer canopy fruit suffered increased wind damage or scarring. Citrus growers in very windy areas should therefore bear this in mind if considering making use of NPN. The use of NPN in this preliminary study did show an exclusionary effect on wild FCM males and a reduction in trap catches of sterile FCM males overall, which indicates that NPN can aid in preventing larger citrus pests from reaching the tree. However, successful exclusion of pests too large to fit through the holes in the netting would have to involve strict application of the NPN such that the edges of the netting are buried beneath the soil surface surrounding the tree row preventing ingress of such pests and constant general net maintenance. The observation that a very

low number of Medfly and sterile FCM males were caught within the netting perhaps indicates the difficulty of achieving total exclusion.

Final conclusion and recommendations

Considering the results from this study, the production practices, and specifically crop manipulations, of 'Nadorcott' mandarin fruit under permanent shade netting would not differ to a large extent, compared to a standard commercial open orchard. Fruit set evaluations were not affected which indicates the normal gibberellic acid (GA₃) fruit set sprays will still be needed. In addition, the use of chemical fruit thinning agents at the normal timing and concentration would still be recommended under shade netting for years when high fruit loads are expected. It should, however, be noted that shade netting may further enhance the effect on fruit size in reaction to these thinning treatments. Furthermore, as the trees under the shade netting exhibited increased vegetative growth, the use of chemical vegetative growth inhibitors such as uniconazole may be a useful manipulation during spring to restrict excessive growth of the summer shoots. Finally, as the trees reach maturity, additional pruning strategies may be required to prevent dense tree canopies and overshadowing of potential bearing units, which may lead to decreased productivity and fruit quality.

To conclude the 20% white shade netting did alter the microclimate of a 'Nadorcott' mandarin orchard in Citrusdal, Western Province, South Africa as well as the leaf physiology associated with photosynthesis especially during heat stress periods during the summer. These changes in microclimate and leaf physiology effected the carbohydrate level in a citrus tree especially in the leaves with an increased availability in non-structural carbohydrate starch. However, less changes in root carbohydrates were observed over the two growing seasons. The increased soil water content and lower solar radiation experienced under the shade net increased the tree water potential. These aspects resulted in an increased the vegetative growth of 'Nadorcott' mandarin without affecting any reproductive parameters. The 20% white shade netting was effective in reducing the sunburn incidence of fruit grown under the net, which in turn lead to a higher packout percentage, without negatively influencing the external and internal quality parameters. The shade nets also had no influence on the fruit development (size, rind colour, internal quality), with similar trends observed over the season for both treatments. Furthermore, the quality of shade netted fruit when subjected to cold storage at either 4 or -0.6 °C was not negatively influenced, with no effect of the shade net evident on the incidence of the physiological disorder, staining, during cold storage. The rind cutting, puncture and fruit compression tests results serves as a guideline value of the threshold force that can be applied before damage is inflicted on 'Nadorcott' mandarin fruit. It may also be of value in adapting the harvest- and packhouse procedures to ensure that a minimum force is subjected on the fruit at all times. However further investigation is needed in order to draw clear conclusions regarding the effect of shade netting on the rind properties of 'Nadorcott' fruit. The study area had very low wild insect pest populations. Movement of sterile FCM was possible under the permanent netting, but recaptures were lower under the nets. NPN does appear to be a barrier to the movement of larger citrus pests such as FCM and fruit fly, but this was not total exclusion as some sterile FCM and fruit fly catches were recorded. Damage to NPN during the season compromises net integrity, therefore maintenance is essential.

Recommendations

Shade netting does offer the citrus producer the potential to improve the microclimate of an orchard, especially during the summer months, when conditions considered stressful to citrus tree physiology arise. Shade netting can alter the potential to increased carbon assimilation during heat stress periods thereby improving fruit growth in the specific period. This aspect should be further evaluated in different climatic regions to establish whether the shade net has the same effect in winter and summer rainfall areas. Due to reduced evaporative demand in summer of the soil-plant-atmosphere continuum the shade net could offer the potential to reduce irrigation volumes without causing water stressed trees. It is important to ensure the correct percentage of shade netting is used for a specific environment, depending on climate, location and cultivar. With emphasis on cultivar, the tree architecture be should considered as it is likely to influence light penetration into the deeper canopy. In addition, research should be conducted on cultivars which generally struggle to attain a good rind colour, to determine the possible impact of shade netting on carotenoid syntheses. The external appearance of fruit is also influenced by pests and diseases, therefore a detailed study should be done on the pest and

disease pressures under the shade net, as well as a full classification of external blemishes occurring on the fruit as caused by these factors.

Future research

The use of shade nets shows promising results and is highly recommended on high value cultivars such as 'Nadorcott' mandarin and 'Cambria' Navel orange which are prone to sunburn. This new technology seems to be the way forward in providing blemish free fruit, however there are many unanswered questions such as pest and disease pressures under the shade net as well as how factors such as various cultivars and geographic location influences the effect shade nets has on fruit quality during both pre- and post-harvest conditions. Research on shade netting on *Citrus* in South Africa is in its infant stages, and further research is needed to aid in this unanswered questions. In order to better understand the impacts of shade netting on insect pests, a further study will be conducted from 2019 in areas with higher pest populations and more varied citrus pests.

Technology transfer

Presentation at local society conference

1. Brown R., Stander O.P.J.², North, J.², and Cronje P.J.R. 2018. The influence of shade netting on citrus fruit thinning agents' efficacy. CRI Bi-annual research symposium. 20-24 Aug 2016. Champagne Castle sport resort, UKZN.
2. Du Toit Prins, Stander OPJ, Barry GH, T Vahrmeyer and & Cronjé PJR. 2018. The impact of shade netting of the micro climate of a mandarin citrus orchard. 20-24 Aug 2016. Champagne Castle sport resort, UKZN.
3. North JJ and PJR Cronje. 2016. Evaluation of a portable and efficient device to measure acidity in citrus. 20-24 Aug 2016. Champagne Castle sport resort, UKZN.
4. Rosalie, R, OPJ Stander², J North² & P.J.R. Cronje. 2016. Impact of shade netting on carbohydrate metabolism of mandarin citrus trees. 20-24 Aug 2016. 20-24 Aug Champagne Castle sport resort, UKZN.
5. Van Niekerk Jan, Jakkie Stander, Tertia van Wyk, Claire Love and Martin Gilbert. 2018. The influence of drape nets on insect pests, spray deposition and certain horticultural aspects in citrus. 19-22 August 2018. Champagne Castle sport resort, UKZN.
6. Botes J, Hoffman L, Zacarias L, PJR Cronje. 2018. The influence of 20% white shade nets on mandarin fruit development and quality. 19-22 August 2018. Champagne Castle sport resort, UKZN.
7. Prins Du Toit, Graham Barry, Jakkie Stander and Paul Cronje. 2018. The impact of 20% white shade netting on the microclimate and citrus tree physiology. 19-22 August 2018. Champagne Castle sport resort, UKZN.
8. Robert Brown, Jakkie Stander and Paul Cronje. 2018. Effect of permanent shade netting on late mandarin tree phenology and productivity. 19-22 August 2018. Champagne Castle sport resort, UKZN.

Presentations at industry extension meetings

- CRI production workshops 2017/8
 - **Shade netting research feedback day: 26 July 2017, Citrusdal.**
 - **Competitiveness of SA citrus** – Prof Johan van Rooyen and Johann Boonzaaier (BFAP-US)
 - Impact of netting on **microclimate** - Du Toit Prins SU
 - **Water use** of citrus under netting – Mathew Banda UP
 - Change in **carbohydrate accumulation** under netting - Dr William Mavengere SU.
 - Impact of netting on **fruit quality** – Johane Botes - SU
 - Change in **insect population** under netting – Claire Love CRI-SU
 - Impact of netting on **yield and return on investment** - Robert Brown – SU

Panel discussion (30-40 min): Paul Cronje (Facilitator), Tim Grout, Martin Gilbert, Jan van Niekerk, Etienne Rabe and Jakkie Stander

Presentations at international symposium

Standar, OPJ, JM Van Niekerk, T Van Wyk, C Love, MJ Gilbert, R Brown, DM Prins, GH Barry and PJR Cronje. 2019. The influence of drape-nets on foliar spray deposition, insect pests, and important tree responses of 'Nadorcott' mandarin. 1st International Symposium on Nettings and Screens in Horticulture. 27 to 31 January 2019 in Tenerife, Canary Islands, Spain.

Barry, GH, DM Prins, R Brown, OPJ Stander and PJR Cronjé. 2019. White shade netting affects the microclimate and tree physiology in a 'Nadorcott mandarin' orchard. 1st International Symposium on Nettings and Screens in Horticulture. 27 to 31 January 2019 in Tenerife, Canary Islands, Spain. 1st International Symposium on Nettings and Screens in Horticulture. 27 to 31 January 2019 in Tenerife, Canary Islands, Spain.

Cronje PJR, Botes, J, Prins DM, R Brown, OPJ Stander, Hoffman, EW, Zacarias, L and Barry, GH. 2019. The influence of 20% white shade nets on fruit quality of 'Nadorcott' mandarin. 1st International Symposium on Nettings and Screens in Horticulture. 27 to 31 January 2019 in Tenerife, Canary Islands, Spain.

Publications

Standar O.P.J., J. North, J.M. Van Niekerk, T. Van Wyk, C. Love, and M.J. Gilbert. 2019. The Influence of Non-Permanent Netting on Foliar Spray Deposition, Insect Pest Prevalence and Production of 'Nadorcott' Mandarin (*Citrus reticulata*). HortScience. 54 (4):667–675..

Postgraduate students

- Funded by RCE programme:
 - Du Toit, P. M., 2018. The impact of shade netting on the microclimate of a citrus orchard and the tree's physiology. MSc Thesis. Dept. Horticultural Science University of Stellenbosch, South Africa.
 - Botes, J., 2018. Impact of shade netting on internal and external quality of 'Nadorcott' mandarin fruit. MSc Thesis. Dept. Horticultural Science University of Stellenbosch, South Africa.
 - Brown, R., 2018. Effect of permanent shade netting on 'Nadorcott' mandarin tree phenology and productivity. MSc Thesis Dept. Horticultural Science University of Stellenbosch, South Africa.
- Non-funded students in collaboration with KUL:
 - Leila Lurquin. 2017. Evaluation of the possible benefits of shade netting on internal quality of Nadorcott mandarin fruit/*Evaluatie van mogelijke positieve effecten van schaduwnetten op de interne kwaliteit van mandarijn vruchten, cv Nadorcott*. Master of Science in de bio-ingenieurswetenschappen: landbouwkunde. KU Leuven, Belgium.

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- Botes, J., 2019. Impact of shade netting on internal and external quality of 'Nadorcott' mandarin fruit. MSc Thesis (*Submitted*) Dept. Horticultural Science University of Stellenbosch. South Africa
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5.3.4 **PROGRESS REPORT: The use of novel soil conditioners to improve citrus P nutrition and tree performance.**

Project 1234 (August 2018 – July 2019) by PJ Raath, (CRI)

Summary

The trial was initiated on two Washington Navel/Carizzo blocks in Addo (August 2018). Nine treatments were applied; all consisted of an application of 40 kg P/ha with different combinations of soil conditioners. Last mentioned included elemental sulphur, a phosphinate polymer and two commercial products that contain strains of either *Bacillus & Pseudomonas spp.* (Kiplant All-Grip®) or *Bacillus & Azospirillum* (Kiplant iNmass®) respectively. The goal was to evaluate to what extent the use of these products enhances availability and uptake of P in high pH soils. The first set of soil analyses (conducted in August 2018) indicated that, contrary to the producer's analyses results, both experimental blocks have exceptionally high soil P concentrations (an average of 409 & 445 mg/kg respectively). Consequently, it was decided to shift the focus of the trial to investigate the effect of the treatments in soil with high P-concentrations only, and since the results obtained after the initial sampling and data collection was similar for both blocks, only one block was used further on.

Leaf samples collected in October 2018 (two months after application of the soil conditioners), showed an increase in leaf P and Ca concentrations only, and for Treatment 10 (strip applied combination of the two commercial microbial inoculants) only when compared to the control (no soil conditioner). Leaf samples taken in March 2019, however, showed no significant difference in the foliar nutrient concentrations between the treatments. The implication is that for soil with high P concentrations, application of P is not required since available soil P is then at sufficient levels even in high pH (e.g. pH_{KCl} 7.2-7.8) soils. Both the nematode profile method and enzyme (B-glucosidase, phosphatase & urease) activities, were used to measure the effect of the soil conditioners on the soil microbiology. Initial results show that the nematode profile method seems less sensitive to express changes in soil microbiology than the enzyme activity. A month after application of the soil conditioners, only the omnivore nematode spp. and total nematode numbers were increased by T5 (a combination of 300 kg/ha elemental S (330 kg Brimstone 90) and 4L/ha as well as phosphinate polymer (Crestharvest 588®)). However, enzyme activities were significantly increased by various treatments containing either elemental S (phosphatase), *Bacillus/Pseudomonas spp.* (B-glucosidase & urease) or *Bacillus/Azospirillum* (B-glucosidase, phosphatase & urease) or a combination of last mentioned two soil conditioners (B-glucosidase). Final soil sampling and one year's harvest and fruit quality data will be collected in June 2019.

Opsomming

Die projek is op twee Washington Navel/Carizzo blokke in Addo begin in Augustus 2018. Nege behandelings is toegepas wat bestaan het uit 'n toediening van 40 kg P/ha saam met verskillende kombinasies van grondkondisioneerders. Behandelings het elementêre swawel, 'n fosfoniet pilomeer en twee kommersiële biologiese onnokulante wat hetsy *Bacillus & Pseudomonas spp.* (Kiplant All-Grip®) of *Bacillus & Azospirillum* (Kiplant iNmass®) bevat. Die oogmerk was om vas te stel tot watter mate hierdie produkte die beskikbaarheid van P in hoë pH gronde bevorder. Die eerste stel grondontelings (gedoen in Augustus 2018) het getoon dat, in teenstelling met die produsent se ontleding, beide die eksperimentele blokke uitsonderlik hoë konsentrasies P bevat (onderskeidelik 'n gemiddeld van 409 & 445 mg/kg). Die klem van die proef is dus verskuif om slegs te fokus op die effek van die behandelings op grond met hoë P-konsentrasies. Verder was die resultate van die eerste monsterneming en data-insameling soortgelyk vir die twee blokke. Gevolglik is besluit om slegs een blok verder te gebruik.

Blaarmonsters wat in Oktober 2018 getrek is (twee maande na toediening van die produkte) het slegs 'n toename in P en Ca getoon, en slegs vir B10 (kombinasie van twee kommersiële innokulante in 'n strook toegedien) teenoor die kontrole (geen grondkondisioneerder). Blaarmonsters wat in Maart 2019 getrek is, het egter geen betekenisvolle verskille in die minerale voedingstofinhoud tussen die behandelings getoon nie. Die implikasie is dat vir grond met hoë P-konsentrasies is toediening van P nie nodig nie, selfs met 'n hoë pH (pH_{KCl} 7.2-7.8 in hierdie geval). Sowel die nematode profielmetode en ensiem (B-glucosidase, fosfetase & urease) aktiwiteit, is gebruik om die effek van die grondkondisioneerders op grond mikrobiologie te evalueer. Inisiële resultate toon dat die nematode profiel metode minder sensitief is om veranderinge in grond mikrobiologie weer te gee as die ensiem aktiwiteit. 'n Maand na toediening van die produkte is gevind dat slegs die omnivoor nematode spp. en die totale aantal nematodes deur slegs B5 (kombinasie van 300 kg/ha S (330 kg Brimstone 90) en 4L/ha fosfoniet polimeer (Crestharvest 588®)) verhoog is. Sover dit ensiem-aktiwiteit aangaan is ensiem-aktiwiteit betekenisvol verhoog deur verskeie van die behandelings wat hetsy elementêre S (fosfetase), *Bacillus/Pseudomonas* spp. (B-glu2osidase & urease) of *Bacillus/Azospirillum* (B-glukosidase, fosfetase & urease) of 'n kombinasie van lg. twee (B-glukosidase) bevat. Finale monsterneming, en een jaar se oes en vrugkwaliteitsdata, sal in Junie 2019 ingesamel word.

5.4 PROGRAMME: CULTIVAR EVALUATION

Programme coordinator: Johan Joubert (CRI)

5.4.1 Programme summary

The lemon requirements, due to the high numbers produced in the citrus industry, are focusing on specific quality characteristics; early picking window (best prices), low seed numbers, and oblong (longer) fruit shape. Eureka remains the number one lemon selection preferred by Citrus growers and consumers for several reasons: good quality fruit with high juice content; fairly long fruit shape; ability to bear a good crop on the trees with two to three fruit sets; and limited thorns on the bearing branches for optimal picking. There are a number of new lemon selections included in the trial sites to challenge Eureka, with a specific goal towards completely seedless fruit along with optimum yield cropping on the trees (5.4.10, 5.4.11).

The demand to plant mandarin selections in the hot citrus production areas remains a priority with the reality of good quality fruit with good colour development early in the season and optimum Brix: acid ratios being critical, but options are limited. Citrus grown in the cool and intermediate production areas remain the best mandarin producing options (5.4.4, 5.4.6, 5.4.7, 5.4.8, 5.4.15, 5.4.16, 5.4.17, 5.4.18) due to specific climatic requirements (better early colour and acids). The focus for future plantings will have to be for earlier or later maturing fruit outside the Tango, ARCCIT9 (Nadorcott LS) and Nadorcott picking windows since very high numbers of these trees have been planted. The best quality fruit produced in this picking window will be in high demand but marginal fruit quality areas will be in trouble. The consumer demands fruit with low seed numbers, or completely seedless fruit, that peel easily, has good colour development and excellent flavour. Numerous new experimental options went into trial sites in the main citrus productions areas to determine the commercial value of these cultivars including the hotter productions regions (early- and late maturing possibilities).

Star Ruby remained the number one grapefruit planted in South Africa due to excellent internal colour development and very good internal quality with high Brix content. There are numerous new red grapefruit selections included in the trial sites with lower naringin (bitter taste) levels to improve flavour and eating experience (5.4.12).

The focus on late maturing Valencia selections increased (5.4.2, 5.4.3, 5.4.5, 5.4.22, 5.4.23) in the suitable citrus production areas (Letsitele) where demand for low seeded or seedless Valencias with good crop production increased. The problem with high chimera numbers on some of the late Valencia selections stimulated the need for alternative options to replace problem orchards or to establish new plantings (Jassie, Malinda, Kobus du Toit Late, Gussocora etc.).

Navel prices dropped considerably in the past season due to the combination of shelf life problems (low acid levels), postharvest performance and open navel ends, all contributing to the decrease in new navel plantings

(5.4.9, 5.4.19, 5.4.20, 5.4.21). The focus will be on mandarin options to fill the requirements in the production cycle of the packhouse programme.

Rootstock evaluations will expand on the range of new rootstock trials, including the mainstream (commercial), semi-commercial range, as well as several Florida options (with possibility of HLB tolerance) to evaluate for production performance and suitability to different soil types (pH, clay content, salinity etc). There is also a range of new Argentinian rootstocks in the new trials (experimental and semi-commercial) to address the need for lemon scion compatibility as well as specific conditions and smaller tree volumes (5.4.5, 5.4.12).

Programopsomming

Die suurlemoen voorkeure, weens die groot volumes wat in die sitrusbedryf aangepalnt en geproduseer word, fokus op spesifieke kwaliteits eienskappe; vroeë plukvenster (beste pryse), lae saad inhoud en meer langwerpige (silindriese) vrugvorm. Eureka bly steeds die nommer een suurlemoenseleksie wat deur Sitrus produsente en verbruikers verkies word om verskillende redes: vrugte van goeie gehalte met 'n hoë sapinhoud; redelike lang vrugvorm; potensiaal om 'n goeie oes op die bome te dra met twee tot drie vrug sette; beperkte dorings op die dra-takke vir optimale pluk. Daar is 'n aantal nuwe suurlemoen seleksies wat by die proefpersele ingesluit word om met Eureka te vergelyk, met die spesifieke doel om totaal saadlose langwerpige vrugte met optimale opbrengs te produseer (5.4.10, 5.4.11).

Die behoefte om mandaryne in die warm sitrus produksie areas te plant bly 'n prioriteit met die realiteit van goeie kwaliteit vrugte wat goeie kleurontwikkeling vroeg in die seisoen lewer; asook optimale Brix: suur verhoudings ontwikkel (kritiese belang), maar die opsies is beperk. Sitrus wat in die koel en intermediêre produksie areas verbou word, bly die beste mandaryn produserende opsies (5.4.4, 5.4.6, 5.4.7, 5.4.8, 5.4.15, 5.4.16, 5.4.17, 5.4.18) as gevolg van spesifieke klimaatvereistes (beter vroeë kleur en sure). Die fokus vir toekomstige aanplantings sal wees op vroer of later rypwordende vrugte buite die Tango, ARCCIT9 (Nadorcott LS) en Nadorcott plukvensters, aangesien groot volumes van hierdie bome geplant is. Die beste kwaliteit vrugte wat in hierdie plukvenster geproduseer word, sal in groot aanvraag wees, maar die gebiede met marginale vrugkwaliteit gaan in die moeilikheid wees. Die verbruikersvraag bly vir vrugte met 'n lae saad inhoud, of totaal saadlose vrugte, wat maklik skil met 'n goeie kleurontwikkeling en 'n uitstekende smaak. Verskeie nuwe eksperimentele opsies word ingesluit in proefpersele in die belangrikste sitrus produkserende areas om die kommersiële waarde van hierdie kultivars te bepaal, insluitend die warmer produksie areas (vroeë en laat rypwordende opsies).

Star Ruby bly die nommer een pomelo wat in Suid-Afrika aangeplant word a.g.v. uitstekende interne kleurontwikkeling en baie goeie interne gehalte met 'n hoë Brix-inhoud. Daar is verskeie nuwe rooi pomelo seleksies wat by die proefpersele ingesluit word met laer naringien vlakke (bitter smaak) om die smaak en eetervaring te verbeter (5.4.12).

Die fokus op die laat rypwordende Valencia-seleksies het toegeneem (5.4.2, 5.4.3, 5.4.5, 5.4.22, 5.4.23) in die geskikte sitrusproduksie areas (Letsitele), waar die vraag na Valencias met 'n lae saadinhoud of totaal saadlose vrugte met goeie oes produksie toegeneem het. Die probleem met 'n hoë chimera voorkoms by sommige van die laat Valencia seleksies, het die behoefte laat ontstaan om alternatiewe opsies vir die vervanging van probleem boorde of nuwe aanplantings te gebruik (Jassie, Malinda, Kobus du Toit Late, Gussocora ens.).

Die nawel pryse het die afgelope seisoen aansienlik gedaal as gevolg van die kombinasie van rakleef tyd probleme (lae suurvlakke), na-oes prestasie en oop nawel-ente, wat alles bygedra het tot die afname in nuwe nawel aanplantings (5.4.9, 5.4.19, 5.4.20, 5.4.21). Die fokus sal wees op mandaryn opsies om die vereistes in die produksiesiklus van die pakhuisprogram aan te spreek.

Onderstam evaluasies sal uitgebrei word met 'n nuwe reeks onderstam proewe, ingesluit die hoofstroom reeks (kommersiële opsies), semi-kommersiële reeks asook verskeie Florida opsies (met moontlike HLB weerstandbiedendheid) om produksie potensiaal te evalueer en geskiktheid vir verskillende grond tipes (pHp, klei inhoud, sout vlakke) te bepaal. Daar is 'n reek Argentynse onderstamme in nuwe proewe ingesluit

(eksperimenteel en semi-kommersieel) om die suurlemoen-bostam verenigbaarheids kwessie aan te spreek asook kleiner boom volumes te bevorder (5.4.5, 5.4.12).

5.4.2 **PROGRESS REPORT: Evaluation of Valencia selections in hot humid inland areas (Onderberg)** Project 75A by J. Joubert (CRI)

Summary

Selections that performed well in this season, in this hot, humid production area, according to optimal maturity from early to late, are as follows: Valearly, one of the new early maturing (internal quality) varieties matures before Turkey. There was a delayed colour development on the fruit by the time of optimum maturity with Valearly, but deeper orange colour compared to the more yellow of Turkey when fully coloured. Turkey will follow, but bear in mind that this selection has a sensitive rind. Do not allow the fruit to hang for too long because the optimal picking period is no longer than 4-6 weeks.

Delta would follow, with good internal quality, production and fruit size, as well as McClean SL representing the middle of the Valencia season for this area. The later selections can broaden the list of choices to extend the season, commencing with Skilderkrans, Kobus du Toit Late and Jassie (optimum fruit size distribution) and followed by Moosrivier Late 1, producing 0.7 seeds per fruit.

Valearly, Jassie and Kobus du Toit Late remain experimental/semi-commercial selections that performed well. These selections could be included in future plantings when more conclusive information becomes available.

Opsomming

Seleksies wat hierdie seisoen, volgens optimum rypheid van vroeg tot laat goed presteer het vir hierdie vrotige warm produksie area, is soos volg. Valearly is een van die nuwe vroeë Valencia opsies (vroeg intern ryp) wat voor Turkey inpas. Daar was 'n vertraagde kleurontwikkeling op die vrugte gewees met optimum rypheid by Valearly, maar wel dieper orange kleur wanneer opgekleur in vergelyking met meer geel by Turkey. Turkey sal dan volg, wees net versigtig om nie die seleksie te lank te hang nie. Baie skil probleme kan ontwikkel, want die optimum oes tydperk strek oor gemiddeld vier weke, ses weke maksimum dan moet die vrugte af wees.

Delta kan dan volg wat goeie interne kwaliteit, produksie en vruggrootte lewer, asook McClean SL wat dan die middel van die Valencia seisoen vir hierdie area verteenwoordig. Die later seleksies wat kan bydra tot die keuse om die seisoen te verleng, kan bestaan uit Skilderkrans, Kobus du Toit Late en Jassie (optimum vruggrootte verspreiding) gevolg deur Moosrivier Late 1, wat 0.7 sade per vrug produseer.

Valearly, Jassie en Kobus du Toit Late is steeds eksperimentele/semi-kommersiele seleksies wat goed presteer. Hierdie seleksies kan in die toekoms ingesluit word soos meer en beter inligting beskikbaar word.

Objective

- To find suitable Valencia selections with superior characteristics for the hot inland citrus production areas.

Materials and methods

Field evaluations and laboratory analyses were conducted on Delta (control), Jassie, Kobus du Toit Late, McClean SL, Moosrivier Late 1, Skilderkrans, Turkey (control) and Valearly at Riverside in Malelane, Mpumalanga.

Table 5.4.2.1. Internal fruit quality minimum export requirements for Valencia types.

Variety	Juice %	Brix	Min Acid	Max Acids	Ratio	Colour
Valencia EU	48	8.5	0.6	1.8%	7.5:1	Colour plate 3 of set no. 34
Midnight	52	9.5	0.85	1.8%	7.5:1	Colour plate 3 of set no. 34
*Turkey	50	10.0	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34

*Interim internal fruit quality standards.

Table 5.4.2.2. List of Valencia selections evaluated at Riverside (Malelane) during 2018.

Selection	Rootstock	Year Planted	No. of trees
Delta	C35/CC/SC	2012	5/5/5
Jassie	C35/CC/SC	2012	5/5/5
Kobus du Toit Late	C35/CC/SC	2012	5/5/5
McClellan SL	C35/CC/SC	2012	5/5/5
Moosrivier Late 1	C35/CC/SC	2012	5/5/5
Skilderkrans	C35/CC	2012	5/5
Turkey	C35/CC/SC	2012	5/5/5
Valearly	C35/CC/SC	2012	5/5/5

Results and discussion

This project is ongoing – all evaluations and tasks have been completed to date. Trees were visually evaluated at Riverside (Malelane) during the 2018 season.

Delta (control)

Delta produced a fair to good crop on the trees, bearing fruit for the fourth time this season. Fruit size was bigger this season and ranged from medium to large/extra-large (count 72 to 48) with good internal quality values, juice levels above 60, Brix of up to 11.2 and acids above 0.8% (except for C35 with 0.7% on one evaluation). Colour development on all three rootstocks were very similar (between T1 and 3). Delta remained completely seedless and complied with the export requirements. Based on the internal quality results in Table 5.4.2.3, maturity will be from the beginning to the middle of July.

Jassie

The trees were bearing their fourth crop this year on all three rootstock combinations, averaging between 60 and 80 kg per tree. The fruit size varied from medium to large/extra-large, count 72 to 48/40 (avg). The rind texture improved this season becoming smoother with time. Seed count per fruit was decreased even more this season and varied from 2.3 to 3.4 seeds per fruit. Internal quality improved with tree age and produced better juice levels (above 54), good Brix (above 11 at maturity) and lower acids (above 0.8). External colour development improved and peaked between T1 and 3 with the final evaluations. Maturity seems to be end of July to middle of August based on the results in Table 5.4.2.3.

Kobus du Toit Late

Kobus du Toit Late was evaluated at the Riverside trial site on three rootstocks (C35, CC, SC) and produced medium to large fruit size (count 88 to 56) on the trees, with 2.5 seeds average. The colour development was very similar on all three rootstocks compared to 2017. The internal quality was very good, juice levels above 57%, Brix up to 12.6, and higher acids earlier in the season (above 1) for the later maturing selection. External colour peaked from T1 to 3. Maturity seems to be middle to end of July according to Table 5.4.2.3.

McClellan SL

The standard McClellan will be included in future trials as a control to compare the SL selection's performance, although McClellan developed high chimera incidences on the fruit (up to 40%) in commercial plantings. McClellan SL produced fairly round fruit with soft fibre strength that peeled easily, containing low rind oil levels. All the fruit evaluated remained completely seedless. Many totally seedless selections have fruit set problems

and bear less fruit, but this does not appear to be the case with this cultivar. The fruit size peaked at medium-large to large/extra-large (count 88-56/48). The internal quality improved from average to good (2017) to very good with high juice levels for the trial site from 59%, Brix up to 13.4 and acceptable acid levels (above 0.85%). There was a slight delay in external colour ranging from T1-3. Based on the internal quality results in Table 4.6.2.3, maturity will be mid to end of July.

Moosrivier Late 1

Moos Late 1 developed a high acid level (avg 1.1%) when the juice (above 55%) and Brix (above 10.5) content was ready for harvesting at Riverside (fourth crop), and the external colour peaked at T1 to 3. Moos Late 1 developed 0.7 seeds (cross pollination) per fruit compared to last season's 1.0. Moos Late 1 had promising performance, developed smooth round fruit with deep yellow internal colour, good flavour, peeled easily and fairly soft rag. Based on the internal quality results in Table 5.4.2.3 estimated maturity for Moos Late 1 will be from the middle to the end of August.

Skilderkrans

Skilderkrans bore fruit on C35, Carrizo and Swingle at the Riverside trial site. Fruit size improved and varied from medium to large/extra-large (count 72-48/40). Internally the Brix content improved (above 10) and the acid level of 0.95 to 1.0% indicated a later maturing Valencia selection. Juice level increased to average 59%; above the minimum required export figure. There was no delay in external colour (T2-3) on any of the rootstocks evaluated. The fruit developed a smooth rind, fibre strength was fairly soft and the fruit shape was round. Ratios were lower this season due to the low Brix and higher acid levels, delaying peak maturity to end of July and mid-August on all three rootstocks (Table 5.4.2.3).

Turkey (Control)

Fruit size decreased this season from count 88 to 48, with medium to large/extra-large fruit size. Fruit characteristics for Turkey were round fruit shape, smooth rind texture, very good flavour, soft rag, fairly thin rind, easy fruit peeling, and higher seed count per fruit ranging from 0.5 to 5.7 seeds per fruit. The internal colour was light yellow, and externally the fruit remained yellow up to over-matured fruit. It should be borne in mind that this selection is not a true Valencia and actually has the qualities of a mid-season orange; for instance, the exceptionally soft rag of the fruit, and the soft rind that can result in rind problems if managed incorrectly. The Turkey should not be harvested over more than four weeks as extending the harvesting season can lead to the development of rind disorders. Based on the internal quality results in Table 5.4.2.3, estimated maturity will be middle to end of May.

Valearly

Valearly, bearing a good crop (60-80kg/tree) on the trees, developed low seed numbers (0.0 to 2.3 seeds per fruit) this season. The internal quality of the fruit was good early in the season with medium high juice (above 50%), Brix above 10 (accept for Swingle with avg 9.6) and acid above 0.6. In comparison with the other early maturing selections, Valearly seems to be at least two weeks earlier, similar to Weipe, with good internal quality but delayed external colour ranging from T1-4. Estimated maturity according to Table 5.4.2.3, seems to be the end of April.

Conclusions

The internal quality for this season for all the selections evaluated, complied with the export standards. These acid levels will decrease towards the end of the season, indicating extended shelf-life of the selection for example Jassie. Jassie also indicated very low chimera fruit numbers on the trees, providing another good late maturing Valencia option to be included in future plantings. There was no Brix: acid ratio below 7.5:1 at peak maturity this season, which is often associated with later maturing selections having higher acid levels. When the acid levels decrease, the ratio increases. There was a better colour development with most of the selections towards peak maturity time, except in the case of Valearly, where the colour development was delayed even after peak quality. The average seed count for this season was fairly low, including Jassie and Turkey (average 2.8 and 2.6 seeds per fruit), indicating lower cross pollination in the mixed trial block. McClean SL remained completely seedless. Jassie and Kobus du Toit Late will be one of the future possibilities to include in new Valencia plantings (optimum Valencia fruit size distribution, high juice levels, low seed counts and late

maturing). Fruit size increased on the trees, between count 72 and up to count 48/40 on selections with lighter yields.

Table 5.4.2.3. Internal fruit quality data for Valencia and late orange selections at Riverside (Malelane) during the 2018 season.

Cultivar	Rootstock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg seed	Fruit external colour
Delta	C35	26/07/2018	75-87	72-48	63.4	11.2	0.95	11.8	0.0	T1-3
Delta	C35	29/08/2018	75-88	72-48	62.4	11.2	0.70	16.0	0.0	T1-3
Delta	CC	26/07/2018	75-85	72-56	59.8	10.5	0.90	11.7	0.0	T1-3
Delta	CC	29/08/2018	73-87	72-48	62.3	10.9	0.80	13.6	0.0	T1-3
Delta	SC	26/07/2018	74-82	72-56	60.5	10.3	0.80	12.9	0.0	T1-3
Delta	SC	29/08/2018	75-88	72-48	64.4	11.1	0.80	13.9	0.0	T1-3
Jassie	C35	25/07/2018	74-88	72-48	62.9	11.4	1.00	11.4	2.3	T1-3
Jassie	C35	29/08/2018	79-91	64-40	59.4	12.2	0.80	15.3	3.3	T1-3
Jassie	CC	25/07/2018	79-93	64-40	58.9	10.9	0.90	12.1	2.9	T1-3
Jassie	CC	29/08/2018	80-90	64-40	57.4	10.3	0.80	12.9	2.3	T1-3
Jassie	SC	25/07/2018	74-87	72-48	54.3	11.2	1.10	10.2	3.4	T1-3
Jassie	SC	29/08/2018	81-90	64-40	58.4	11.0	0.80	13.8	2.6	T1-3
K du Toit Late	C35	26/07/2018	70-88	88-48	62.2	12.6	0.80	15.8	0.6	T1-3
K du Toit Late	C35	29/08/2018	70-80	88-64	59.9	12.4	0.65	19.1	0.9	T1-3
K du Toit Late	CC	26/07/2018	71-79	88-64	60.9	10.7	1.10	9.7	4.8	T1-3
K du Toit Late	CC	29/08/2018	75-84	72-56	57.8	11.1	0.85	13.1	1.6	T1-3
K du Toit Late	SC	26/07/2018	72-80	88-64	61.4	10.8	1.10	9.8	5.6	T1-3
K du Toit Late	SC	29/08/2018	70-85	88-56	58.4	11.1	0.80	13.9	1.4	T1-3
McClellan SL	C35	26/07/2018	71-83	88-56	60.4	11.6	1.00	11.6	0.0	T1-3
McClellan SL	C35	29/08/2018	72-86	88-48	59.9	13.4	0.85	15.8	0.0	T1-3
McClellan SL	CC	26/07/2018	75-87	72-48	63.0	11.6	0.90	12.9	0.0	T1-3
McClellan SL	CC	29/08/2018	72-90	88-40	60.4	12.8	0.85	15.1	0.0	T1-3
McClellan SL	SC	26/07/2018	71-84	88-56	60.7	11.4	0.90	12.7	0.0	T1-3
McClellan SL	SC	29/08/2018	77-93	72-40	63.9	11.4	0.60	19.0	0.0	T1-3
Moos Late 1	C35	26/07/2018	78-91	64-40	70.3	10.5	1.20	8.8	0.8	T1-3
Moos Late 1	C35	29/08/2018	76-90	72-40	63.1	11.6	1.05	11.0	0.4	T1-3
Moos Late 1	CC	26/07/2018	75-85	72-56	63.2	11.0	1.10	10.0	1.4	T1-3
Moos Late 1	CC	29/08/2018	76-85	72-56	63.4	12.6	0.95	13.3	0.6	T1-3

Moos Late 1	SC	26/07/2018	76-91	72-40	58.2	11.0	1.05	10.5	0.3	T1-3
Moos Late 1	SC	29/08/2018	75-89	72-48	55.4	11.1	0.95	11.7	0.8	T1-3
Skilderkrans	C35	26/07/2018	79-93	64-40	60.1	10.2	0.95	10.7	0.0	T1-4
Skilderkrans	C35	29/08/2018	80-93	64-40	61.6	10.3	0.75	13.7	1.3	T1-3
Skilderkrans	CC	26/07/2018	76-86	72-48	56.3	10.7	1.00	10.7	0.0	T1-3
Skilderkrans	CC	29/08/2018	69-88	88-48	57.1	10.8	0.80	13.5	0.0	T1-3
Turkey	C35	30/04/2018	77-85	72-56	60.8	8.8	0.90	9.8	0.5	T5
Turkey	C35	28/5/2018	78-88	64-48	57.5	10.2	0.60	17.0	1.2	T2-3
Turkey	C35	15/6/2018	87-93	48-40	58.2	11.2	0.65	17.2	1.9	T1-2
Turkey	CC	30/04/2018	72-83	88-56	57.2	6.5	1.10	5.9	4.7	T5
Turkey	CC	28/5/2018	75-83	72-56	47.4	10.4	0.75	13.9	2.4	T2-3
Turkey	CC	15/6/2018	80-92	64-40	55.9	10.8	0.60	18.0	5.7	T1-3
Turkey	SC	30/04/2018	72-80	88-64	55.3	6.4	0.95	6.7	2.0	T5
Valearly	C35	30/04/2018	77-80	72-64	50.4	8.9	0.80	11.1	0.7	T5
Valearly	C35	28/05/2018	75-82	72-56	55.8	10.8	0.75	14.4	0.1	T3-4
Valearly	C35	15/6/2018	80-85	64-56	52.3	11.1	0.60	18.5	0.3	T1-3
Valearly	CC	28/05/2018	70-80	88-64	57.5	10.5	0.60	17.5	0.0	T3-4
Valearly	CC	15/6/2018	76-85	72-56	53.0	10.6	0.70	15.1	0.0	T1-4
Valearly	SC	28/5/2018	73-88	72-48	58.0	10.7	0.85	12.6	0.8	T2-3
Valearly	SC	15/6/2018	80-94	64-40	55.9	9.6	0.65	14.8	2.3	T1-3

5.4.3 PROGRESS REPORT: Evaluation of Valencia selections in the hot dry inland areas (Letsitele and Hoedspruit)

Project 75B by J. Joubert (CRI)

Summary

The season starts with early selections and proceeds to the late maturing selections suitable for this hot-dry production areas. Recommendations have therefore been made accordingly. Valearly will start the season as an early maturing Valencia. Turkey will follow, producing large fruit size with good internal quality and soft fibre. The optimal picking window will be within the first four weeks of peak maturity. Bennie 1 and 2 follow after Turkey with good production and medium to large fruit size. Delta as a control fits in before Gusocora. Gusocora and McClean SL follows next with completely seedless fruit and very good Brix: acid ratios. Midnight 1 and 2 cover the middle of the Valencia season with good internal quality fruit, large fruit size, smooth rind and low seed counts per fruit. Du Roi follows with excellent crop on the trees and medium to medium-large fruit size (count 88 to 56). Valencia Late and Lavalle are currently the latest maturing Valencia selections that are being planted commercially, developing excellent fruit size and yield.

A series of experimental/semi-commercial selections has also been included in the hot production areas. The selection range follows from early, mid-, to late-maturing options. The season starts with Valearly, competing with Turkey to be the earliest maturing Valencia. Kobus Du Toit late mature more towards the end of the Valencia season with medium to large fruit size; followed by Skilderkrans. Late in the season; Jassie with optimum fruit size and good internal quality as well as Moosrivier Late 1 could possibly be added to the options, when more information becomes available from future evaluations.

Opsomming

Die seisoen begin met vroeg rypwordende seleksies en duur voort met die laat rypwordende seleksies in die warm droë produksie areas en aanbevelings is daarvolgens gebaseer. Valearly kan die seisoen begin as 'n vroeg rypwordende Valencia. Turkey kan nou volg, wat groot vrugte produseer met goeie interne kwaliteit en sagte vesel. Optimum plukvenster is binne die eerste vier weke van piek rypheid. Bennie 1 en 2 volg na Turkey met goeie produksie en medium tot groot vuggrootte. Delta as kontrole pas in voor Gusocora. Gusocora en McClean SL volg dan met totaal saadlose vrugte en goeie Brix: suur verhoudings. Midnight 1 en 2 vul die middel van die Valencia seisoen met goeie interne kwaliteit vrugte, groot vuggrootte, gladde skille en lae saadtellings per vrug. Du Roi is volgende met uitstekende oeste op die bome en medium tot medium/groot vrugte (telling 88 tot 56). Lavalley is huidiglik die laatste rypwordende Valencia seleksie wat semi-kommersieel aangeplant word, met uitstekende vuggrootte en produksie.

Daar is 'n reeks eksperimentele/semi-kommersiele seleksies wat ook vir die warm produksie areas ingesluit is. Hier volg die seleksies van vroeg, middle, tot laat rypwordend. Die seisoen kan begin word met Valearly wat meeding met Turkey as die vroegste seleksie. Du Toit Laat word meer aan die einde van die Valencia seisoen ryp met medium tot groot vuggrootte, gevolg deur Skilderkrans. Laat in die seisoen kan aangevul word met Jassie en Moosrivier Late 1, soos meer inligting beskikbaar word uit verdere evaluasies.

Objective

- To find suitable Valencia selections with superior characteristics for the hot inland citrus production areas.

Materials and methods

Field evaluations and laboratory analyses were conducted on Alpha, Bennie 1&2, Delta, Du Roi, Kobus Du Toit Late, Gusocora, Jassie, Lavalley 1, McClean, McClean SL, Midnight 1, 2, Moosrivier Late 1, Skilderkrans, Turkey, Valearly and Val Late at Bosveld Citrus (Letsitele), Groep 91 (Letsitele) and Moriah Citrus (Hoedspruit).

Table 5.4.3.1. List of Valencia selections evaluated at Bosveld Citrus (Letsitele) during the 2018 season.

Selection	Rootstock	Planted
Alpha	SC	2009
Bennie 1	SC	2009
Delta (control)	C35/CC/SC	2011
Du Roi	SC	2009
Kobus Du Toit Late	C35/CC/SC	2011
Gusocora	SC	2009
Jassie	C35/CC/SC	2011
Lavalley 1	SC	2009
McClean SL	CC/SC	2011
Midnight 1	SC	2009
Midnight 2	SC	2009
Moosrivier Late 1	C35/CC/SC	2011
Skilderkrans	C35/CC/SC	2011
Turkey	C35/CC/SC	2011
Val Late	SC	2009

Table 5.4.3.2. List of Valencia selections evaluated at Groep 91 (Letsitele) during the 2018 season.

Selection	Rootstock	Planted
Bennie 1	CC/SC	2006
Benny 2	CC	2006
Kobus du Doit Late	SC/Sunki 812/X639	2013

Jassie	CC/SC	2013
McClean	SC/Sunki 812/X639	2013
McClean SL	SC/Sunki 812/X639	2013
Moosrivier Late 1	CC/SC	2006
Skilderkrans	CC/SC	2006
Turkey	C35/CC/SC	2006
Valearly	SC/Sunki 812/X639	2013

Table 5.4.3.4. List of Valencia selections evaluated at Moriah Citrus (Hoedspruit) during the 2018 season.

Selection	Rootstock	Top-worked
Bennie 2	MxT	2011
Gusocora	MxT	2011
Midnight 1 (I15)	MxT	2011
Midnight 2 (F17)	MxT	2011

Results and discussion

Alpha

Fruit production on the Alpha trees this season varied between 60 to 80 kg per tree. Alpha was planted on C35 and Swingle at the Bosveld trial site to compare tree development (vigour) and yield production (trees on C35 almost a third smaller). The internal quality was still very good compared to 2017, juice levels peaked at 60%, Brix was above 10 and acids were fairly high (between 1.0 and 1.5%). Fruit size increased slightly and varied from count 72 to 56/48, still excellent for Valencia production and export. External colour peaked from T1 to T3. Maturity seems to be end of June to middle of July (Table 5.4.3.5).

Bennie 1 and 2

Bennie was evaluated at all three trial sites this season: Bosveld, Groep 91 and Moriah. There was a good crop on the Benny trees at Group 91; with the remaining drought conditions and severe high temperatures. There was a good crop on both selections and fruit size peaked between count 88 and 56 (very good for Valencia production). The internal colour of the fruit was deep yellow, fruit shape round, rind texture fairly smooth, medium rag content and medium rind thickness. Benny 1 and 2 internally produced similar juice levels (average 54%), Brix (average 12.8), acid (1.3%) and seed counts (average 5.3 seeds per fruit). External colour on both selections by the time of harvest varied between T1 and T3 (better colour on SC this season). Based on ratios, Benny 1 and 2 mature end of June to beginning of July (Table 5.4.3.5).

Delta (control)

Delta on all three rootstocks (C35, CC, SC), as control variety, produced completely seedless fruit and a good yield on the trees. Fruit size peaked between count 72 and 48 (lighter crop) with good internal quality, reaching juice levels of 57%, Brix of 13.3 and acid content of 1.0%. The external colour of the fruit was between T1 and T3. Maturity is middle to the end of June (Table 5.4.3.5).

Du Roi

Du Roi was planted on two rootstocks, C35 and Swingle at the Bosveld trial, and for this season the Swingle combination was evaluated as a control selection due to C35's severe susceptibility to Blight in the Letsitele production area. There was a good yield and fruit size peaked between count 88 and 56. The external colour peaked between T1 and T3 and the average seed count was 0.8 seeds per fruit (lower). Swingle developed a juice content of 59%, Brix of 11.8 and acids of 1.3%. Maturity is end of July to middle August (Table 5.4.3.5).

Kobus Du Toit Late

Kobus Du Toit Late was evaluated at the Bosveld trial site on three rootstocks (C35, CC, SC) and Groep 91 on four rootstock (CC, SC, Sunki 812, X639) and produced small, medium and large fruit size (count 105 to 56) on the trees (bigger fruit size at Groep 91), with 4.0 seeds average (higher compared to 2017). The colour development was very similar on all the rootstock combinations this season. The internal quality was good,

juice levels above 50%, Brix up to 14 and good acids for the later maturing selection. External colour peaked from T2 to 4. Maturity seems to be middle to end of July to middle August according to Table 5.4.3.5.

Gusocora

Gusocora was evaluated at Bosveld Citrus this year on Swingle rootstock. The fruit was completely seedless and developed a good internal quality where juice (56%), Brix (10) and acid (0.9) complied with export requirements. The external colour varied from T1 to T4, correlating with the internal quality and Brix:acid ratio of 12. Fruit size was smaller and peaked between counts 88 and 72, optimal fruit size for export Valencias (medium to large). There was a good crop on the trees, bearing in mind that Swingle induce good yields and internal quality. It is apparent that Gusocora's maturity is middle to end of July (Table 5.4.3.5).

Jassie

Fruit size at Bosveld on Carrizo and Swingle was bigger (peaked between count 88 and 56) compared to Mahela (semi-commercial block) with count 88 and 64, but smaller on C35 (peaked between count 105 and 72). Production was good on all the rootstock combinations. Internal quality was good with juice levels of 55%, Brix up to 14 (C35) and average acid levels of 1.2% (average). Seed count was slightly higher this year and varied from 2.4 to 6.9 (avg. 3.9) seeds per fruit. Fruit shape was round, with a smooth rind texture, internal colour was light yellow, and juice flavour was good. Fibre strength was fairly soft, rind thickness was medium and the fruit peeled easily. Jassie bore high numbers of fruit inside the tree (good quality and colour). Maturity is middle of July to beginning of August in this area (Table 5.4.3.5).

Lavalle 1

The seed production this season remained the same and Lavalle produced 0.4 seeds per fruit. The internal quality complied with export requirements and acid level was above 1.0% at the first evaluation at the end of July. Keep in mind that Lavalle is a late Valencia selection with good shelf life and the optimal harvest time will be in August. The navel end on some fruit seems to develop a button and there were split fruit on some of the trees evaluated, but this varies from season to season (seen only 2013). From the ratio on this date it is apparent that Lavalle 1 maturity is end of August to middle of September (Table 5.4.3.5).

McClellan and McClellan SL

McClellan was planted and evaluated this season on Carrizo, Swingle, X639 and Sunki 812 at Groep 91 to compare with McClellan SL. McClellan SL was planted on C35, Carrizo and Swingle at the Bosveld trial site and Carrizo, Swingle, X639 and Sunki 812 at Groep 91, with a good crop production and remained completely seedless/very low seeded similar to all the other trial sites where the selection was included. Fruit quality improved at the Groep 91 site due to trees bearing their second crop now; the Sunki 812 combination being the best for 2018. Fruit size peaked from count 88 to 56/48 (excellent for Valencia production). External colour varied from T1-3 (optimum). At both sites juice was 50% and above (as high as 69% on Sunki 812), Brix improved to as high as 14.8 (over mature) and acids were above 1.0% (peak maturity) towards the end of the season, resulting in good Brix:acid ratios (above 12:1). Maturity seems to be end of June to middle of July (Table 5.4.3.5).

Midnight 1 & 2

Midnight 1 and 2 bore an average to good yield of between 80 and 100 kg per tree on the two rootstock combinations where MxT was the smallest. The fruit size varied between count 88 and 56, juice content was around 57%, Brix levels lower around 11 (peak maturity) and acids around 1.0%. Midnight 2 outperformed Midnight 1 this season with a better Brix level. Midnight 1 and 2 developed low seed numbers in the fruit, ranging from 0.1 up to 0.5 seeds per fruit. The characteristic Midnight die-back was more visible on Midnight 1 compared to Midnight 2. Fruit shape was round, rind texture was fairly smooth, and fruit was raggy with a medium rind thickness which peeled moderately. Maturity seems to be middle of July to the end of July (Table 5.4.3.5).

Moosrivier Late 1

This season Moos Late 1 developed a wider fruit size distribution in general (small, medium to large size count 125-56) on Carrizo and C35, with medium to large fruit on Swingle (count 88 to 64). Crop production for Moos Late 1 was good. Moos Late 1 performed well on all three rootstocks, developing internal qualities that met

export standards and high acids (up to 1.25% towards peak maturity) indicating a late maturing Valencia selection. The seed count per fruit varied from 1.2 to 4.2. When internal quality was taken into consideration; estimated maturity end of July to middle August. (Table 5.4.3.5).

Skilderkrans

Skilderkrans at Group 91 will be the back-up site (not evaluated) and the trial block at Bosveld bore fruit on C35, Carrizo and Swingle. Fruit size varied from medium to large-extra-large (count 88-40). Internally the Brix content was good (up to 14) and the acid level of 0.9 to 1.2% (peak maturity) indicated a later maturing Valencia selection. Juice level increased to average 55.5% later in the season; above the minimum required export figure. There was no delay in external colour on any of the rootstocks evaluated (between T1 and 3). The fruit developed a smooth rind, fibre strength was fairly soft and the fruit shape was round. Ratios were lower this season due to the higher acid levels accept on Carrizo, delaying peak maturity to end of July and mid-August on Swingle and C35 (Table 5.4.3.5).

Turkey

Turkey was planted on three rootstocks: Carrizo, Swingle and C35, to determine the compatibility status. All three combinations performed well; yield was the best in combination with Carrizo relative to tree size. Fruit size distribution ranged from medium and large/extra-large (count 88-48/40), good Brix content (average 11.5), lower acid levels and higher Brix:acid ratio. The average seed count per fruit increased with 0.3 and peaked at 3.0 seeds. The external colour (between T1 and T3) at the middle of June was similar on all three rootstocks. Yield production and tree size showed Carrizo to be the best rootstock combination for Turkey. C35 developed the smallest tree size (2.5 m) in combination with Turkey. The Swingle trees were declining and die-back was visible. Based on the ratios, maturity will be end of May to middle June (Table 5.4.3.5).

Valearly

Valearly, bearing an average to good crop on the trees, was low-seeded this season (0.6 seeds per fruit). The internal quality of the fruit was good with juice levels above 50% early in the season, higher Brix on Carrizo (avg.14.8) compared to C35 (13.6) and acid of 0.84. In comparison with the other early maturing selections (Turkey and Weipe), Valearly seems to be at least two weeks earlier, similar to Weipe, with slightly delayed external colour development. Estimated maturity according to Table 5.4.3.5 seems to be 2nd week to the end of May.

Valencia Late (control)

The Valencia Late was included as one of the control selections in this trial at Bosveld Citrus. Yield production on the trees improved this season (70 kg per tree) and fruit size peaked from medium to large (count 88 to 64), optimal Valencia export quality. Acid levels were above 1.0% when the second evaluation was completed, indicating the late maturity qualities of the selection. The juice content improved this season to 59% and Brix 11 with last evaluation. Seed count went down from 1.0 seeds per fruit to 1.2. Maturity will be late in the season and according to Table 5.4.3.5, peak middle to end of August.

Conclusion

Alpha performed similarly to the 2017/8 evaluation, developing a good crop on the trees. The internal quality was good (juice levels lower) and fruit size peaked between counts 72 to 56/48 (picked up one count size).

Bennie 1 and 2 produced similar fruit qualities this season, as well as yield production and small to large fruit size (peaked from count 88 to 56). Delta was the control variety for the trial; fruit size peaked between count 72 and 48 (increase due to lighter crop) with good internal quality, reaching juice levels of 57%, Brix of 13.3 and acid content of 1.0%.

Du Roi was evaluated on Swingle this season with bigger fruit size ranging from count 88 to 56 (smaller compared to 2017 season). Kobus du Toit Late performed well with good fruit size and promising juice and Brix levels. Gusocora performed well on Swingle, meeting the export standards (acid levels improved).

Jassie produced an excellent internal quality (high Brix and acid) on Carrizo and Swingle, with small to large fruit size (count 88-56) due to the crop load on the trees. Lavalle 1 was ultra-late, maturing in August/September (acid above 1.0%) on swingle rootstock.

McClellan SL remained completely seedless (very low seeded) at the trial site with good internal quality and optimum fruit size (count 88-56).

Fruit quality on Midnight 1 was better, with higher Brix than Midnight 2 (Bosveld trial site). External colour was similar on both Midnight selections this season (slight delay on Midnight 2).

Turkey performed best in combination with Carrizo when Brix:acid ratio and yield production were considered. Valearly produced a better crop on the trees with a delayed colour development compared to Turkey this season. Future evaluations will determine the value of this cultivar for the citrus industry.

Table 5.4.3.5. Internal fruit quality data for Valencia orange selections at Bosveld Citrus (Letsitele), Groep 91 (Letsitele) and Moriah Citrus (Hoedspruit) during the 2018 season.

Cultivar	Rootstock	Date harvested	Site	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg seed	Fruit external colour
Alpha	SC	20/6/2018	Bosveld	74-81	88-64	55.1	10.8	1.45	7.4	0.7	T2-4
Alpha	SC	02/08/2018	Bosveld	70-86	88-48	57.2	10.9	1.25	8.7	0.3	T1-3
Alpha	SC	05/09/2018	Bosveld	73-85	72-56	60.1	11.8	1.00	11.8	1.5	T1-3
Bennie 1	SC	20/6/2018	Bosveld	74-85	72-56	55.7	11.2	0.90	12.4	0.9	T2-5
Bennie 1	SC	2/8/2018	Bosveld	73-85	72-56	59.0	11.1	0.75	14.8	2.7	T1-3
Bennie 1	SC	5/9/2018	Bosveld	75-86	72-48	61.2	11.0	0.70	15.7	1.6	T1-3
Delta	C35	20/6/2018	Bosveld	75-84	72-56	51.4	12.7	1.15	11.0	0.0	T2-5
Delta	C35	2/8/2018	Bosveld	75-86	72-48	52.4	14.5	1.10	13.2	0.0	T1-3
Delta	C35	5/9/2018	Bosveld	74-82	72-56	55.3	14.3	0.80	17.9	0.0	T1-3
Delta	CC	20/06/2018	Bosveld	68-86	88-48	50.2	12.6	1.35	9.3	0.0	T2-5
Delta	CC	02/08/2018	Bosveld	72-89	88-48	60.9	13.4	1.00	13.4	0.0	T1-3
Delta	CC	05/09/2018	Bosveld	68-84	88-56	55.6	14.2	0.80	17.8	0.0	T1-3
Delta	SC	20/06/2018	Bosveld	70-77	88-72	55.8	13.2	1.25	10.6	0.0	T2-6
Delta	SC	20/06/2018	Bosveld	68-78	88-64	53.9	10.3	0.95	10.8	0.0	T3-5
Delta	SC	02/08/2018	Bosveld	70-86	88-48	54.1	13.9	1.05	13.2	0.0	T1-3
Delta	SC	05/09/2018	Bosveld	70-85	88-56	57.7	14.3	0.90	15.9	0.0	T1-3

Du Roi	SC	20/06/2018	Bosveld	70-77	88-72	57.3	11.3	1.55	7.3	0.8	T1-4
Du Roi	SC	02/08/2018	Bosveld	71-79	88-64	58.6	10.9	1.20	9.1	0.0	T1-4
Du Roi	SC	05/09/2018	Bosveld	72-84	88-56	61.9	11.4	1.00	11.4	1.6	T1-3
K du Toit Late	C35	02/08/2018	Bosveld	65-79	105-64	59.7	14.3	1.10	13.0	5.3	T1-3
K du Toit Late	C35	05/09/2018	Bosveld	65-78	105-64	57.8	13.1	0.90	14.6	6.3	T1-3
K du Toit Late	CC	2/8/2018	Bosveld	67-80	105-64	54.2	13.9	1.00	13.9	4.3	T1-3
K du Toit Late	CC	05/09/2018	Bosveld	70-79	88-64	57.1	13.0	0.80	16.3	4.1	T1-3
K du Toit Late	SC	02/08/2018	Bosveld	69-84	88-56	53.4	13.1	1.00	13.1	5.3	T1-3
K du Toit Late	SC	05/09/2018	Bosveld	65-80	105-64	57.5	12.8	0.85	15.1	3.7	T1-3
Gusocora	SC	20/06/2018	Bosveld	68-75	88-72	56.6	10.3	0.95	10.8	0.0	T2-5
Gusocora	SC	02/08/2018	Bosveld	69-75	88-72	56.7	10.7	0.80	13.4	0.0	T1-4
Gusocora	SC	05/09/2018	Bosveld	70-85	88-56	57.4	11.1	0.65	17.1	0.0	T1-3
Jassie	C35	13/07/2018	Bosveld	67-78	105-64	55.9	14.2	1.10	12.9	2.9	T1-4
Jassie	C35	02/08/2018	Bosveld	65-77	105-72	53.7	14.0	1.30	10.8	4.7	T1-3
Jassie	C35	05/09/2018	Bosveld	65-82	105-56	57.3	14.3	1.05	13.6	6.8	T1-3
Jassie	CC	13/07/2018	Bosveld	72-80	88-64	56.8	13.9	1.10	12.6	3.2	T1-3
Jassie	CC	02/08/2018	Bosveld	71-86	88-48	55.9	13.3	1.10	12.1	4.9	T1-3
Jassie	CC	05/09/2018	Bosveld	78-82	64-56	56.7	13.8	0.80	17.3	3.3	T1-3
Jassie	SC	13/07/2018	Bosveld	72-83	88-56	57.9	12.8	1.30	9.8	3.2	T1-3
Jassie	SC	02/08/2018	Bosveld	70-77	88-72	67.5	12.6	1.00	12.6	4.0	T1-3
Jassie	SC	05/09/2018	Bosveld	69-84	88-56	54.6	13.0	0.80	16.3	2.4	T1-3
Lavalle	SC	02/08/2018	Bosveld	77-90	72-40	57.1	12.9	1.00	12.9	0.3	T1-4
Lavalle	SC	05/09/2018	Bosveld	75-86	72-48	63.2	11.2	0.70	16.0	0.6	T1-5
McCleane SL	C35	20/06/2018	Bosveld	71-79	88-64	52.5	13.6	1.35	10.1	0.0	T2-4

McClea n SL	C35	02/08/201 8	Bosveld	73- 82	72-56	53.4	13.8	1.10	12.5	0.0	T1-3
McClea n SL	C35	05/09/201 8	Bosveld	71- 82	88-56	59.2	14.7	0.90	16.3	0.0	T1-3
McClea n SL	CC	20/06//20 18	Bosveld	68- 80	88-64	51.0	13.6	1.20	11.3	0.0	T2-5
McClea n SL	CC	02/08/201 8	Bosveld	70- 79	88-64	55.3	14.2	1.00	14.2	0.0	T1-3
McClea n SL	CC	05/09/201 8	Bosveld	70- 77	88-72	60.0	14.8	0.90	16.4	0.0	T1-3
McClea n SL	SC	20/06/201 8	Bosveld	72- 87	88-48	53.0	12.9	1.15	11.2	0.0	T2-5
McClea n SL	SC	02/08/201 8	Bosveld	73- 86	72-48	52.8	13.7	1.15	11.9	0.0	T1-3
McClea n SL	SC	5/9/2018	Bosveld	69- 86	88-48	61.7	11.5	0.60	19.2	0.0	T1-2
Midknig ht 1	SC	20/06/201 8	Bosveld	70- 85	88-56	62.2	11.6	1.25	9.3	0.0	T1-4
Midknig ht 1	SC	02/08/201 8	Bosveld	71- 89	88-48	57.3	11.9	1.00	11.9	0.0	T1-3
Midknig ht 1	SC	05/09/201 8	Bosveld	76- 85	72-56	62.9	12.5	0.85	14.7	0.0	T1-3
Midknig ht 2	SC	20/06/201 8	Bosveld	73- 82	72-56	57.5	10.3	1.20	8.6	0.0	T2-4
Midknig ht 2	SC	02/08/201 8	Bosveld	74- 82	72-56	58.7	10.9	1.05	10.4	0.5	T1-4
Midknig ht 2	SC	05/09/201 8	Bosveld	73- 85	72-56	63.9	10.5	0.70	15.0	0.0	T1-3
Moos Late 1	C35	02/08/201 8	Bosveld	64- 82	125- 56	57.0	14.4	1.40	10.3	1.6	T1-3
Moos Late 1	C35	05/09/201 8	Bosveld	65- 82	105- 56	59.8	14.1	1.00	14.1	3.7	T1-3
Moos Late 1	CC	02/08/201 8	Bosveld	64- 71	125- 88	59.3	13.9	1.35	10.3	1.9	T1-3
Moos Late 1	CC	05/09/201 8	Bosveld	65- 72	105- 88	60.3	13.7	1.05	13.0	1.5	T1-3
Moos Late 1	SC	05/09/201 8	Bosveld	69- 85	88-56	56.1	13.4	1.05	12.8	0.8	T1-3
Moos Late 1	SC	02/08/201 8	Bosveld	70- 78	88-64	58.2	13.0	1.25	10.4	1.2	T1-3
Skilderk rans	C35	02/08/201 8	Bosveld	72- 84	88-56	52.8	13.9	1.20	11.6	0.1	T1-3
Skilderk rans	C35	05/09/201 8	Bosveld	70- 92	88-40	55.1	14.1	1.05	13.4	0.0	T1-3
Skilderk rans	CC	02/08/201 8	Bosveld	75- 82	72-56	55.7	13.5	1.20	11.3	0.0	T1-3
Skilderk rans	CC	05/09/201 8	Bosveld	73- 78	72-64	58.2	13.6	1.00	13.6	0.0	T1-3
Skilderk rans	SC	02/08/201 8	Bosveld	76- 94	72-40	54.8	13.4	1.15	11.7	0.0	T1-3
Skilderk rans	SC	05/09/201 8	Bosveld	76- 85	72-56	56.4	14.6	0.90	16.2	0.0	T1-3
Turkey	C35	04/05/201 8	Bosveld	75- 84	72-56	55.5	11.8	1.00	11.8	1.5	T5

Turkey	C35	20/06/2018	Bosveld	76-90	72-40	52.5	12.8	0.85	15.1	0.9	T1-3
Turkey	CC	04/05/2018	Bosveld	78-85	64-56	60.1	10.6	1.05	10.1	0.0	T5
Turkey	CC	20/06/2018	Bosveld	76-88	72-48	51.0	12.3	0.70	17.6	0.5	T1-3
Turkey	SC	20/06/2018	Bosveld	74-90	72-40	53.7	11.8	0.85	13.9	1.2	T1-3
Valearly	C35	04/05/2018	Bosveld	68-78	88-64	53.2	12.7	0.95	13.4	0.0	T5
Valearly	C35	20/06/2018	Bosveld	71-82	88-56	48.8	14.5	1.00	14.5	0.2	T1-4
Valearly	CC	04/05/2018	Bosveld	72-78	88-64	60.0	12.5	1.00	12.5	1.3	T5
Valearly	CC	20/06/2018	Bosveld	69-86	88-48	49.4	13.7	0.80	17.1	0.0	T1-4
Valearly	SC	04/05/2018	Bosveld	73-82	72-56	43.1	11.7	0.95	12.3	0.7	T5
Valencia late	SC	20/06/2018	Bosveld	74-80	72-64	57.6	10.8	1.20	9.0	1.0	T3-6
Valencia late	SC	2/8/2018	Bosveld	74-80	72-64	57.1	11	1.00	11.0	1.1	T1-4
Valencia late	SC	05/09/2018	Bosveld	72-81	88-64	59.8	10.7	0.65	16.5	1.2	T1-3
Bennie 1	CC	20/06/2018	G91	68-76	88-72	54.3	12.8	1.50	8.5	5.2	T2-4
Bennie 1	CC	02/08/2018	G91	72-80	88-64	59.2	13.1	1.30	10.1	5.8	T1-3
Bennie 1	SC	20/06/2018	G91	70-76	88-72	54.5	12.1	1.65	7.3	4.3	T2-4
Bennie 1	SC	02/08/2018	G91	68-82	88-56	51.1	13.9	1.45	9.6	4.8	T1-3
Bennie 2	CC	20/06/2018	G91	75-82	72-56	53.0	12.3	1.15	10.7	6.8	T1-4
Bennie 2	CC	02/08/2018	G91	74-85	72-56	55.6	12.7	1.05	12.1	5.8	T1-3
Bennie 2	SC	20/06/2018	G91	73-80	72-64	53.0	12.4	1.15	10.8	5.2	T2-4
Bennie 2	SC	02/08/2018	G91	71-82	88-56	52.8	12.8	1.15	11.1	4.1	T1-3
K du Toit Late	CC	02/08/2018	G91	71-82	88-56	56.3	10.7	1.05	10.2	3.4	T1-3
K du Toit Late	CC	05/09/2018	G91	73-82	72-56	59.0	11.6	0.85	13.6	4.0	T1-3
K du Toit Late	SC	02/08/2018	G91	72-87	88-48	50.6	11.0	1.00	11.0	4.8	T1-3
K du Toit Late	SC	05/09/2018	G91	73-85	72-56	56.3	11.2	0.75	14.9	3.2	T1-3

K du Toit Late	Sunki 812	02/08/2018	G91	73-82	72-56	57.5	12.4	1.10	11.3	2.8	T1-3
K du Toit Late	Sunki 812	05/09/2018	G91	75-84	72-56	57.1	12.2	0.90	13.6	4.1	T1-3
K du Toit Late	X639	02/08/2018	G91	75-83	72-56	54.8	11.2	0.96	11.7	1.3	T1-3
K du Toit Late	X639	05/09/2018	G91	72-84	88-56	55.6	11.1	0.85	13.1	4.5	T1-3
Jassie	CC	02/08/2018	G91	70-80	88-64	53.8	12.9	1.10	11.7	5.9	T1-3
Jassie	SC	02/08/2018	G91	64-80	125-64	50.5	14.1	1.50	9.4	5.9	T1-3
McClea n	CC	02/08/2018	G91	70-87	88-48	57.2	11.6	1.20	9.7	1.4	T1-3
McClea n	CC	05/09/2018	G91	75-89	72-48	56.1	11.1	0.85	13.1	0.4	T1-3
McClea n	SC	02/08/2018	G91	77-95	72-40	52.4	10.2	1.05	9.7	1.9	T1-3
McClea n	SC	05/09/2018	G91	75-86	72-48	57.3	11.9	1.00	11.9	1.1	T1-3
McClea n	Sunki 812	02/08/2018	G91	79-90	64-40	53.7	12.2	1.15	10.6	3.3	T1-3
McClea n	Sunki 812	05/09/2018	G91	72-88	88-48	64.9	12.8	1.00	12.8	0.8	T1-3
McClea n	X639	02/08/2018	G91	79-90	64-40	59.0	11.3	0.95	11.9	1.5	T1-3
McClea n	X639	05/09/2018	G91	74-85	72-56	58.5	11.8	0.90	13.1	1.9	T1-3
McClea n SL	CC	02/08/2018	G91	73-86	72-48	54.4	11.0	0.90	12.2	0.0	T1-3
McClea n SL	CC	05/09/2018	G91	72-83	88-56	56.3	11.6	0.70	16.6	0.1	T1-2
McClea n SL	SC	02/08/2018	G91	77-90	72-40	49.9	12.2	0.90	13.6	0.0	T1-3
McClea n SL	SC	5/9/2018	G91	73-85	72-56	54.7	12.1	0.85	14.2	0.0	T1-3
McClea n SL	Sunki 812	02/08/2018	G91	76-99	72-36	53.0	12.4	1.10	11.3	1.0	T1-3
McClea n SL	Sunki 812	05/09/2018	G91	72-85	88-56	69.0	13.2	0.90	14.7	0.0	T1-3
McClea n SL	X639	02/08/2018	G91	72-83	88-56	54.2	11.5	0.95	12.1	1.0	T1-3
McClea n SL	X639	05/09/2018	G91	74-85	72-56	60.7	12.2	0.80	15.3	0.0	T1-3
Moos Late 1	CC	02/08/2018	G91	68-77	88-72	59.7	13.1	1.70	7.7	4.2	T1-3
Moos Late 1	SC	02/08/2018	G91	65-74	105-72	58.0	13.3	1.90	7.0	0.0	T1-3
Skilderk rans	CC	02/08/2018	G91	72-80	88-64	51.8	13.1	1.20	10.9	0.0	T1-3

Skilderkrans	SC	02/08/2018	G91	63-85	125-56	51.8	13.9	1.50	9.3	0.6	T1-3
Turkey	C35	04/05/2018	G91	70-75	88-72	57.8	11.3	1.10	10.3	6.5	T5
Turkey	CC	04/05/2018	G91	68-74	88-72	63.9	11.1	1.20	9.3	2.3	T5
Turkey	SC	04/05/2018	G91	73-80	72-64	59.5	11.0	1.25	8.8	6.2	T5
Benny 2	MxT	21/6/2018	Moriah	76-89	72-48	54.4	10.5	1.15	9.1	0.1	T1-2
Midknig ht I15	MxT	21/6/2018	Moriah	78-84	64-56	56.8	11.9	1.05	11.3	0.1	T1-4
Midknig ht F17	MxT	21/6/2018	Moriah	71-85	88-56	57.7	11.1	0.85	13.1	0.1	T1-4

5.4.4 PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the hot inland areas (Letsitele and Malelane) Project 75C by J. Joubert (CRI)

Summary

Etna, Sirio and Tango matures first according to the results of the 2017 season for the warm production areas, and Tango developed the smallest fruit size and good internal quality. Samba and Leanri fits in before Furr, that follows and developing the highest seed count per fruit for this trial. Next will be African Sunset and Orah, developing the second highest seed count per fruit. The mid-maturing mandarins are represented by Valley Gold and Mor 26, which developed the highest Brix levels compared to the other selections (up to Brix of 12). Yosemite Gold and Gold Nugget matured next, towards the mid-late period of the Mandarin Hybrid range evaluated at this trial site, with good internal quality (Brix: acid ratio of 12.5:1) as well as good external colour (T1). Tahoe Gold followed, with the highest juice level of 71% for this season. Shasta Gold was the second last selection to mature at the middle to end of July and was completely seedless. Tambor and Tanor Late, followed by Sugar Belle (1.8% acid) was the last selections to mature at these trial sites, ending the Mandarin Hybrid season. Picking periods should not be longer than 3 - 4 weeks to maintain good internal quality and to avoid rind disorders.

Opsomming

Etna, Sirio en Tango word die vroegste ryp volgens resultate van die 2018 seisoen vir hierdie warm produksie area, met Tango die kleinste vruggrootte en goeie interne kwaliteit. Samba en Leanri pas in voor Furr, wat daarna volg met die hoogste saadtelling per vrug vir hierdie proef. Volgende is African Sunset en Orah, met die tweede hoogste saad telling per vrug. Die middel van die mandaryne word verteenwoordig deur Valley Gold en Mor 26 met die hoogste Brix vlakke in vergelyking met die ander seleksies (tot Brix van 12). Yosemite Gold en Gold Nugget was volgende om ryp te word en verteenwoordig die mid-laat van die Mandaryn Hibried reeks, ge-evalueer met 'n goeie interne kwaliteit vrug (Brix:suur verhouding van 12.5:1), asook goeie eksterne kleur ontwikkeling (T1). Tahoe Gold volg, met van die hoogste sap inhoud van 71% vir hierdie seisoen. Shasta Gold was die tweede laaste seleskie gereed vir oes teen middle tot einde Julie, en was totaal saadloos. Tambor en Tanor Late, gevolg deur Sugar Belle (1.8% suur) was die laaste seleksie om ryp te word op hierdie proef persele, wat ook die Mandaryn Hibried seisoen afsluit vir hierdie proef. Daar word aanbeveel om nie die oesperiode langer as 3 tot 4 weke te verleng nie om goeie interne kwaliteit te verseker met minimum skil probleme.

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 hot production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Mandarin Hybrid selections from Bosveld Citrus (Letsitele), Overbrug (Hoedspruit), Mahela Citrus (Letsitele), Moriah Citrus (Hoedspruit) and Riverside (Malelane) from the Limpopo region.

The following varieties were evaluated:

Table 5.4.4.1. List of Mandarin Hybrid selections evaluated at Bosveld Citrus (Letsitele) during the 2018 season.

Selection	Rootstock	Planted	Topwork
African Sunset (B24)	SC	2009	
Mor 26	SC	2009	
Samba	CC		2015
Shasta Gold	CC	2010	
Sugar Belle	CC		2015
Valley Gold (B17)	SC	2009	

Table 5.4.4.2. List of Mandarin Hybrid selections evaluated at Overbrug (Hoedspruit) during the 2018 season.

Selection	Rootstock	Planted
African Sunset (B24)	C35	2011
ARCCIT 9 (ARC Nadorcott LS)	CC	2011
Edit x Nova (Dina)	CC	2011
Nadorcott LS	CC	2011
Valley Gold	CC	2011

Table 5.4.4.3. List of Mandarin Hybrid selections evaluated at Mahela (Letsitele) during the 2018 season.

Selection	Rootstock	Planted
Etna	CC	2014
Gold Nugget	CC	2013
Saint André	CC	2014
Samba	CC	2014
Sirio	CC	2014
Tango	CC	2013
Tanor Late	CC	2014
Tahoe Gold	CC	2013
Yosemite Gold	CC	2013

Table 5.4.4.3. List of Mandarin Hybrid selections evaluated at Moriah (Hoedspruit) during the 2018 season.

Selection	Rootstock	Topwork
Africa Sunset	MxT	2011
Furr (Clemcott)	MxT	2011

Mor 26	MxT	2011
Orah	MxT	2011

Table 5.4.4.4. List of Mandarin Hybrid selections evaluated at Riverside (Malelane) during the 2018 season.

Selection	Rootstock	Planted
Gold Nugget	CC	2011
Shasta Gold	CC	2011
Tahoe Gold	CC	2011
Tango	CC	2011
Yosemite Gold	CC	2011

Results and discussion

For Mandarin Hybrid selections, a ratio of 11:1 is considered to be the build-up towards peak maturity of 12:1. After reaching the peak, the ratio increases to 13:1, after which it is considered over-mature. This process from the 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.

African Sunset (B24)

There was enough fruit on the trees to complete one evaluations at the Bosveld, one at Moriah and two evaluations at the Overbrug trial site, due to a fairly light crop. The large to very large fruit size (count 1XXX) is also a selection quality, but the light crop contributes to this scenario. African Sunset developed a protruding navel-end on most of the fruit; the bigger the fruit size the more visible the navel-end. The internal quality was better at the Overbrug site (high juice (63%), Brix 14, and fairly low acid levels (0.85%) compared to Bosveld with low juice (average 52.9%), Brix 10.4, and fairly low acid levels (average 0.65%). Fruit was completely seedless at both sites. External colour improved ranging from T1 to T2, bearing in mind the hot production areas. Based on the internal quality results in Table 5.4.4.5, estimated maturity will be middle to the end of May.

Etna

Etna bore a good crop with large to extra-large fruit (count 1X to 1XX) and good internal quality for a hot production area. External colour was better at peak maturity (T2). Average seed count was 0.8 seeds per fruit this season due to cross-pollination (lower compared to 2017). Maturity seems to be middle of April for the hot production areas, according to the information in Table 5.4.4.5.

Furr (Clemcott)

Furr developed large to extra-large fruit size (count 1XX – 1XXX) on the trees at Moriah Estate, one of the characteristics of the cultivar, as well as an excellent crop on the trees (60 to 80 kg/tree). The external colour development on the fruit was good for the Hoedspruit area (T1-2). Internally the fruit quality was very good, developing high juice (up to 60%) and Brix (up to 12.8) levels with fairly low acids. Another quality of the fruit is the high seed count of between 6.5 and 16.3 seeds per fruit (high self-and cross pollination). Maturity seems to be middle to the end of May to middle of June for the hot production areas, according to the information in Table 5.4.4.5.

Gold Nugget

Gold Nugget developed a very upright tree shape (V shape) with long aggressive growing shoots in the middle, bearing no crop at all. The crop will be on the other bearing branches towards the middle of the tree, and correctional pruning on this variety is crucial. Remove the long shoots to set the crop lower, as well as to set more smooth textured fruit on the lower branches. Gold Nugget is known for its rough textured fruit with coarse rinds, but in the evaluations it came to light that the lower fruit was smoother compared to the fruit on the aggressive long upright branches. The internal quality of the fruit improved with tree age and developed good juice (up to 65%), high Brix (up to 11.7) and lower acid (above 0.7%) levels and an improved external colour (T1). Future evaluations will determine the feasibility of this selection in the hot areas. Fruit was completely

seedless at both trial sites. Based on the internal quality results in Table 5.4.4.5, estimated maturity will be the end of May to middle of June.

Mor 26

Mor 26 produced a light (Bosveld) to average (Moriah) crop on the trees for the 2018 season. The fruit size was erratic and peaked between count 1 and 1XXX, medium to large/extra-large fruit. The external colour development was yellow and peaked at T1-2. The internal quality was good with high juice levels of up to 60%, Brix up to 14 and acceptable acid levels (0.85 to 0.9%). There were on average 2.4 seeds in the fruit at Bosveld and no seeds at Moriah. Based on the internal quality results in Table 5.4.4.5, estimated maturity will be the end of May to the beginning of June.

Nadorcott ARC & ARCCIT 9 LS

There was a good crop on the ARC trees this season to evaluate (management improvement). The fruit shape was similar to the Nadorcott selection. Rind texture was very smooth with a natural shine (similar to packhouse waxing). Both selections produced a fruit size that peaked at count 1XX even with the better crop load on the trees. Nadorcott ARC and ARCCIT 9 LS produced low seed numbers in the fruit (up to 0.8 seeds per fruit) this season. Maturity seems to be two weeks earlier on the ARCCIT 9 LS selection, according to Table 5.4.4.5, but information was limited due to only two evaluations being done (beginning to middle of June).

Orah

Orah, producing a good crop on the trees with medium to large/extra-large (avg. count 1-1XX/1XXX) fruit size. The average seed count in the fruit picked up from 2017 to 11.5 seeds per fruit, one of the characteristics of the selection. Internal quality was good, the Brix levels were above 12.5 by time of harvest, as well as good juice levels (above 60%) and acceptable acids (0.85%). Early external colour development ranged from T1 to T4 (only two evaluation). Based on the internal quality results in Table 5.4.4.5, estimated maturity will be end of May (degreening) to the middle of June.

Samba

Samba on Carizzo rootstock produced a good second crop with good internal quality on the large fast growing thornless trees at Mahela and Bosveld (Letsitele). Colour development was early in the season (deep orange) with a smooth rind texture on the fruit. Fruit were completely seedless to low-seeded (0.8 to 1.6 seeds per fruit) this season in the combined trial block (future evaluations will confirm low seed numbers) and peaked from medium to large fruit size (average count 1 to 1XX). Based on the internal quality results in Table 5.4.4.5, estimated maturity will be middle to the end of April.

Shasta Gold

Shasta developed ribbing on most of the fruit, as well as sunburn. The fruit was fairly flat on the trees at all the trial sites. Rind texture on the fruit became smoother as the trees matured. Tree size compared to the other selections was medium with only Tahoe Gold developing into a smaller tree, with more compact bearing branches. There was no fruit on the trees at Mahela this year. The fruit quality at the Riverside was very similar compared to the previous season. The flavour improved with high juice (up to 57%) and the oil content in the rind was fairly high. Shasta produced fruit with soft fibre strength that peels easily, and all the fruit evaluated were completely seedless. The fruit size remained the same this season and peaked from large to very large (count 1XX-1XXX). The internal quality was good with juice levels up to 57%, Brix above 13 and acceptable acid levels (above 1.0%). Based on the internal quality results in Table 5.4.4.5 maturity will be middle of July to end of July.

Tahoe Gold

This selection developed the smallest tree size when compared to the other UCR 5 varieties (compact tree). Tahoe Gold produced a good crop on all the trial trees at Riverside (no fruit at Bosveld site – alternate bearing). The fruit size decreased due to the heavy crop and peaked from large to extra-large (count 1-1XX) and the fruit shape was similar to that of a Minneola tangelo fruit. The external colour was slightly delayed between T3-4 when the internal quality was optimum. Tahoe produced fruit with soft fibre strength that peels easily, and there was average 0.3 seeds per fruit evaluated (crosspollination in mixed orchard). The internal quality was good with juice levels of as high as 70%, Brix averaged 12.2 and acid levels were acceptable by the time of

harvest. Based on the internal quality results in Table 5.4.4.5, estimated maturity will be middle to the end of June.

Tambor

Tambor is an addition to the late maturing mandarin selections for the hot production areas, producing 0.7 seeds per fruit, fairly low compared to the Furr and Orah selections. The external colour was on the yellow side at peak maturity, but with good internal quality, developing juice levels above 64%, Brix above 11 and acids above 1.4 at the last evaluation. Fruit size peaked from count 1XX to count 1XXX, very large for mandarin varieties. Based on the internal quality results in Table 5.4.4.5, estimated maturity will be end of July to the middle of August.

Tango

There was a good crop on the trees at all the trial sites this season compared to the average last year. Tango was completely seedless at all sites (in 2016 there was 0.2 seeds at Mahela). The fruit shape was similar to the Nadorcott selection. Rind texture was very smooth with a natural shine (similar to packhouse waxing). The Tango trees were thorn less with an upright growth pattern and tree shape. The fruit was firm and the rind thin, fibre was soft and peeled very easily. Internally the fruit was high in juice content (above 52%), Brix levels lower (average 9.2) and peaked at 12.1 for the Bosveld trial (lowest Brix at Mahela site – 8.0), acid levels were on the lower side early in the season (indicating a shorter shelf life), and deep orange coloured fibre. Fruit size peaked at count 2 to 1XX (medium to large). Based on the internal quality results in Table 5.4.4.5, estimated maturity will be end of April to the middle of May with delayed external colour development.

Valley Gold (B17)

Valley Gold was evaluated at the Bosveld and Overbrug trial site. The internal quality was good with Brix averaging 12.9 and acid levels around 0.9% and external colour between T1 and 2 when the second and third evaluation was completed. Fruit size peaked from count 1 to 1XX (large to very large) due to a fairly light crop on the trees. There was an increase in fruit split on the trees at Bosveld and high fruit split was present on the trees at Overbrug resulting in up to 30 to 60% fruit drop. Maturity is estimated to be end of May to middle of June for these hot production areas.

Yosemite Gold

The fruit set on Yosemite Gold remained light to very light at the Riverside and Mahela trial sites. Additional measures may be necessary to increase the crop on the trees, for example Gibb sprays or girdling. Yosemite Gold developed a very promising soft citrus type fruit shape (similar to Minneola tangelo). The fruit was firm, rind texture was smooth, and the fibre was soft, peeled very easily and completely seedless this season. Yosemite developed the biggest tree size compared to the other UCR 5 selections. This aggressive growth characteristic will be one of the reason for the poor crop on the trees (vegetative growth), and must be redirected into fruit set and crop on the trees (dwarfing rootstocks option). Fruit size decreased and varied from large to very large (count 1X-1XX), similar to Tahoe Gold, due to the light crop on the trees. The internal quality improved this season with higher juice (above 59%), good Brix (up to 61%) and good acid levels. External colour developed along with the internal quality towards the end of the evaluations (T1). Based on the internal quality results in Table 5.4.4.5, estimated maturity will be mid-June to mid-July.

Additional selection

The internal quality of Tasty (Bruce) was average with lower juice levels (49.7%), higher Brix (above 11) and acids were lower (0.95%), indicating the early maturing characteristics of the selection with completely seedless fruit. The fruit size peaked from count 1 to count 1XX.

Sirio produced large to extra-large fruit (count 1XX to 1XXX) on the trees due to a fair crop (large fruit size in hot areas and young trees) with average internal quality in the hot production areas. The fruit was low seeded with one evaluation (rest seedless).

Edit x Nova (Dina) bore fruit on the Overbrug (Hoedspruit) trial trees this year. The tree shape is very up-right with a dark bark colour. Fruit size varied from count 2 to 1XX, very good for mandarin production and almost

seedless fruit. The internal quality was good on the young trees and acid levels remained on the low but stable side.

Leanri cropped the first fruit on the trees this season at Moriah, the fruit size varied from large/extra-large (1XX-1XXX) with good internal quality.

One of the new ultra-late selections to include at the Bosveld trial site will be Sugar Belle, bearing fruit for the first time this year. Middle July the acids were still above 1.8% keeping the Brix: acid ratio below 9.0 at peak colour development (completely seedless fruit).

Tanor Late cropped fruit at Mahela in Letsitele and was at peak maturity by the end of July to middle August (late maturing selection). Fruit size was large to extra-large (count 1XX to 1XXX) with completely seedless fruit.

Conclusion

There was an improvement in the external colour delay in the hot areas that were a problem in the past; future evaluations will clarify the situation. Degreening may be an option for the Gold Nugget and TDEs (fruit colour development was yellow with degreening), but ethylene reacted slowly with Tango (W. Murcott selection) and Nadorcott. Furr, Tambor, Orah, Gold Nugget and Yosemite Gold may be a possibility to consider for the hot areas due to stronger fruit with optimal fruit size, and good internal quality when external colour becomes more intense (T1-2). In the hot areas it will be crucial to cover the mandarin orchards with shade net, to minimise sunburn and improve pack out percentage of the fruit. There was severe sunburn on the Shasta Gold fruit compared to the cooler production areas.

African Sunset, Tahoe Gold, Shasta Gold, Yosemite Gold, Furr, Gold Nugget and Mor 26 and had the larger fruit size, followed by Tambor, Samba and then Orah. The smaller fruit size was produced on Tango. Furr and Orah developed the highest number of seeds, followed by Mor 26 and Tambor. There were similar seed numbers this season in Tahoe Gold and Yosemite Gold, as well as Valley Gold.

Edit x Nova, Tanor Late, Sugar Belle and Leanri was evaluated for the first time; and Etna, Sirio and Tasty 1 for the second time this season; future evaluations will continue to determine suitability for this production area.

Table 5.4.4.5. Internal fruit quality data for Mandarin hybrid selections at Bosveld (Letsitele), Overbrug (Hoedspruit), Mahela (Letsitele), Moriah (Hoedspruit) and Riverside (Malelane) during the 2018 season.

Cultivar	Rootstock	Date harvested	Site	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg seed	Fruit external colour
African Sunset	SC	20/06/2018	Bosveld	82-94	1XXX-1XXX	52.9	10.4	0.65	16.0	0.0	T2-5
African Sunset	MxT	31/05/2018	Moriah	77-89	1XX-1XXX	61.8	11.5	0.85	13.5	0.0	T1
African Sunset	CC	01/06/2018	Overbrug	66-76	1-1XX	60.2	14.2	0.95	14.9	1.0	T1
African Sunset	CC	19/6/2018	Overbrug	74-83	1XX-1XXX	65.0	13.8	0.75	18.4	0.0	T1-2
Edit x Nova	CC	29/03/2018	Overbrug	65-69	1-1X	52.5	14.7	1.00	14.7	0.2	T6
Edit x Nova	CC	17/04/2018	Overbrug	61-69	2-1X	54.3	14.7	0.75	19.6	0.0	T5
Edit x Nova	CC	01/06/2018	Overbrug	61-76	2-1XX	73.8	15.1	0.75	20.1	0.0	T1

Etna	CC	28/03/2018	Mahela	67-75	1-1XX	64.6	8.2	0.90	9.1	1.4	T2
Etna	CC	17/04/2018	Mahela	76-89	1XX-1XXX	51.8	6.5	0.43	15.3	0.0	T4
Etna	CC	03/05/2018	Mahela	76-84	1XX-1XXX	53.0	6.1	0.55	11.1	0.8	T3
Furr	MxT	17/04/2018	Moriah	74-80	1XX-1XXX	60.7	10.7	0.70	15.3	16.3	T5
Furr	MxT	31/05/2018	Moriah	80-84	1XXX-1XXX	60.9	12.8	0.85	15.1	6.5	T1-2
Furr	MxT	21/6/2018	Moriah	75-90	1XX-1XXX	59.4	11.6	0.70	16.6	10.8	T1-3
Gold Nugget	CC	20/06/2018	Mahela	65-78	1-1XXX	63.4	11.2	0.90	12.4	0.0	T1-4
Gold Nugget	CC	28/05/2018	Riverside	67-77	1-1XXX	56.4	10.5	0.65	16.2	0.0	T1
Gold Nugget	CC	15/06/2018	Riverside	68-76	1X1XX	65.3	11.7	0.67	17.5	0.0	T1
Leanri	C35	29/03/2018	Moriah	70-79	1X-1XXX	50.0	8.7	0.75	11.6	0.0	T5
Leanri	C35	17/04/2018	Moriah	67-83	1-1XXX	61.7	13.5	0.73	18.6	0.5	T6
Leanri	C35	31/05/2018	Moriah	73-82	1XX-1XXX	64.0	12.8	0.75	17.1	0.0	T1-3
Leanri	C35	21/6/2018	Moriah	80-90	1XXX-1XXX	60.0	12.0	0.62	19.4	0.3	T1-3
Mor 26	SC	20/06/2018	Bosveld	64-77	1-1XX	60.3	14.1	0.85	16.6	2.4	T1-4
Mor 26	MxT	31/05/2018	Moriah	70-80	1X-1XXX	60.3	12.9	0.90	14.3	0.0	T1-2
Nadorcott ARC	CC	29/03/2018	Overbrug	55-66	3-1	50.7	14.3	2.05	7.0	0.3	T3
Nadorcott ARC	CC	17/04/2018	Overbrug	62-68	2-1X	53.7	14.0	1.15	12.2	0.0	T4
Nadorcott ARC	CC	01/06/2018	Overbrug	60-69	2-1X	62.2	14.6	1.25	11.7	0.2	T1
Nadorcott ARC	CC	19/06/2018	Overbrug	61-72	2-1XX	62.0	15.0	1.00	15.0	0.1	T1-3
Nadorcott ARC CIT 9 LS	CC	29/03/2018	Overbrug	59-67	2-1	38.2	14.1	1.90	7.4	0.3	T8
Nadorcott ARC CIT 9 LS	CC	17/04/2018	Overbrug	55-67	2-1	54.9	13.5	1.15	11.7	0.0	T6
Nadorcott ARC CIT 9 LS	CC	1/6/2018	Overbrug	58-66	3-1	64.5	14.3	1.05	13.6	0.1	T1
Nadorcott ARC CIT 9 LS	CC	19/06/2018	Overbrug	56-75	3-1XX	63.3	14.5	1.00	14.5	0.8	T1-4
Orah	MxT	31/05/2018	Moriah	67-78	1-1XXX	61.4	13.3	0.80	16.6	5.8	T1
Orah	MxT	21/6/2018	Moriah	65-77	1-1XX	62.8	12.1	0.85	14.2	17.2	T1-4
Saint Andre	CC	03/05/2018	Mahela	72-78	1XX-1XXX	60.0	12.7	0.65	19.5	0.3	T2

Saint Andre	CC	17/04/2018	Mahela	67-80	1-1XXX	60.7	13.2	0.70	18.9	1.3	T3
Samba	CC	04/05/2018	Bosveld	64-75	1-1XX	58.7	12.0	0.70	17.1	0.0	T3
Samba	CC	01/06/2018	Bosveld	71-78	1X-1XXX	51.4	11.0	0.75	14.7	0.0	T1
Samba	CC	28/03/2018	Mahela	65-73	1-1XX	53.3	13.7	0.85	16.1	0.8	T2
Samba	CC	17/04/2018	Mahela	65-73	1-1XX	61.2	14.2	0.70	20.3	1.6	T3
Samba	CC	03/05/2018	Mahela	68-74	1X-1XX	66.0	12.7	0.70	18.1	0.0	T1
Shasta Gold	CC	25/07/2018	Riverside	76-100	1XX-1XXX	58.6	13.1	1.20	10.9	0.0	T1-3
Shasta Gold	CC	28/08/2018	Riverside	80-97	1XXX	57.1	13.3	1.00	13.3	0.0	T1-3
Sirio	CC	28/03/2018	Mahela	66-71	1-1X	50.5	9.1	1.00	9.1	0.0	T5
Sirio	CC	17/04/2018	Mahela	72-78	1XX-1XXX	68.3	8.8	0.78	11.4	0.0	T6
Sirio	CC	03/05/2018	Mahela	72-85	1XX-1XXX	50.4	12.3	0.80	15.4	0.8	T3
Sugar Belle	CC	04/05/2018	Bosveld	65-71	1-1X	56.0	12.6	2.05	6.1	0.0	T4
Sugar Belle	CC	01/06/2018	Bosveld	64-75	1-1XX	63.0	14.6	2.00	7.3	0.0	T1
Sugar Belle	CC	20/06/2018	Bosveld	64-73	1-1XX	55.6	14.8	1.75	8.5	0.0	T1
Sugar Belle	CC	13/07/2018	Bosveld	63-72	2-1XX	58.5	16.4	1.85	8.9	0.0	T1-3
Tahoe Gold	CC	30/04/2018	Riverside	62-73	2-1XX	64.6	13.6	1.28	10.7	0.8	T4
Tahoe Gold	CC	28/05/2018	Riverside	66-81	1-1XXX	70.7	10.9	0.85	12.8	0.0	T3-4
Tahoe Gold	CC	15/06/2018	Riverside	64-75	1-1XX	63.8	12.0	0.75	16.0	0.0	T1
Tambor	MxT	31/05/2018	Moriah	77-93	1XX-1XXX	64.1	11.3	1.35	8.4	0.7	T1
Tango	CC	28/03/2018	Mahela	66-71	1-1XX	56.9	8.1	0.86	9.4	0.0	T5
Tango	CC	17/04/2018	Mahela	69-75	1X-1XX	59.7	8.0	0.80	10.0	0.0	T6
Tango	CC	03/05/2018	Mahela	64-75	1-1XX	67.1	8.4	0.70	12.0	0.0	T5
Tango	CC	20/06/2018	Mahela	66-76	1-1XX	57.5	11.1	1.35	8.2	0.0	T1-5
Tango	CC	05/04/2018	Riverside	64-75	1-1XX	58.5	8.1	0.83	9.8	0.0	T5
Tango	CC	30/04/2018	Riverside	63-72	2-1XX	62.3	8.0	0.80	10.0	0.0	T4
Tango	CC	28/05/2018	Riverside	67-77	1-1XXX	60.6	9.6	0.65	14.8	0.0	T1-2
Tango	CC	15/06/2018	Riverside	64-73	1-1XX	52.0	12.1	0.75	16.1	0.0	T1
Tanor Late	CC	21/06/2018	Mahela	73-89	1XX-1XXX	51.1	11.7	1.50	7.8	0.0	T1-4
Tanor Late	CC	02/08/2018	Mahela	74-82	1XX-1XXX	57.1	14.7	1.30	11.3	0.0	T1-3
Tasty 1	CC	03/05/2018	Mahela	65-77	1-1XX	49.7	11.9	0.95	12.5	0.0	T4
Valley Gold	SC	20/06/2018	Bosveld	67-71	1-1X	56.3	11.6	0.95	12.2	1.7	T1-4
Valley Gold	CC	01/06/2018	Overbrug	73-84	1XX-1XXX	55.2	13.4	0.80	16.8	0.0	T1-2

Valley Gold	CC	19/06/2018	Overbrug	69-75	1X-1XX	62.2	13.8	0.95	14.5	0.8	T1-2
Yosemite	CC	20/06/2018	Mahela	68-79	1X-1XX	61.7	12.8	0.95	13.5	0.1	T1-2
Yosemite Gold	CC	28/05/2018	Riverside	71-81	1X-1XXX	61.2	10.7	1.00	10.7	0.3	T1
Yosemite Gold	CC	15/06/2018	Riverside	73-79	1XX-1XXX	59.4	13.7	0.90	15.2	0.0	T1

5.4.5 PROGRESS REPORT: Evaluation of Valencia selections in the hot dry production areas (Weipe and Tshipise)

Project 899A by J. Joubert (CRI)

Summary

This was the fourth season to evaluate the NGB trial site and third season for the Alicedale site due to fruit numbers on the trees, and meaningful data collected. Valearly will start the season as the earliest maturing Valencia with a colour delay on the over mature fruit, followed by Weipe with low acid levels and wind damage on the rind. Delta will be next in line, followed by Alpha, Skilderkrans and McClean SL with delayed colour and completely seedless fruit. Next to mature will be McClean with advanced colour and 1.5 seeds per fruit. Kobus du Toit Late follows as part of the middle maturing Valencia section, Moos Late 1 and Rhode Red. Gusocora with seedless fruit, as well as Henrietta and Bennie will be next, followed by Louisa, and Jassie, towards the late Valencia section, with excellent internal quality and optimal colour development (96.9 kg per tree). Lavalle will end of the Valencia season in the warm dry production areas.

Opsomming

Hierdie was die vierde seisoen wat die NGB proef ge-evalueer is en die derde seisoen vir Alicedale as gevolg van voldoende vrugte aan die bome, betekenisvolle data kon versamel word. Valearly begin die seisoen as die vroegste Valencia met 'n vertraagde vrugkleur op oorryp vrugte, gevolg deur Weipe met lae suurvlakke en wind skade op die skil. Delta sal volgende in lyn wees, gevolg deur Alpha, Skilderkrans en McClean SL met later vrugkleur en totaal saadlose vrugte. Kobus du Toit Laat volg as deel van die mid-rypwordende Valencia gedeelte, met Moos Late 1 en Rhode Red. Gusocora met saadlose vrugte, asook Henrietta en Bennie is volgende, gevolg deur Louisa en Jassie, nader aan die laat Valencia periode met uitstekende interne kwaliteit en optimum kleur ontwikkeling (102 kg per boom). Lavalle sal die Valencia seisoen afsluit in die warm droë produksie areas.

Objective

- To find suitable Valencia selections with superior characteristics for the hot dry inland citrus production areas.

Materials and methods

Field evaluations and laboratory analyses were conducted on Bennie, Delta, Gusocora, Henrietta, Jassie, Kobus du Toit Late, Lavalle, Louisa, McClean SL, Mooslate 1, Rhode Red, Skilderkrans, Turkey, Valearly and Weipe at Alicedale (Tshipise) and NGB (Weipe).

Table 5.4.5.1. List of Valencia selections evaluated at Alicedale (Tshipise) during 2018.

Selection	Rootstock	Planted
Alpha	C35/Sunki 812/RL/X639	2013
Bennie	C35/Sunki 812/RL/X639	2013

Delta	C35/Sunki 812/RL/X639	2013
Gusocora	C35/Sunki 812/RL/X639	2013
Henrietta	C35/Sunki 812/RL/X639	2013
Jassie	C35/Sunki 812/X639	2013
Kobus du Toit Late	C35/Sunki 812/RL/X639	2013
Lavalle	C35/Sunki 812/RL/X639	2013
Louisa	C35/Sunki 812/RL/X639	2013
McClellan SL	C35/Sunki 812/RL/X639	2013
Rhode Red	C35/Sunki 812/RL/X639	2013
Skilderkrans	C35/Sunki 812/RL/X639	2013
Turkey	C35/Sunki 812/RL/X639	2013
Weipe	C35/Sunki 812/RL/X639	2013

Table 5.4.5.2. List of Valencia selections evaluated at NGB (Weipe) during 2018.

Selection	Rootstock	Topwork
Delta	X639	2012
Jassie	X639	2012
Kobus du Toit Late	X639	2012
McClellan SL	X639	2012
Moosrivier Late 1	X639	2012
Rhode Red	X639	2012
Skilderkrans	X639	2012
Valearly	X639	2012

Results and discussion

The Alicedale trial site at Tshipise bore fruit on all the cultivars on different rootstocks and evaluations were done accordingly. There was a good fruit set on the trees for 2018 (determine yield production) and all cultivar combinations will be evaluated in the next season.

Alpha

The fruit was completely seedless on all four rootstock combinations with medium to large fruit size (count 64 to 40). Rough lemon and Sunki 812 matured first Brix: acid ratio above 10 and high juice content (above 62%). It is apparent that Alpha's maturity is middle to end of July (Table 5.4.5.3).

Bennie

There was a good crop on Bennie (best with X639 – 134 kg/tree) and the fruit size peaked between count 64 and 40 (very good for Valencia production). The internal colour of the fruit was deep yellow, fruit shape round, rind texture fairly smooth, medium rag content and medium rind thickness. Bennie produced good juice levels (average 61%), Brix (average 10.0) and acid (1.0%) and fairly low seed counts (average 0.8 seeds per fruit). External colour by the time of harvest varied between T1 and T4. Based on ratios, Bennie end of June to beginning of July (Table 5.4.5.3).

Delta

The Delta (control) trees was topworked at NGB in the Weipe area on X639 rootstock because of high pH soils in the area and planted at Alicedale on four rootstocks. Fruit size distribution was uniform and range from count 88 to 40, medium to large/extra-large fruit and optimum Valencia requirements. Internal quality was good with high juice (up to 57%), Brix (10.3) and fairly low acid levels through the season (average 0.85%). Based on the internal quality results in Table 5.4.5.3, estimated maturity will be mid-June to mid-July.

Gusocora

Gusocora was evaluated at Alicedale this year on C35, RL, Sunki 812 and X639 rootstocks. The fruit was completely seedless and developed a good internal quality where juice (up to 59%), Brix (up to 12.5) and acid

above 0.8 complied with export requirements. The external colour (delayed) varied from T1 to T5, correlating with the internal quality and Brix:acid ratio (8:1 for maturity). Fruit size was bigger and peaked between counts 64 and 40/36, optimal fruit size for export Valencias (medium to large). There was a good crop on the trees (best on RL – 131 kg/tree). It is apparent that Gusocora's maturity is middle to end of July (Table 5.4.5.3).

Henrietta

Henrietta was evaluated on all four rootstock combinations at Alicedale, Tshipise this season. Juice levels peaked above 60% average with higher Brix (up to 10.4) and acids 1.2 (Sunki 812). The external colour development was good and peaked between T2 and T4 for the season. Average seeds per fruit total decreased to 0.4 seeds per fruit (1.8 seeds for 2017). Based on the internal quality results in Table 5.4.5.3, estimated maturity will be mid-July to mid-August.

Jassie

Jassie seems to be one of the most promising new Valencia selections being tested and evaluated in the different citrus production and climatic areas. The best crop production (102 kg/tree) on the new trees was a combination with Jassie (RL) at the Alicedale trial site (precocious bearing). Fruit size distribution was excellent even with the high yield on the trees; the counts were from 88 to 40. Fruit quality was good with high juice (average 59), Brix of up to 12.8 (X639) and fairly high acid levels (above 0.8%) at the final evaluation, indicating the late characteristics of the cultivar. The seed counts varied from 0.6 up to 6.8 seeds per fruit (average 4.0 seeds per fruit). Based on the internal quality results in Table 5.4.5.3, estimated maturity will be mid-July to mid-August.

Kobus du Toit Late

There was an external colour delay improved on the fruit during the season, ranging from T1 to T4/5 up until the last evaluation. Fruit average size varied from medium to large, count 88 to 48. Internal quality was good depending on the age of the trees and the rootstock combinations. Kobus du Toit Late performed the best on X639 at NGB, Weipe. Seed production remained the same this season but was still fairly low for a seeded selection (average 3.3 seeds per fruit). Acids levels was above 0.9% the entire season, except for on X639 at NGB. Maturity, based on the internal quality results in Table 5.4.5.3, is estimated to be the end of June to middle of July for these hot production areas.

Lavalle

Lavalle was evaluated on all four rootstocks this season. There was a decrease in seed production this season and Lavalle produced completely seedless fruit. The internal quality complied with export requirements and acid level was above 1.2% at the second evaluation at the middle of July (Alicedale harvested fruit to determine final crop). Keep in mind that Lavalle is a late Valencia selection with good shelf life and the optimal harvest time will be in August. The navel end on some fruit seems to develop a button and there were split fruit on some of the trees evaluated, but this varies from season to season. From the ratio on this date it is apparent that Lavelle's maturity is end of August to middle of September (Table 5.4.5.3).

Louisa

There was a good crop on all four rootstock combinations at Alicedale with RL cropping 120 kg per tree. The fruit was completely seedless and internal quality was average to good with improved juice (55 to 58%) and higher Brix levels (up to 10.9). The fruit colour was fairly yellow by the time of peak maturity between T2 and T5. Fruit size peaked from medium to large, count 72 to count 40. Based on the internal quality results in Table 5.4.5.3, estimated maturity will be middle to the end of July.

McClellan SL

When compared to all the other Valencia trial sites, McClellan SL remained completely seedless fruit (except one evaluation at NGB on X639 – 0.7 seeds). This year all the combination with McClellan SL was bearing fruit (98 to 123 kg per tree) at Alicedale to evaluate, indicating potential for the future (crop improvement due to Gibb spray). The fruit size peaked between count 88 and 48/40 (medium to large/very large) with good internal quality (juice up to 60%, Brix 12, acid 0.8). Maturity (Table 5.4.5.3) is estimated to be the end of June to middle of July.

Moosrivier Late 1

Moosrivier produced fruit with small, medium to large fruit size (count 105 to 64) at the NGB trial site on X639 rootstock. Internal quality improved and was very good with high juice (average 58.0%), average Brix (11.5) and improved acids (above 1.0) after completion of the evaluations. Seed count per fruit varied from 3.3 to 4.3 seeds and colour development peaked between T1 and T4. Based on the internal quality results in Table 5.4.5.3, estimated maturity will be middle to the end of July.

Skilderkrans

Skilderkrans was evaluated at Alicedale and NGB in the hot production areas. Fruit size varied from medium to large/extra-large (count 88-48/40). Internally the Brix content was good (up to 11.7) and the acid level of 0.85 to 1.2% (peak maturity) indicated a later maturing Valencia selection. Juice level increased to average 58.6% later in the season; above the minimum required export figure. There was a slight delay in external colour at Alicedale on all four of the rootstocks evaluated (T2-6). The fruit developed a smooth rind, fibre strength was fairly soft and the fruit shape was round. Ratios were lower this season due to the higher acid levels, delaying peak maturity to end of July and mid-August on Swingle and C35 (Table 5.4.5.3).

Turkey

Turkey cropped fruit on all four rootstock combinations at Alicedale performing well on RL, X 639, Sunki 812 and C35 with high juice (above 57%) and average Brix (above 9.1) levels, as well as acceptable acids (above 0.8). Colour development was delayed throughout the season (T3-4) and seed number remained fairly low (between 0.7 and 4.2 seeds per fruit). Maturity (Table 5.4.5.3) is estimated to be the end of May to middle of June for these hot production areas.

Weipe

The Weipe selection was developed to replace the Limpopo SL as an early maturing Valencia. Weipe was evaluated for the third time at the Alicedale trial site and was planted on C35, Sunki 812, RL and X639. There was a good crop on the trees due to bigger mature trees (52 to 83 kg/tree). Fruit size was medium to large/extra-large (count 72-48), internal quality was good (juice above 50%, acid 0.80%) with higher Brix level (up to 10). Colour development ranged from T2 to T3. Maturity is estimated to be middle to end of May (Table 5.4.5.3).

Additional selections

Valearly was only evaluated twice early this season due to a low acid level of 0.85%, juice above 52% and a good Brix (up to 11.2). The fruit size varied from count 88 to 56 (medium to large) and there were 0.7 seeds per fruit.

Rhode Red developed high numbers of Chimeras on the fruit and future evaluations will determine the potential of the cultivar due to instability.

Conclusions

Bennie matures well on the trees and to reduce rind pitting problems the recommendation will be to harvest the fruit from middle July onwards (stronger rind). Gusocora seems to have optimal colour development at peak maturity and degreening might be an option.

Valearly's colour was delayed at peak maturity (T5) and resulted in low acids (0.85) for the cultivar later in the season as well as poor shelf life. Rhode Red and Skilderkrans developed high numbers of Chimeras on the fruit this season, questioning the stability of the selection.

All the selections evaluated developed seeds in their fruit, except for Alpha, Delta, Gusocora, Louisa, Lavallo, McClean seedless, Skilderkrans, Valearly and Weipe. All the selections comply with the minimum export standards. The ideal fruit size distribution for Valencia exports was achieved and peaked from count 88 to count 56 (excellent).

Table 5.4.5.3. Internal fruit quality data for Valencia orange selections at Alicedale (Tshipise) and NGB (Weipe) during the 2018 season.

Selection	Root-stock	Date harvested	Site	Size mm	Count	Juice (%)	Brix °	Acid (%)	Ratio	Ave. seed	Colour
Alpha	RL	12/7/2018	Alicedale	80-93	64-40	62.7	9.7	0.90	10.8	0.0	T1-4
Alpha	X639	12/7/2018	Alicedale	82-94	56-40	61.3	9.9	1.05	9.4	0.1	T1-4
Alpha	Sunki 812	12/7/2018	Alicedale	82-97	56-36	62.2	10.1	1.00	10.1	0.1	T1-4
Alpha	C35	12/7/2018	Alicedale	79-99	64-36	60.3	10.0	1.10	9.1	0.0	T1-5
Bennie	RL	12/7/2018	Alicedale	80-95	64-40	61.3	9.1	1.00	9.1	0.0	T1-4
Bennie	X639	12/7/2018	Alicedale	80-95	64-40	57.5	9.3	0.95	9.8	0.8	T1-4
Bennie	Sunki 812	12/7/2018	Alicedale	84-93	56-40	64.5	10.7	1.05	10.2	1.6	T1-4
Bennie	C35	12/7/2018	Alicedale	82-95	56-40	62.2	10.2	1.05	9.7	0.6	T1-4
Delta	RL	12/7/2018	Alicedale	82-100	56-36	57.8	9.0	0.80	11.3	0.0	T3-6
Delta	X639	12/7/2018	Alicedale	77-92	72-40	56.1	9.4	0.80	11.8	0.0	T3-6
Delta	Sunki 812	12/7/2018	Alicedale	77-97	72-36	56.2	10.8	0.85	12.7	0.0	T3-6
Delta	C35	12/7/2018	Alicedale	78-92	64-40	56.7	10.0	0.80	12.5	0.0	T3-5
Delta	X639	11/07/2018	NGB	70-83	88-56	57.0	12.3	1.00	12.3	0.7	T1-4
Gusocora	RL	12/7/2018	Alicedale	78-100	64-36	57.7	9.0	0.90	10.0	0.0	T2-6
Gusocora	X639	12/7/2018	Alicedale	79-93	64-40	55.9	10.0	0.80	12.5	0.0	T2-5
Gusocora	Sunki 812	12/7/2018	Alicedale	79-97	64-36	58.4	10.2	0.95	10.7	0.0	T2-5
Gusocora	C35	12/7/2018	Alicedale	76-85	72-56	59.8	10.7	0.90	11.9	0.0	T1-5
Henrietta	RL	12/7/2018	Alicedale	75-89	72-48	59.5	8.4	1.00	8.4	0.0	T2-5
Henrietta	X639	12/7/2018	Alicedale	74-90	72-40	58.7	9.4	1.05	9.0	0.3	T2-6
Henrietta	Sunki 812	12/7/2018	Alicedale	75-88	72-48	62.4	10.4	1.20	8.7	0.0	T2-4
Henrietta	C35	12/7/2018	Alicedale	82-96	56-40	60.8	8.8	1.10	8.0	1.5	T1-4
Jassie	X639	12/7/2018	Alicedale	75-93	72-40	59.9	10.0	0.95	10.5	0.6	T1-4
Jassie	Sunki 812	12/7/2018	Alicedale	77-92	72-40	60.3	10.8	1.05	10.3	3.3	T1-4
Jassie	C35	12/7/2018	Alicedale	82-99	56-36	61.2	10.3	0.95	10.8	1.8	T1-4
Jassie	X639	11/07/2018	NGB	74-77	72-72	53.8	12.2	1.00	12.2	6.8	T1-3
Jassie	X639	04/09/2018	NGB	69-80	88-64	59.7	12.8	0.80	16.0	7.4	T1-3
K du Toit Late	RL	12/7/2018	Alicedale	76-87	72-48	58.1	9.1	0.90	10.1	2.8	T1-5
K du Toit Late	X639	12/7/2018	Alicedale	77-87	72-48	60.1	9.6	1.00	9.6	1.5	T1-5
K du Toit Late	Sunki 812	12/7/2018	Alicedale	73-85	72-56	60.6	10.5	1.10	9.5	3.6	T1-5
K du Toit Late	C35	12/7/2018	Alicedale	75-86	72-48	63.2	9.6	1.10	8.7	1.1	T1-5
K du Toit Late	X639	11/07/2018	NGB	64-76	125-72	56.6	12.1	1.00	12.1	4.8	T1-4
K du Toit Late	X639	04/09/2018	NGB	70-82	88-56	62.5	12.0	0.70	17.1	5.8	T1-3
Lavalle	RL	12/7/2018	Alicedale	78-91	64-40	58.8	9.1	1.30	7.0	0.0	T1-5
Lavalle	X639	12/7/2018	Alicedale	80-92	64-40	58.6	10.0	1.25	8.0	0.0	T1-5

Lavalle	Sunki 812	12/7/2018	Alicedale	81-93	64-40	57.1	11.3	1.55	7.3	0.0	T2-5
Lavalle	C35	12/7/2018	Alicedale	80-99	64-36	58.1	9.3	1.30	7.2	0.0	T2-5
Louisa	RL	12/7/2018	Alicedale	78-90	64-40	55.8	8.8	1.00	8.8	0.0	T2-5
Louisa	X639	12/7/2018	Alicedale	72-89	72-48	58.1	9.2	0.95	9.7	0.0	T2-5
Louisa	Sunki 812	12/7/2018	Alicedale	78-89	64-48	58.4	12.0	1.10	10.9	0.0	T2-5
Louisa	C35	12/7/2018	Alicedale	81-93	64-40	56.7	9.7	0.95	10.2	0.0	T2-5
McClellan SL	RL	12/7/2018	Alicedale	77-90	72-40	59.4	8.4	0.80	10.5	0.0	T2-6
McClellan SL	X639	12/7/2018	Alicedale	76-87	72-48	59.9	9.0	0.80	11.3	0.0	T2-5
McClellan SL	Sunki 812	12/7/2018	Alicedale	73-87	72-48	60.0	10.5	0.90	11.7	0.0	T2-5
McClellan SL	C35	12/7/2018	Alicedale	78-87	64-48	60.3	11.0	0.90	12.2	0.0	T2-5
McClellan SL	X639	11/07/2018	NGB	75-78	72-64	55.9	12.0	0.80	15.0	0.7	T2-3
McClellan SL	X639	04/09/2018	NGB	70-79	88-64	59.8	12.1	0.70	17.3	0.0	T1-3
Moos Late 1	X639	11/07/2018	NGB	67-75	105-72	53.3	11.4	1.00	11.4	3.3	T1-4
Moos Late 1	X639	04/09/2018	NGB	69-80	88-64	61.6	11.6	1.05	11.0	4.3	T1-3
Rhode Red	RL	12/7/2018	Alicedale	72-85	88-56	56.9	8.5	0.80	10.6	0.9	T2-6
Rhode Red	X639	12/7/2018	Alicedale	70-88	88-48	60.4	8.9	0.90	9.9	0.8	T2-6
Rhode Red	Sunki 812	12/7/2018	Alicedale	75-93	72-40	60.8	9.7	1.00	9.7	0.9	T2-6
Rhode Red	C35	12/7/2018	Alicedale	73-87	72-48	62.7	9.4	1.00	9.4	0.6	T2-6
Rhode Red	X639	11/07/2018	NGB	68-77	88-72	46.2	11.6	1.30	8.9	4.8	T1-4
Rhode Red	X639	04/09/2018	NGB	72-82	88-56	58.9	11.6	0.85	13.6	3.3	T1-3
Skilderkrans	RL	12/7/2018	Alicedale	75-94	72-40	58.4	8.8	0.95	9.3	0.0	T3-6
Skilderkrans	X639	12/7/2018	Alicedale	74-88	72-48	60.6	8.8	1.05	8.4	0.0	T2-6
Skilderkrans	Sunki 812	12/7/2018	Alicedale	74-92	72-40	57.8	10.5	1.10	9.5	0.0	T3-6
Skilderkrans	C35	12/7/2018	Alicedale	75-94	72-40	59.6	10.7	1.10	9.7	0.0	T3-6
Skilderkrans	X639	11/07/2018	NGB	70-82	88-56	57.3	11.5	1.15	10.0	0.6	T1-4
Skilderkrans	X639	04/09/2018	NGB	73-84	72-56	58.0	11.7	0.85	13.8	0.0	T1-3
Turkey	RL	3/5/2018	Alicedale	75-82	72-56	57.1	9.3	0.95	9.8	0.7	T5
Turkey	X639	3/5/2018	Alicedale	73-85	72-56	59.0	9.3	0.90	10.3	0.7	T5
Turkey	Sunki 812	3/5/2018	Alicedale	75-80	72-64	58.3	10.9	1.15	9.5	0.7	T5
Turkey	C35	3/5/2018	Alicedale	72-80	88-64	59.8	11.1	0.95	11.7	1.0	T5
Turkey	RL	30/5/2018	Alicedale	73-80	72-64	62.4	9.1	0.95	9.6	0.7	T3-4
Turkey	X639	30/5/2018	Alicedale	73-80	72-64	59.2	10.7	0.75	14.3	1.3	T3-4
Turkey	Sunki 812	30/5/2018	Alicedale	75-82	72-56	60.7	10.9	0.95	11.5	4.2	T3-4
Turkey	C35	30/5/2018	Alicedale	75-85	72-56	63.5	10.5	0.90	11.7	4.0	T3-4
Turkey	X639	02/05/2018	NGB	75-82	72-56	60.5	11.3	1.05	10.8	2.3	T5
Val early	X639	02/05/2018	NGB	72-83	88-56	56.5	9.9	0.85	11.6	0.5	T5
Val early	X639	30/05/2018	NGB	70-79	88-64	51.8	11.2	0.60	18.7	0.8	T2-3
Weipe	RL	3/5/2018	Alicedale	75-80	72-64	51.7	8.6	0.70	12.3	0.0	T5
Weipe	X639	3/5/2018	Alicedale	75-82	72-56	50.0	9.2	0.70	13.1	0.0	T5
Weipe	Sunki 812	3/5/2018	Alicedale	74-83	72-56	50.8	10.2	0.90	11.3	0.0	T5
Weipe	C35	3/5/2018	Alicedale	75-85	72-56	54.9	10.0	0.90	11.1	0.0	T5
Weipe	RL	30/5/2018	Alicedale	74-80	72-64	64.6	10.3	0.80	12.9	0.0	T2-3
Weipe	X639	30/5/2018	Alicedale	75-84	72-56	56.3	10.0	0.70	14.3	0.0	T3-4
Weipe	Sunki 812	30/5/2018	Alicedale	77-84	72-56	56.0	10.8	0.90	12.0	0.0	T3-4

Weipe	C35	30/5/2018	Alicedale	78-89	64-48	56.4	10.6	0.70	15.1	0.0	T2-3
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5.4.6 **PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the intermediate production areas (Marble Hall)**

Project 941C by J. Joubert (CRI)

Summary

The quality of the Mandarin Hybrid fruit improved in this climatic region (intermediate areas). The results indicated that RHM with high seed counts and low acid levels, followed by Leanri matures first. Tango was next in line with smaller fruit size and fair to good internal quality. Tango, Gold Nugget, Tahoe Gold and Yosemite Gold were completely seedless this season. I22 also indicated to be a fairly early maturing selection with a light crop on the trees. Edit x Nova and Meirav 63 seem to fit in with the mid-maturing selections with deep orange rind colour. Yosemite Gold was next to mature, followed by Tahoe Gold. Tahoe Gold developed large to extra-large fruit size for this season. Gold Nugget, followed by Shasta Gold was the last selection to mature, at the end of June, ending off the Mandarin Hybrid season for this trial. Picking periods should not be longer than 3 - 4 weeks to maintain good internal quality and to avoid rind disorders.

Opsomming

Die kwaliteit van die Mandaryn Hibried vrugte het verbeter in die klimaatsonne (intermediere areas). Die resultate het aangedui dat RHM met hoë saadtellings en lae suurvlakke, gevolg deur Leanri die vroegste ryp geword het. Tango is volgende, met kleiner vruggrootte en gemiddelde tot goeie interne kwaliteit. Tango, Gold Nugget, Tahoe Gold en Yosemite Gold was total saadloos gewees hierdie seisoen. I22 het ook as een van die vroeër seleksies ingepas met 'n ligte oes op die bome. Edit x Nova en Meirav 63 pas hier in saam met die mid seleksies met diep oranje skilkleur Yosemite Gold was volgende gereed vir oes, gevolg deur Tahoe Gold. Tahoe Gold het groot tot baie groot vrugte vir hierdie seisoen geproduseer. Gold Nugget, gevolg deur Shasta Gold was die laaste seleksie gereed vir oes, teen einde Junie, wat die Mandaryn Hibried seisoen afsluit vir hierdie proef. Daar word aanbeveel om nie die oesperiode langer as 3 tot 4 weke te verleng nie om goeie interne kwaliteit te verseker met minimum skil probleme.

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 intermediate, inland production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Mandarin Hybrid selections from Moosrivier Estate (Marble Hall).

Table 5.4.6.1. List of Mandarin Hybrid selections evaluated at Moosrivier Estate (Marble Hall) during the 2018 season.

Selection	Rootstock	Planted
African Sunset (B24)	Sunki 812	2013
Edit x Nova	Sunki 812	2013
Gold Nugget	CC/C35/X639	2013
I 22	Sunki 812	2013
IRM 1 & 2	Sunki 812	2013

Leanri	Sunki 812	2013
Meirav 63	Sunki 812	2013
Meirav 119	Sunki 812	2013
Michal 6/47	Sunki 812	2013
Michal 89/64	Sunki 812	2013
Mor 2	Sunki 812	2013
Mor 15	Sunki 812	2013
Mor 26	Sunki 812	2013
RHM	CC/Sunki 812	2013
Shani SL	Sunki 812	2013
Tahoe Gold	CC/C35/X639	2013
Tango	CC/C35/X639	2013
Valley Gold	Sunki 812	2013
Yosemite	CC/C35/X639	2013

Results and discussion

The trial site at Moosrivier was relocated to a new site and trees were established for future evaluations due to cold damage and soil quality at the old site. All the trees at Moosrivier bore their third crop for this season with improved fruit numbers and more mature tree internal quality and fruit size characteristics.

For Mandarin Hybrid selections, a ratio of 11:1 is considered to be the build-up towards peak maturity, with a ratio of 12:1. After reaching the peak, the ratio increases to 13:1, 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 a greater instance of quality and rind issues.

African Sunset (B24)

The very large fruit size (count 1XXX) is a selection quality and the light crop contributes to this scenario. African Sunset developed a protruding navel-end on most of the fruit; the bigger the fruit size the more visible the navel-end. The internal quality improved with average juice (54%), Brix above 10 and acceptable acid levels. External colour peaked at T1 to T4 for this intermediate production area.

Gold Nugget

Gold Nugget developed a very upright tree shape (V shape) with long aggressive growing shoots in the middle, bearing no crop at all. The crop will be on the other lower bearing branches towards the middle of the tree, and correctional pruning on this variety is crucial. Remove the long shoots to set the crop lower on the tree with this variety, and to set more smooth textured fruit on the lower branches. Gold Nugget is known for its rough textured fruit with coarse rinds, but in the evaluations it came to light that the lower fruit was smoother compared to the fruit on the aggressive long upright branches. The fruit on all the trees at Moosrivier were completely seedless, and fruit size at Moosrivier was large (count 2-1XXX) due to young trees. The internal quality was average, low juice (from 42 % up to 51%) was captured throughout the season; Brix and acid levels were better (avg. 11.7 and 1.0%). The external colour improved this season and peaked between T1 and T4. Based on the internal quality results in Table 5.4.6.2, estimated maturity will be the middle of June.

IRM 1&2

IRM 2 developed better external colour (orange) earlier in the season and more ribbing on the fruit compared to IRM 1 at other trial sites. IRM 1&2 produced good to very good internal quality fruit with 58.2% juice, Brix above 11 and acids above 1.0%. There was a slight colour delay on the IRM 2 fruit at peak maturity. Seed counts very low and peaked at 4.6 compared to IRM 1 with 3.0 seeds per fruit in the combined trial block (cross pollination).

Mor 2, 15 and 26

Mor 2 and 15 was planted as control for the 26 selection at Moosrivier. Mor 2 had no fruit on the trees this season. The fruit size was erratic and peaked between count 1 and 1XXX, large to extra-large fruit. The

external colour development was yellow and peaked between T2 and T4. The internal quality improved to good with juice levels of up to 60% (peak maturity), Brix up to 14 and fairly high acid levels (avg. 1.0%). There were on average 2.2 seeds in the fruit at the Moosrivier site. Based on the internal quality results in Table 5.4.6.2, estimated maturity will be the end of May to the beginning of June.

Tahoe Gold

Tahoe developed a small tree size (compact tree) when compared to the other UC5 varieties. The tree bears fruit in bundles in a similar way to grapefruit. The fruit size peaked from large to very large (count 1-1XXX) and the fruit shape was similar to that of Minneola tangelo. There was no delay this season in the external colour development at Moosrivier (T1-4). Tahoe produced fruit with soft fibre strength that peeled fairly easily, and all the fruit evaluated were completely seedless. The internal quality improved from good to very good; juice up to 61%, Brix up to 13 and acceptable acids (Table 5.4.6.2). Estimated maturity is end of May to middle June.

Tango

Tango remained completely seedless at the trial site except for one evaluation with 0.3 seeds per fruit. There was a good to very good crop on the trees at Moosrivier and the fruit shape was similar to the Nadorcott selection. Rind texture was very smooth with a natural wax shine on the fruit. The Tango trees were thorn less with V-tree shape. The fruit was firm and the rind thin, fibre was soft and peeled very easily. Internally the fruit was high in juice content (up to 62%), Brix was average/good (up to 12) and the acid levels stabilizing below 1.0 towards the end of the season (X639 outperformed C35 and Sunki 812 this season). Fruit size varied on young trees and peaked at count 2 to 1XXX (medium to large/extra-large). Based on the internal quality results in Table 5.4.6.2, estimated maturity will be end of April to middle of May (delayed external colour).

Valley Gold (B17)

Valley Gold was evaluated at the new trial site located at Moosrivier. The internal quality was good with Brix averaging 10.4 and acid levels around 1.0% and external colour between T1 and 3 when the third evaluation was completed. Fruit size peaked from count 1 to 1XXX (large to very large) due to a fairly light crop on the trees. There was an increase in fruit split on the trees and high fruit split was present resulting in up to 30 to 60% fruit drop. Maturity is estimated to be middle to end of June for this intermediate production area.

Yosemite Gold

Yosemite Gold cropped a light yield on C35, CC and X639 (only one evaluation possible), and additional measures may be necessary to increase the crop on the trees (Gibb sprays or girdling). Yosemite developed a very promising soft citrus fruit shape. The fruit was firm, rind texture was smooth and the fibre was soft. It peeled very easily and the fruit was completely seedless. Yosemite Gold developed the biggest tree size compared to the other TDE selections at all the different mandarin trial sites. This aggressive growth characteristic is the reason for the poor crop on the trees (vegetative growth), and must be channelled into fruit set and crop. Fruit size varied from large to very large (count 1XX-1XXX), similar to Shasta Gold and Tahoe Gold. The internal quality was average to good developing higher juice and acid levels (above 1.0%) with improved Brix for the season (above 12). External colour developed along with the internal quality towards the end of the evaluations (T1 to T3). Based on the internal quality results in Table 5.4.6.2, estimated maturity will be the middle to end of June.

Additional selections

The internal quality of Edit x Nova was average this season with lower juice levels (average 48%) and no granulation problems in the fruit compared to Nova. Brix (average 12.4) and higher acids (1.1%), indicating the mid maturing characteristics of the selection in the intermediate productions areas, with low seeded fruit (average 0.1 seeds per fruit). The fruit size peaked from count 2 to count 1XX (bigger compared to 2017).

I22 cropped a poor yield on the trees and two evaluations was possible. The colour development was delayed compared to the internal qualities and over mature Brix: acid ratio of the fruit.

Leanri developed a fairly large fruit size between count 1 and 1XXX, slightly smaller compared to the hot production areas. 2018 was the first crop on the trees and internal quality was very good on Sunki 812 (average juice 57%, Brix 13.2, acid 1.0%). Seed numbers were fairly low, between 1.6 and 3.0 seeds per fruit.

Meirav 63 and 119 (experimental) developed a deep orange rind colour (T1 with peak maturity). Internal quality improved and was good with high juice content (average above 50%), Brix of 11.9 and acids above 1.1%. The fruit evaluated was low seeded (average 1.7) at the Moosrivier trial site.

Shani SL bore a fair crop on the trees with large to extra-large fruit size (count 1 to count 1X1XXX) as well as deep orange rind colour (T1-T5). Internal quality was good early in the season with good juice (up to 62%), Brix (12) and acids (average 1.3%) levels. The fruit was low seeded and ranged from 0.7 to 1.6 seeds per fruit).

RHM cropped fruit for the first time this season with high seed numbers (average 13.2 seeds per fruit) and are prone to crosspollination. There was a delayed colour development (T8) from the first evaluation with low acids (0.80%), indicating peak maturity Brix: acid ratio (CC – above 10 and Sunki 812 above 14). Future evaluations will determine optimum quality of the fruit evaluated.

Conclusion

The delay in external colour development improved this season due to age of the trees (more mature); future evaluation will confirm this. Gold Nugget improved considerably with smoother fruit, large fruit size and good internal quality. Yosemite Gold had the largest fruit size (light crop), followed by Tahoe Gold, and then Gold Nugget. The smaller fruit size was produced on Tango, reaching up to 1XX, with a lighter crop on the young trees at Moosrivier. There were no incidences of seed in the UCR 5 fruit at the trial site (very low – 0.2 seeds per fruit).

This was the second evaluation of IRM 1&2, Leanri, Meirav 119, Mor 2, 15, 26 and UC 5; the third evaluation of Edit x Nova and Meirav 63 at Moosrivier; so information is limited and future evaluations will improve recommendations on these varieties (management improvement). The highest seed numbers were on RHM, IRM 1&2 and the Meirav selections this season, followed by the Mor selections. All the other selections developed very low seed numbers in the fruit. Meirav 63 and 119 performed well and internally the fruit was high in juice and Brix content with deep orange colour development, as well as Exit x Nova (Dina).

Table 5.4.6.2. Internal fruit quality data for Mandarin hybrid selections at Moosrivier (Marble Hall) during the 2018 season.

Cultivar	Rootstock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg seed	Fruit external colour
African Sunset (B24)	Sunki 812	07/06/2018	86-100	1XXX	56.9	12.5	1.28	9.8	1.5	T2-3
African Sunset (B24)	Sunki 812	20/06/2018	85-100	1XXX	50.3	10.8	0.95	11.4	0.8	T1-4
Edit x Nova	Sunki 812	17/05/2018	63-82	2-1XX	50.0	12.4	1.10	11.3	0.0	T3
Edit x Nova	Sunki 812	07/06/2018	72-86	2-1XX	45.2	11.8	1.00	11.8	0.0	T1
Edit x Nova	Sunki 812	20/06/2018	66-81	1-1XX	47.9	13.1	1.20	10.9	0.3	T1-2
Gold Nugget	C35	07/06/2018	67-85	1-1XXX	49.3	10.9	1.05	10.4	0.0	T5-6
Gold Nugget	C35	20/06/2018	72-85	1XX-1XXX	49.6	13.9	0.85	16.4	0.0	T3-4
Gold Nugget	C35	20/07/2018	67-99	1-1XXX	42.2	12.3	1.44	8.5	0.0	T1-3

Gold Nugget	CC	07/06/2018	68-79	1X-1XXX	42.7	10.5	0.70	15.0	0.0	T5-6
Gold Nugget	CC	19/07/2018	70-80	1X-1XXX	47.4	12.8	1.00	12.8	0.0	T1-4
Gold Nugget	X639	07/06/2018	73-89	1XX-1XXX	48.0	10.0	1.00	10.0	0.0	T5-6
Gold Nugget	X639	20/06/2018	72-83	1XX-1XXX	50.9	10.6	0.90	11.8	0.0	T3-4
Gold Nugget	X639	19/07/2018	61-84	2-1XXX	46.1	12.4	1.00	12.4	0.0	T1-3
I 22	Sunki 812	17/05/2018	60-69	2-1X	47.5	9.6	0.95	10.1	1.5	T6
I 22	Sunki 812	20/06/2018	66-81	1-1XXX	52.4	10.4	0.80	13.0	4.2	T3-5
IRM 1	Sunki 812	07/06/2018	69-83	1X-1XXX	58.9	10.7	1.00	10.7	1.5	T3-4
IRM 1	Sunki 812	20/06/2018	68-77	1X-1XX	58.2	9.6	0.85	11.3	5.0	T2-4
IRM 1	Sunki 812	19/07/2018	65-80	1-1XXX	59.8	12.5	1.00	12.5	2.4	T1-3
IRM 2	Sunki 812	07/06/2018	68-79	1X-1XXX	57.6	11.3	1.00	11.3	4.5	T2-3
IRM 2	Sunki 812	20/06/2018	70-85	1X-1XXX	57.0	11.7	0.95	12.3	4.8	T2-4
IRM 2	Sunki 812	04/07/2018	71-87	1X-1XXX	59.6	11.6	0.75	15.5	4.3	T1-3
IRM 2	Sunki 812	19/07/2018	71-82	1X-1XXX	58.0	11.9	1.00	11.9	4.7	T1-3
Leanri	Sunki 812	08/05/2018	68-77	1X-1XX	59.3	13.6	1.05	13.0	1.6	T3
Leanri	Sunki 812	17/05/2018	76-85	1XX-1XXX	57.9	12.2	0.90	13.6	2.3	T2
Leanri	Sunki 812	07/06/2018	71-88	1X-1XXX	52.9	14.1	1.00	14.1	3.0	T1-2
Leanri	Sunki 812	20/06/2018	71-80	1X-1XXX	57.2	12.8	0.90	14.2	2.4	T1-3
Meirav 63	Sunki 812	08/05/2018	61-73	2-1XX	58.0	10.2	1.25	8.2	3.5	T3
Meirav 63	Sunki 812	17/05/2018	62-70	2-1X	54.1	12.8	1.10	11.6	0.7	T3
Meirav 63	Sunki 812	07/06/2018	61-67	2-1	50.0	15.1	1.20	12.6	0.8	T1
Meirav 63	Sunki 812	20/06/2018	59-70	2-1X	47.3	15.0	1.06	14.2	2.4	T1-3
Meirav 119	Sunki 812	13/04/2018	62-73	2-1XX	43.0	8.1	0.88	9.3	2.0	T8
Meirav 119	Sunki 812	17/05/2018	66-75	1-1XX	53.1	11.9	0.90	13.2	2.3	T2-3
Meirav 119	Sunki 812	07/06/2018	61-75	2-1XX	43.6	13.4	1.00	13.4	1.0	T1
Meirav 119	Sunki 812	20/06/2018	68-81	1X-1XXX	47.4	13.5	0.80	16.9	2.3	T1-2
Meirav 119	Sunki 812	04/07/2018	68-77	1X-1XX	44.6	13.8	0.90	15.3	1.7	T1-2

Michal 6/47	Sunki 812	13/04/2018	55-65	3-1	55.0	13.8	0.75	18.4	2.4	T8
Michal 6/47	Sunki 812	08/05/2018	55-66	3-1	61.7	13.3	0.65	20.5	1.2	T4
Michal 6/47	Sunki 812	17/05/2018	56-63	3-2	35.7	12.3	0.75	16.4	0.0	T3-4
Michal 6/47	Sunki 812	07/06/2018	60-65	2-1	56.0	13.3	0.75	17.7	2.0	T1
Michal 6/47	Sunki 812	20/06/2018	58-66	3-1	55.9	15.6	0.95	16.4	2.3	T1-2
Michal 89/64	Sunki 812	17/05/2018	61-70	2-1X	53.3	11.8	0.80	14.8	1.0	T4
Michal 89/64	Sunki 812	07/06/2018	61-75	2-1XX	52.5	12.6	0.85	14.8	1.4	T1
Michal 89/64	Sunki 812	20/06/2018	56-62	3-2	45.1	15.2	1.04	14.6	3.9	T1-2
Mor 15	Sunki 812	20/06/2018	70-80	1X-1XXX	60.3	10.0	0.90	11.1	3.1	T2-4
Mor 26	Sunki 812	07/06/2018	65-75	1-1XX	55.5	11.5	1.15	10.0	3.6	T2-3
Mor 26	MxT	21/06/2018	66-79	1-1XXX	64.4	13.9	0.95	14.6	0.0	T1-2
RHM	CC	13/04/2018	60-70	2-1X	54.9	9.1	0.88	10.4	18.3	T8
RHM	CC	08/05/2018	61-74	2-1XX	52.1	10.1	0.60	16.8	10.5	T6
RHM	CC	17/05/2018	63-69	2-1X	56.2	12.2	0.65	18.8	11.0	T3-4
RHM	CC	07/06/2018	70-77	1X-1XX	58.1	11.6	0.60	19.3	15.3	T1-2
RHM	CC	20/06/2018	65-77	1-1XX	59.4	13.1	0.62	21.1	16.8	T1-2
RHM	Sunki 812	13/04/2018	56-68	3-1X	54.4	10.7	0.75	14.3	16.5	T8
RHM	Sunki 812	08/05/2018	60-74	2-1XX	54.2	12.7	0.60	21.2	10.0	T6
RHM	Sunki 812	17/05/2018	64-70	1-1X	56.0	11.9	0.80	14.9	9.9	T3-4
RHM	Sunki 812	07/06/2018	64-79	1-1XXX	59.9	14.4	0.70	20.6	13.4	T1-2
RHM	Sunki 812	20/06/2018	65-73	1-1XX	59.8	13.0	0.65	20.0	10.2	T1-2
Shani SL	Sunki 812	17/05/2018	67-78	1-1XXX	62.8	12.3	1.40	8.8	0.7	T5-6
Shani SL	Sunki 812	07/06/2018	66-82	1-1XXX	54.9	11.9	1.50	7.9	1.6	T2-3
Shani SL	Sunki 812	20/06/2018	68-82	1X-1XXX	56.3	12.7	0.95	13.4	1.6	T2-4
Shani SL	Sunki 812	19/07/2018	70-85	1X-1XXX	56.6	12.6	1.25	10.1	0.8	T1-5
Tahoe Gold	C35	17/05/2018	67-76	1-1XX	59.4	10.4	1.05	9.9	0.0	T5-6
Tahoe Gold	C35	20/06/2018	67-85	1-1XXX	57.2	9.9	0.90	11.0	0.0	T1-5

Tahoe Gold	CC	17/05/2018	64-72	1-1XX	59.2	11.6	1.35	8.6	0.0	T5-6
Tahoe Gold	CC	20/06/2018	70-78	1X-1XXX	61.2	12.6	1.10	11.5	0.0	T1-2
Tahoe Gold	X639	17/05/2018	53-75	4-1XX	61.1	10.2	1.15	8.9	0.0	T5-6
Tahoe Gold	X639	20/06/2018	67-81	1-1XXX	57.1	11.2	0.80	14.0	0.2	T1-4
Tango	C35	13/04/2018	68-77	1X-1XX	49.2	4.7	0.68	7.0	0.0	T8
Tango	C35	08/05/2018	64-83	1-1XXX	49.1	4.5	0.75	6.0	0.0	T6
Tango	C35	17/05/2018	71-80	1X-1XXX	48.7	8.3	0.65	12.8	0.0	T5
Tango	C35	07/06/2018	73-84	1XX-1XXX	46.1	9.7	0.60	16.2	0.0	T3-4
Tango	C35	20/06/2018	68-85	1X-1XXX	49.7	9.8	0.75	13.1	0.0	T2-4
Tango	CC	13/04/2018	65-75	1-1XX	53.1	5.0	0.83	6.1	0.0	T8
Tango	CC	17/05/2018	70-80	1X-1XXX	56.6	8.7	0.65	13.4	0.0	T6
Tango	CC	07/06/2018	70-77	1X-1XX	58.4	9.4	0.55	17.1	0.0	T4-5
Tango	X639	13/04/2018	63-72	2-1XX	53.7	6.4	1.03	6.2	0.0	T8
Tango	X639	08/05/2018	67-80	1-1XXX	62.9	5.4	0.75	7.2	0.0	T6
Tango	X639	17/05/2018	69-78	1X-1XXX	51.4	9.2	0.65	14.2	0.0	T6
Tango	X639	07/06/2018	67-74	1-1XX	53.1	10.8	0.80	13.5	0.0	T5-6
Tango	X639	20/06/2018	62-75	2-1XX	56.6	12.2	0.90	13.6	0.3	T1-3
Valley Gold (B17)	Sunki 812	17/05/2018	64-70	1-1X	53.5	9.7	1.05	9.2	3.2	T5
Valley Gold (B17)	Sunki 812	07/06/2018	86-97	1XXX	51.1	9.5	0.80	11.9	1.3	T4-5
Valley Gold (B17)	Sunki 812	20/06/2018	67-78	1-1XXX	56.1	12.0	1.10	10.9	2.4	T1-3
Yosemite Gold	C35	19/07/2018	72-99	1XX-1XXX	52.0	13.1	1.50	8.7	0.2	T1-3
Yosemite Gold	CC	19/07/2018	74-90	1XX-1XXX	46.1	10.8	1.40	7.7	0.0	T1-4
Yosemite Gold	X639	20/07/2018	73-82	1XX-1XXX	50.5	12.9	1.00	12.9	0.0	T1-3

5.4.7 PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the hot dry inland areas (Tshipise and Weipe)

Project 899B by J. Joubert (CRI)

Opsomming

Die kwaliteit van die Mandaryn Hibried vrugte het aansienlik verskil tussen die verskillende produksie areas, wat 'n baie belangrike punt uitlig wanneer dit by die keuse van kultivars vir aanplantings kom, sowel as die onderstam wat gebruik word. Die resultate van die 2018 seisoen vir hierdie warm produksie areas het steeds aangedui dat Tango die vroegste ryp geword het met kleiner vruggroottes en goeie interne kwaliteit (suurvlakke daal vinnig in begin van seisoen). Daarna het Tahoe Gold gevolg, met beter eksterne vrugkleur. Tango, Tahoe Gold, Yosemite Gold, Gold Nugget en Shasta Gold was totaal saadloos gewees hierdie seisoen. Gold Nugget en Yosemite Gold was volgende om ryp te word, nader aan die einde van die Mandaryn Hibried reeks, met 'n gemiddelde tot goeie interne kwaliteit, asook goeie eksterne kleurontwikkeling. Shasta Gold was die laatste seleksie gereed vir oes, teen einde Junie tot middel Julie, met die hoogste suurvlakke vir hierdie seisoen, wat die Mandaryn Hibried seisoen afsluit vir hierdie proef.

Etna het eerste ryp geword van die nuwe addisionele seleksies, gevolg deur Sirio met lae-saad vrugte vir die seisoen. Saint André, Nova en Nova SL het gevolg met goeie kleurontwikkeling en lae tot saadlose vrugte. Volgende om ryp te word sal Samba wees, gevolg deur Furr met hoë saadtellings in die vrugte. Mor 26 volg nou, met 'n ligte oes op die bome (tussenstam opsie) en goeie interne kwaliteit. Tambor 1, 2 en Tanor Late word laaste ryp by hierdie proef perseel en eindig die mandaryn seisoen vir die warm produksie area.

Daar word aanbeveel om nie die oesperiode langer as 3 tot 4 weke te verleng nie om goeie interne kwaliteit te verseker met minimum skilprobleme.

Summary

The quality of the Mandarin Hybrid fruit between the different production areas was very different, indicating how important it is to decide what cultivar to plant where, as well as the suitable rootstock for that area. The results of the 2018 season still indicated that for the warm production areas Tango matures first with smaller fruit size and good internal quality (acid levels drop early in season). Tahoe Gold followed, with improved external colour. Tango, Tahoe Gold, Yosemite Gold, Gold Nugget and Shasta Gold were completely seedless this season. Gold Nugget and Yosemite Gold matured next towards the end of the Mandarin Hybrid range evaluated at these trial sites, with average to good internal quality, as well as good external colour development. Shasta Gold was the last selection to mature at the end of June to middle of July, with the highest acids for this season, ending off the Mandarin Hybrid season for this trial.

Etna was first to mature from the new additional selections, followed by Sirio with low-seeded fruit this season. Saint André, Nova and Nova SL follows with good external colour development and low seeded to seedless fruit. Next to mature will be Samba, followed by Furr with high seed numbers in the fruit. Mor 26 follows, cropping light yields on the trees (interstock option) and good internal quality. Tambor 1, 2 and Tanor Late matures last at this trial site, ending of the mandarin season for the hot areas.

Picking periods should not be longer than 3 - 4 weeks to maintain good internal quality and to avoid rind disorders.

Objectives

- To select Mandarin Hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new Mandarin Hybrid cultivars and to determine the climatic suitability of these cultivars in hot, dry production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Mandarin Hybrid selections from Alicedale (Tshipise) and NGB (Weipe) in the Limpopo region.

Table 5.4.7.1. List of Mandarin Hybrid selections evaluated at Alicedale (Tshipise) during the 2018 season.

Selection	Rootstock	Topworked
Edit x Nova (Dina)	X639	2015
Etna	X639	2013/2014
Furr (Clemcott)	X639	2013/2014
Gold Nugget	X639	2010
IRM 2	X639	2015
Leanri	X639	2015
Meirav 119	X639	2015
Mor 26	X639	2013/2014
Nadorcott ARCCIT 9	X639	2015
Nadorcott	X639	2015
Nova	X639	2013/2014
Nova SL	X639	2013/2014
Page	X639	2013/2014
RHM	X639	2015
Saint Andre	X639	2013/2014
Samba	X639	2013/2014
Shasta Gold	X639	2010
Sirio	X639	2013/2014
Tahoe Gold	X639	2010
Tambor 1&2	X639	2014
Tango	X639	2010
Tanor Late	X639	2013/2014
Tasty 1	X639	2013/2014
Tasty 2	X639	2013/2014
Winola	X639	2013/2014
Yosemite Gold	X639	2010

Table 5.4.7.2. List of Mandarin Hybrid selections evaluated at NGB (Weipe) during the 2018 season.

Selection	Rootstock	Topworked
Shasta Gold	X639	2011
Tahoe Gold	X639	2011
Yosemite Gold	X639	2011
Gold Nugget	X639	2011
Tango	X639	2011

Results and discussion

More information was available at Alicedale (new and existing trial site) due to better crops on the topworked mandarin trees. There was limited fruit on the Edit x Nova (Dina), IRM 2 and Orri trees this season. Tanor Late fruit was picked earlier in the season before late maturing evaluations started and there was no fruit to sample. Evaluations were completed on trees bearing fruit.

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 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 a greater instance of quality and rind issues.

Gold Nugget

Gold Nugget developed a very upright tree shape (V shape) with long aggressive growing shoots in the middle, bearing no crop at all. The crop will be on the other lower bearing branches towards the middle of the tree,

and correctional pruning on this variety is crucial. Remove the long shoots to set the crop lower on the tree with this variety, and to set more smooth textured fruit on the lower branches. Gold Nugget is known for its rough textured fruit with coarse rinds, but in the evaluations it came to light that the lower fruit was smoother compared to the fruit on the aggressive long upright branches. Fruit size at Alicedale and NGB decreased this season and peaked between medium and large (count 2-1X) and the fruit on all the trees were completely seedless. The internal quality of the fruit improved from fair (2017) to good/very good and developed juice (avg 62.8%), Brix (11.2) and acid levels below 1.0% avg and an external colour between T2 and T3. Future evaluations will determine the feasibility of Gold Nugget in the hot areas. Based on the internal quality results in Table 5.4.7.3, estimated maturity will be the middle to end of June.

Shasta Gold

Shasta Gold developed fairly round fruit (Minneola tangelo type) on the trees at the trial site. There was ribbing on most of the fruit, as well as sunburn. The tree size remained on the smaller and compact side. There were a lot of thorns on the bearing branches of the trees. Rind texture was rough (scale 4-5). The flavour was fair with high rind oil content. Shasta produced fruit with soft fibre strength that peels easily, and all the fruit evaluated was completely seedless at both locations. The fruit size peaked at very large (count 1XXX). The internal quality was good with high juice (avg 68%), Brix (12) levels and acid levels (above 1.0% final evaluation). Based on the internal quality results in Table 5.4.7.3, maturity will be middle of June to the end of July.

Tahoe Gold

Tahoe Gold produced a good crop on the trees at Alicedale and NGB. This selection developed a small tree size when compared to the other UC5 varieties (compact tree). The tree bears fruit in bundles in a similar way to grapefruit. The fruit size was smaller this season and peaked from medium to large (count 2-1X) and the fruit shape was similar to that of Minneola tangelo. There was an improvement in the external colour when the internal quality was optimal. Tahoe Gold produced fruit with soft fibre strength that peeled fairly easily, and all the fruit evaluated were completely seedless at both sites. The internal quality was fair to good this season with juice levels averaging 66%, Brix averaging 12.3 and acid levels were acceptable (0.9%). Based on the internal quality results in Table 5.4.7.3, estimated maturity was the middle of May to middle of June.

Tango

Tango remained completely seedless at NGB and Alicedale this season (low seed numbers last year at Alicedale – 0.1 seeds per fruit). The fruit shape was similar to the Nadorcott selection. Rind texture was very smooth with a natural wax shine on the fruit. The Tango trees were thorn less and an upright V-shape. The fruit was firm and the rind thin, fibre was soft and peeled very easy. Internally the fruit was high in juice content (above 52%), Brix improved for this selection (average 10.6 with final evaluation), acid levels (below 1.1) decreased early in the season (indicating a short shelf life) and deep orange coloured fibre. Fruit size increased and peaked at count 2 to 1XX (medium to large). Based on the internal quality results in Table 5.4.7.3, estimated maturity will be middle of April.

Yosemite Gold

Yosemite Gold cropped a better yield on X639 at Alicedale and NGB, additional measures will be necessary to increase the crop on the trees (Gibb sprays or girdling). Yosemite developed a very promising soft citrus fruit shape. The fruit was firm, rind texture was smooth and the fibre was soft. It peeled very easily and was completely seedless. Yosemite Gold developed the biggest tree size compared to the other TDE selections at Alicedale and NGB. This aggressive growth characteristic may be the reason for the poor crop on the trees (vegetative growth), and must be channelled into fruit set and crop (dwarfing rootstocks). Fruit size varied from large to very large (count 1X-1XXX), similar to Shasta Gold. The internal quality was good developing higher Brix (11.3), acid levels (1.0%) and juice for the season (average 55.2%). External colour developed along with the internal quality towards the end of the evaluations. Based on the internal quality results in Table 5.4.7.3, estimated maturity will be the middle to end of June.

Additional selections (third crop)

Etna bore a good crop with medium to very large fruit (count 2 to 1XX) and average/good internal quality (high juice, low Brix and average acids). External colour was delayed at peak maturity (T3 to T4) and all the fruit

evaluated was seedless. Furr (Clemcott) was included as a control for the hot production areas and to compare with Leanri. Fruit size was large to very large (count 1X-1XXX), good internal quality with acceptable acids (avg 0.9%) early in the season and very high number of seeds in the fruit (avg. 15.4 seeds). Mor 26 cropped very low numbers of fruit on the trees (count 2 to 1XX). External colour was delayed (T5) with optimum maturity and the fruit was seedless this season.

Nova was included as a control for Nova SL and Saint André in the trial, the fruit peels fairly difficult and low numbers of seed developed in the fruit. External colour was late and the fruit size varied between count 3 and count 1 (medium to large). Nova SL (ARC) produced a coarse rind texture on the fruit with medium to large fruit size (count 3 to count 1). The acid levels in the fruit was similar compared to Nova (control) and the external colour development delayed this season at peak maturity. Fruit was completely seedless this season.

Orri trees were very aggressive growing, producing a light crop on the trees this season and one evaluation was possible. Internal quality was good; high juice (61%), average Brix (10) and acceptable acids (0.85%). Page had a fair yield on the trees (60 kg/tree) with medium to large fruit size (count 2 to 1XX). Internal quality was fair to good, acid levels dropped early in the season (0.9 with first evaluation end of March) with delayed colour development (T2 to T5). The fruit was low seeded compared to last season`s seedless fruit and the rind texture was very smooth with deep orange colour development.

Saint Andre originated from a Furr orchard in the Eastern Cape. The fruit matures after Nova (2 weeks) with good internal quality juice (53.4%) and acids (average 1.1%), but fairly low Brix (above 8). Seed counts were higher (0.3 seeds per fruit) compared to Nova and Nova SL (completely seedless this season) and fruit size varied between count 2 and count 1X. Samba produced an average second crop on the large fast growing thornless trees. Colour development was early in the season (deep orange) with a smooth rind texture on the fruit. Seed counts were very low, completely seedless this season in the mixed block and the fruit size peaked from count 2 to count 1XX. Sirio produced erratic fruit sizes from medium to very large coarse fruit (count 2 to 1XXX) with very good internal quality; juice 55%, Brix 13.5 and acids above 1.0% and delayed colour development on the trees (T5).

Tambor 1 cropped fruit for the second time and Tambor 2 for the first time this season. Fruit size peaked between count 1 and 1XXX (large to very large) with 2.2 seeds per fruit. The internal quality was good; high juice of 64.4%, Brix of 11.2 and high acids early in the season of 1.6 and colour development peaked between T1 and T3. Tasty 1 and 2 bore fruit this season. Fruit size was smaller this season and varied from large to very large (count 1 to 1XX) fruit and the internal quality was fair to average (good juice, low Brix and acids) with completely seedless fruit.

Additional selections (first crop)

Nadorcott and Nadorcott ARCCIT 9 cropped the best yield on the young trees, followed by Leanri with large fruit size and good colour development. RHM and Meirav 119 cropped sufficient fruit to comply two evaluations. The acid levels from the RHM fruit was very low early in the season and Meirav 119 developed very good colour on the young trees. The fruit on Edit x Nova (Dina) and IRM 2 were very limited this season and will improve next year. The Edit x Nova trees were very upright in shape and pruning will be crucial to develop proper bearing branches.

Conclusion

The external colour delay (internal quality improved with more mature trees) in the hotter areas remained a problem; future evaluations will confirm this. Degreening may be an option for the Gold Nugget and TDEs, but ethylene reacted slowly or not at all for Tango (W. Murcott selection) and Nadorcott. Shasta Gold may be a possibility to consider for the hot areas due to higher acid levels late in the season, when external colour becomes more intense (T1-2) due to temperature drop (winter time). The appearance of Shasta Gold's fruit in the Tshipise and Weipe area (hot) may be a problem. In the hot areas it will become crucial to cover the mandarin orchards with shade net, to minimise sunburn and improve pack-out percentage (Shasta had severe sunburn at Alicedale and NGB). Gold Nugget improved considerably with good internal quality, better production and medium to large fruit size (improved colour development). Shasta Gold had the largest fruit

size, followed by Yosemite Gold and then Tango. The smallest fruit size was produced on Tahoe Gold and Gold Nugget.

This was the third evaluation of Etna, Furr (control), Mor 26, Nova (Control), Nova SL, Page, Saint André, Samba, Sirio, Tambor 1 (control) and 2, Tasty 1 and 2, information is progressing with trees maturing and better fruit quality; so future evaluations will improve recommendations on these varieties. The promising selections at this early stage was Page (good colour development and low seeded fruit), Saint André (bigger fruit size and later maturing) and Samba with good internal quality fruit, good colour development and crop on the trees. Seed numbers on these selections was very low to completely seedless in the combination trial block with cross pollinating cultivars included. Furr developed the highest seed numbers per fruit, typical characteristic of the selection with good colour development and internal quality.

This was the first evaluation (trees three years old – topworked end of 2015) of Nadorcott, Nadorcott ARCCIT 9, Edit x Nova (Dina), IRM 2, Leanri, Meirav 119 and RHM.

Table 5.4.7.3. Internal fruit quality data for Mandarin hybrid selections at Alicedale (Tshipise) and NGB (Weipe) during the 2018 season.

Cultivar	Root stock	Date harvested	Site	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg seed	Fruit external colour
ARCCIT 9	X639	27/03/2018	Alicedale	65-74	1-1XX	52.7	6.7	0.91	7.4	0.0	T8
ARCCIT 9	X639	3/5/2018	Alicedale	64-75	1-1XX	50.6	5.4	0.50	10.8	0.0	T6
ARCCIT 9	X639	30/05/2018	Alicedale	68-78	1X-1XX X	54.6	9.3	0.60	15.5	0.0	T3-4
Edit x Nova	X639	27/03/2018	Alicedale	61-63	2-2	69.7	8.2	1.03	8.0	0.0	T8
Etna	X639	27/03/2018	Alicedale	60-67	2-1	56.7	8.1	0.85	9.5	0.0	T8
Etna	X639	03/05/2018	Alicedale	64-75	1-1XX	58.6	6.9	0.70	9.9	0.0	T4
Furr	X639	03/05/2018	Alicedale	71-77	1X-1XX	54.5	14.3	0.85	16.8	7.3	T5
Furr	X639	30/05/2018	Alicedale	69-80	1X-1XX X	58.1	12.5	0.90	13.9	5.3	T2-3
Gold Nugget	X639	30/05/2018	Alicedale	63-69	2-1X	57.3	10.8	0.95	11.4	0.0	T2-3
Gold Nugget	X639	30/05/2018	NGB	62-70	2-1X	68.4	11.5	0.80	14.4	0.0	T2-3
IRM 2	X639	03/05/2018	Alicedale	67-75	1-1XX	55.3	8.1	0.55	14.7	0.0	T5
Leanri	X639	27/03/2018	Alicedale	67-71	1-1X	57.6	8.6	1.13	7.6	0.0	T5
Leanri	X639	03/05/2018	Alicedale	70-82	1X-1XX X	56.2	11.1	0.70	15.9	0.3	T4
Leanri	X639	30/05/2018	Alicedale	70-82	1X-1XX X	46.2	10.8	0.65	16.6	0.0	T1-3

Meirav 119	X639	03/05/2018	Alicedale	56-70	3-1X	58.3	13.5	0.75	18.0	0.8	T3-4
Meirav 119	X639	30/05/2018	Alicedale	56-70	3-1X	56.6	12.7	0.75	16.9	0.3	T1-2
Mor 26	X639	27/03/2018	Alicedale	62-70	2-1X	45.7	8.4	1.25	6.7	0.0	T5
Mor 26	X639	03/05/2018	Alicedale	63-72	2-1XX	56.5	12.8	0.70	18.3	0.0	T5
Mor 26	X639	30/05/2018	Alicedale	65-74	1-1XX	63.1	12.0	0.70	17.1	0.0	T3-4
Nadorco tt	X639	27/03/2018	Alicedale	63-74	2-1XX	55.2	7.3	0.88	8.3	0.1	T8
Nadorco tt	X639	03/05/2018	Alicedale	64-71	1-1X	52.3	6.7	0.45	14.9	0.0	T5
Nadorco tt	X639	30/05/2018	Alicedale	64-70	1-1X	54.0	10.0	0.55	18.2	0.1	T3-4
Nova	X639	27/03/2018	Alicedale	56-65	3-1	54.6	8.3	1.11	7.5	0.0	T8
Nova ARC	X639	27/03/2018	Alicedale	58-65	3-1	57.5	8.2	1.07	7.7	0.0	T8
Orri	X639	30/05/2018	Alicedale	64-71	1-1X	61.1	9.9	0.85	11.6	0.3	T2-3
Page	X639	27/03/2018	Alicedale	65-69	1-1X	54.4	8.7	0.90	9.7	0.3	T5
Page	X639	03/05/2018	Alicedale	62-73	2-1XX	53.2	13.7	0.70	19.6	0.0	T2
RHM	X639	27/03/2018	Alicedale	60-63	2-1	53.8	8.2	0.69	12.0	0.0	T8
RHM	X639	03/05/2018	Alicedale	63-76	2-1XX	56.0	7.3	0.40	18.3	0.0	T7
Saint Andre	X639	27/03/2018	Alicedale	60-68	2-1X	53.4	8.1	1.13	7.2	0.3	T5
Samba	X639	27/03/2018	Alicedale	60-67	2-1	55.8	10.4	0.92	11.4	0.0	T3
Samba	X639	03/05/2018	Alicedale	62-74	2-1XX	59.1	9.0	0.70	12.9	0.0	T1
Shasta Gold	X639	11/07/2018	NGB	79-87	1XX X	68.4	11.9	1.00	11.9	0.0	T1-3
Sirio	X639	27/03/2018	Alicedale	63-72	2-1XX	55.4	13.2	1.15	11.5	0.0	T5
Sirio	X639	03/05/2018	Alicedale	72-77	1XX-1XX	54.0	14.2	1.00	14.2	0.0	T3-4
Tahoe Gold	X639	30/05/2018	NGB	61-69	2-1X	66.0	12.3	0.90	13.7	0.2	T2-4
Tambor 1	X639	12/07/2018	Alicedale	70-78	1X-1XX X	63.4	11.3	1.40	8.1	2.8	T1-3
Tambor 2	X639	30/05/2018	Alicedale	67-74	1-1XX	65.9	10.6	1.55	6.8	0.7	T3-4
Tambor 2	X639	12/07/2018	Alicedale	69-78	1X-1XX X	63.8	11.7	1.20	9.8	3.2	T1-3
Tango	X639	27/03/2018	Alicedale	62-67	2-1	65.4	8.2	1.03	8.0	0.1	T8

Tango	X639	27/03/2018	NGB	63-67	2-1X	51.5	8.9	1.46	6.1	0.0	T3
Tango	X639	02/05/2018	NGB	62-70	2-1X	55.3	13.1	0.85	15.4	0.0	T4
Tango	X639	30/05/2018	NGB	62-75	2-1XX	60.3	12.3	1.00	12.3	0.0	T1
Tasty 1	X639	03/05/2018	Alicedale	65-72	1-1XX	57.5	7.7	0.58	13.4	0.0	T4
Tasty 2	X639	03/05/2018	Alicedale	64-74	1-1XX	57.0	12.1	0.75	16.1	0.0	T4
Winola	X639	03/05/2018	Alicedale	65-78	1-1XX X	56.6	13.6	1.75	7.8	0.0	T4-5
Winola	X639	30/05/2018	Alicedale	60-70	2-1X	60.1	13.0	1.80	7.2	0.1	T1
Yosemite Gold	X639	30/05/2018	Alicedale	69-77	1X-1XX	57.3	10.8	1.10	9.8	0.2	T1
Yosemite Gold	X639	11/07/2018	NGB	77-88	1XX-1XX X	53.1	11.8	0.80	14.8	0.3	T1-3

5.4.8 PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the intermediate production areas (Karino)

Project 963C by J. Joubert (CRI)

Summary

The quality of the Mandarin Hybrid fruit was similar in the two different production areas (Nelspruit and Marble Hall), due to the similar climatic region (intermediate areas) and tree age (2011). The results indicated that in the Nelspruit production area, Nadorcott ARCCIT9 LS matures first (two weeks before ARC selection), followed by Nadorcott ARC with medium to large fruit size for this season and excellent colour development. Edit x Nova and Meirav 63 also indicated to be fairly early maturing selections. IRM 2 and Shani SL matures next towards the middle of the mandarin season with less ribbing on the fruit compared to IRM 1 and Phoenix at the other trial sites. All the fruit evaluated this season had very low seed numbers due to cross pollination impact from the seeded varieties close by, except for IRM 2.

Opsomming

Die kwaliteit van die Mandaryn Hibried vrugte het ooreengestem tussen die twee produksie areas (Nelspruit and Marble Hall), a.g.v. die klimaatsone (intermediêre areas) en boom ouderdom (2011). Die resultate vir die Nelspruit produksie area het aangedui dat Nadorcott ARCCIT9 LS eerste gereed was vir die oesproses (twee weke voor die ARC seleksie), gevolg deur Nadorcott ARC met medium tot groot vrugte vir hierdie seisoen en baie goeie kleur ontwikkeling. Edit x Nova en Meirav 63 het ook onder die vroeë seleksies ingepas. IRM 2 en Shani SL was volgende gereed vir oes gewees meer na die middel van die mandaryn seisoen; met minder ribbing op die vrugte in vergelyking met IRM 1 by die ander proef persele. Al die vrugte geëvalueer het baie lae saadinhoud aangedui a.g.v. die kruisbestuivings impak van aangrensende varieteite met saad, behalwe vir IRM 2.

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 intermediate, inland production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Mandarin Hybrid selections from Karino-koöp (Nelspruit) in the Mpumalanga region and Ngonini Estate (Jeppes Reef) in Swaziland.

Table 5.4.8.1. List of Mandarin Hybrid selections evaluated at Karino-koöp (Nelspruit) during the 2018 season.

Selection	Rootstock	Planted
Edit x Nova	CC	2011
Irradiated I22	CC	2011
IRM 2	CC	2011
Meirav 63	CC	2011
Meirav 119	CC	2014
Michal 6/47	CC	2014
Michal 89/64	CC	2014
Nadorcott ARC	CC	2011
Nadorcott ARCCIT9 LS	CC	2011
Shani SL	CC	2011
Tango	CC	2014
Tahoe	CC	2014
Vallei Gold	CC	2011
Winola	CC	2014

Table 5.4.8.2. List of Mandarin Hybrid selections evaluated at Ngonini Estate (Swaziland) during the 2018 season.

Selection	Rootstock	Topworked
Etna	C35	2015
Nova	C35	2015
Orri	C35	2015
Page	C35	2015
Saint André	C35	2015
Samba	C35	2015
Sugar Belle	C35	2015

Results and discussion

The trees at Karino-koöp were evaluated for the fourth time this season; this having an impact on the quality and quantity of the fruit. More information was available and future evaluations will be conducted. There was a new trial site planted on the Karino premises and topworked at Ngonini Estate to expand the evaluation opportunities and the trees will bear fruit for the first time in 2017.

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 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 a greater instance of quality and rind issues.

IRM 2

The tree shape of the IRM 2 was very upright (V-shaped) with no thorns and aggressive growing. IRM 2 produced an alternative crop on the trees and fruit size peaked from medium to large/extra-large (count 2 to

1XXX). The seed numbers decreased slightly this season from 3.1 seeds to 2.7 seeds per fruit, peels easily with some ribbing on the fruit (typical Murcott characteristic). Juice levels increased compared to 2017 and averaged above 62%, Brix was very good (up to 14.8) and acids were above 1.4%. The external colour was deep orange and peaked between T1 and 3. Based on the internal quality results in Table 5.4.8.3, estimated maturity will be the middle to end of June

Nadorcott ARC & ARCCIT9 LS

The fruit shape was similar to the Nadorcott selection. Rind texture was very smooth with a natural shine (similar to packhouse waxing). Both selections developed good internal quality with high juice levels (from 59 to 64%), Brix averaging 11 and acceptable acids (avg. 0.8%). Nadorcott ARCCIT9 LS produced a better crop on the trees compared to the ARC selection and the fruit size was bigger this season apart from a good crop load; varied from count 2-1XXX. Both selections evaluated were completely seedless at the Karino trial site. Maturity seems to be two weeks earlier on the ARCCIT9 LS selection, but information was limited due to the fourth year of evaluation (end of April to the end of May), according to Table 5.4.8.3.

Additional selection (Karino)

The internal quality of Edit x Nova was good with high juice levels above 56%, no granulation problems in the fruit compared to Nova. Brix (above 13 – second and third evaluation) and lower acids (0.8%), indicating the early to mid-maturing characteristics of the selection in the intermediate production areas, with completely seedless fruit. The fruit size peaked from count 2 to count 1XX.

Irradiated I22 bore a light crop on the tree this season and two evaluations were possible. The fruit size was between count 1 and count 1XXX (large fruit due to light crop); high juice content and completely seedless fruit.

Meirav 63 developed a deep orange rind colour (T1 with peak maturity). Internal quality was good with high juice content (above 57%), Brix of 11.6 and good acids (average 1.0%). The seed content increased back from 0.3 to 1.3 seeds per fruit evaluated at the Karino trial site (cross pollination).

Shani SL bore a fair crop on the trees with small/medium to large fruit size (count 3 to count 1) as well as deep orange rind colour (T2-T3). Internal quality was good early in the season with good juice (67%), Brix (14) and acids (average 1.2%) levels. The fruit was completely seedless (0.1 seeds).

Meirav 119, Michal 6/47 & 89/64, Tango, Tahoe and Winola was included in the Karino trial site in 2014 and bore their first limited fruit numbers on the trees this season. Fruit size peaked from large to extra-large on Meirav 119 and Michal 89/64 (count 1X to 1XXX), but smaller fruit was cropped on Michal 6/47 (between count 4 and 1X) with low-seeded fruit on all three selections. Tango cropped completely seedless fruit and Tahoe as well as Winola developed juice levels above 60%.

Additional selection (Ngonini)

Etna cropped very limited fruit numbers (first crop) this year with average internal quality and delayed colour development (T5). Nova was included as control variety for the Nona SL (ARC) and Saint André selection. Saint André produced a better crop on the trees with delayed colour development (2 weeks later).

Page, Samba and Orri was included this season at the trial site topworked in 2015 on C35 rootstock. Page matured before Samba with Orri being the later selection of the three and seed numbers were low (between 0.3 and 2.3 seeds per fruit). Sugar Belle bore a fairly light first crop on the trees with late colour development and high acid levels; high juice and Brix (62% and 14).

Conclusion

This was the third evaluation of Edit x Nova, Irradiated I22, Meirav 63 and Shani SL; and the fourth evaluation of IRM 2, Nadorcott ARC and Nadorcott ARCCIT9 LS; and first evaluation of Meirav 119, Michal 6/47 & 89/64, Tango, Tahoe and Winola at the Karino site, so information is limited and future evaluations will improve recommendations on these varieties. All the selections developed very low seed numbers in the fruit compared

to the previous season, where they were completely seedless for this trial, except for IRM2 with average 2.7 seeds per fruit. There was a good external colour development on the Nadorcott, Meirav 63&119, Michal 6/47 & 89/64 and Shani SL selections (deep orange).

The Ngonini site in Swaziland cropped fruit for the first time and more information will become available with trees becoming more mature in future. Saint André seems to be an improved Nova selection that will mature 2 weeks later with bigger fruit size. Samba performed well and will contribute to the list of available early options for new plantings.

Table 5.4.8.3. Internal fruit quality data for Mandarin hybrid selections at Karino- koöp (Nelspruit) and Ngonini (Swaziland) during the 2018 season.

Cultivar	Root-stock	Date harvested	Site	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Avg seed	Fruit external colour
Edit x Nova	CC	06/04/2018	Karino	62-69	2-1X	56.0	9.0	0.75	12.0	0.0	T8
Edit x Nova	CC	30/04/2018	Karino	69-73	1X-1XX	61.5	13.8	0.75	18.4	0.0	T5
Edit x Nova	CC	15/06/2018	Karino	66-71	1-1X	61.6	13.6	0.75	18.1	0.0	T1
Etna	C35	05/04/2018	Ngonini	62-76	2-1XX	57.0	13.0	0.63	20.8	0.0	T5
I22	CC	28/05/2018	Karino	65-75	1-1XX	56.2	8.5	0.65	13.1	0.0	T3-4
I22	CC	15/06/2018	Karino	66-79	1-1XXX	59.7	9.8	0.60	16.3	0.0	T1-2
IRM2	CC	28/05/2018	Karino	64-67	1-1	52.2	11.4	1.65	6.9	2.3	T2
IRM2	CC	15/06/2018	Karino	60-72	2-1XX	72.1	13.6	1.40	9.7	2.5	T1
IRM2	CC	25/07/2018	Karino	61-80	2-1XXX	64.2	14.8	1.70	8.7	3.3	T1-3
Meirav 63	CC	30/04/2018	Karino	53-66	4-1	57.0	10.7	1.15	9.3	0.7	T6
Meirav 63	CC	28/05/2018	Karino	58-67	3-1	59.7	11.9	0.90	13.2	1.6	T3-4
Meirav 63	CC	15/06/2018	Karino	59-62	2-2	66.7	12.3	0.95	12.9	1.5	T1
Meirav 119	CC	28/05/2018	Karino	71-80	1X-1XXX	56.1	10.4	0.55	18.9	0.7	T1-2
Meirav 119	CC	15/06/2018	Karino	72-82	1XX-1XXX	52.0	12.2	0.75	16.3	0.3	T1-2
Michal 6/47	CC	06/04/2018	Karino	53-58	4-3	56.8	9.0	0.55	16.4	0.0	T4
Michal 6/47	CC	30/04/2018	Karino	56-59	3-2	58.2	13.9	0.55	25.3	0.2	T1
Michal 6/47	CC	28/05/2018	Karino	54-65	4-1	61.1	13.0	0.60	21.7	0.0	T1
Michal 6/47	CC	15/06/2018	Karino	65-68	1-1X	59.8	13.5	0.60	22.5	0.3	T1
Michal 89/64	CC	30/04/2018	Karino	65-72	1-1XX	53.1	8.7	0.50	17.4	0.5	T4

Michal 89/64	CC	28/05/2018	Karino	68-76	1X-1XX	55.9	10.1	0.50	20.2	0.5	T1
Michal 89/64	CC	15/06/2018	Karino	72-89	1XX-1XXX	46.8	13.4	0.50	26.8	0.0	T1
Nadorcott ARC	CC	30/04/2018	Karino	63-77	2-1XX	64.6	8.4	0.50	16.8	0.0	T5
Nadorcott ARC	CC	28/05/2018	Karino	60-65	2-1	60.2	11.7	0.85	13.8	0.0	T2-3
Nadorcott ARC	CC	15/06/2018	Karino	65-80	1-1XXX	63.0	12.1	0.80	15.1	0.0	T1
ARCCIT 9 LS	CC	30/04/2018	Karino	63-70	2-1X	59.4	8.8	0.95	9.3	0.0	T5
ARCCIT 9 LS	CC	28/05/2018	Karino	59-86	2-1XXX	62.2	11.9	0.80	14.9	0.0	T2-3
ARCCIT 9 LS	CC	16/06/2018	Karino	76-80	1XX-1XXX	63.1	12.2	0.75	16.3	0.0	T1
Nova	C35	05/04/2018	Ngonini	68-77	1X-1XX	59.3	13.8	0.61	22.8	4.0	T6
Nova	C35	30/04/2018	Ngonini	67-76	1-1XX	60.1	8.8	0.60	14.7	1.7	T6
Orri	C35	30/04/2018	Ngonini	63-72	2-1XX	54.4	11.3	0.75	15.1	1.5	T3
Page	C35	05/04/2018	Ngonini	60-67	2-1	60.5	10.8	0.50	21.6	1.0	T3
Page	C35	30/04/2018	Ngonini	59-68	2-1X	56.1	13.3	0.60	22.2	0.3	T1
Saint Andre	C35	05/04/2018	Ngonini	65-80	1-1XXX	56.5	10.9	0.63	17.4	4.3	T8
Saint Andre	C35	30/04/2018	Ngonini	69-80	1X-1XXX	58.2	13.5	0.55	24.5	3.0	T5
Samba	C35	05/04/2018	Ngonini	62-71	2-1X	58.3	13.3	0.55	24.2	2.3	T3
Samba	C35	30/04/2018	Ngonini	61-72	2-1XX	58.0	11.3	0.60	18.8	1.5	T1
Shani SL	CC	28/05/2018	Karino	57-65	3-1	61.4	13.6	1.15	11.8	0.0	T3
Shani SL	CC	15/06/2018	Karino	55-65	3-1	73.8	14.2	1.20	11.8	0.1	T2-3
Sugar Belle	C35	30/04/2018	Ngonini	60-75	2-1XX	62.4	13.6	1.90	7.2	7.0	T6
Tango	CC	28/05/2018	Karino	69-76	1X-1XX	53.7	10.1	0.70	14.4	0.0	T3
Tango	CC	15/06/2018	Karino	74-82	1XX-1XXX	54.0	10.6	0.65	16.3	0.0	T1
Tahoe	CC	28/05/2018	Karino	66-80	1-1XXX	60.8	11	1.15	9.6	0.3	T3-4
Tahoe	CC	15/06/2018	Karino	79-86	1XXX	59.5	11.4	1.02	11.2	0.0	T1
Tasty 1	C35	30/04/2018	Ngonini	70-78	1X-1XXX	37.3	8.5	0.50	17.0	2.7	T5
Valley Gold	CC	28/05/2018	Karino	65-70	1-1X	48.7	12.5	1.15	10.9	0.7	T3-4
Valley Gold	CC	15/06/2018	Karino	61-74	2-1XX	65.8	13.9	1.20	11.6	1.7	T1-2

Winola	CC	28/05/20 18	Karino	72-80	1XX- 1XXX	62.4	10.6	1.25	8.5	0.1	T1-2
Winola	CC	15/06/20 18	Karino	75-84	1XX- 1XXX	63.5	10.9	0.95	11.5	0.0	T1

5.4.9 PROGRESS REPORT: Evaluation of Navel selections in the intermediate production areas (Karino)

Project 963B by J. Joubert (CRI)

Summary

In the Nelspruit production area, M7 matures first with the best internal quality (lower acids) for the trial, followed by Fukumoto 2 with medium to large fruit size for this season and acceptable colour development. Newhall matures next with good juice levels and delayed external colour on the fruit. Clark, Fischer and Dream matured next, towards the middle of the navel orange range, with medium to large fruit size and high Brix levels (average 10). Due to limited evaluation data and poor internal quality (low juice and acids) this season, maturity estimates were not possible for Hutton.

Opsomming

In die Nelspruit produksie area word M7 eerste ryp met die beste interne kwaliteit (laer sure) vir hierdie proef, gevolg deur Fukumoto 2 met medium tot groot vruggrootte vir hierdie seisoen en aanvaarbare kleur ontwikkeling. Newhall word volgende ryp met goeie sap vlakke en vertraagde eksterne kleur op die vrugte. Clark, Fischer en Dream volg, om die middel van die nawel soetlemon reeks te vul, met medium tot groot vruggrootte en hoë Brix vlakke (gemiddeld 10). Hutton se rypwordings inligting was beperk a.g.v. die evaluasie data beskikbaar en swak interne kwaliteit van die vrugte (lae sap en suur).

Objectives

- To select navel cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (juice, 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 intermediate production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on navel selections from Karino-koöp (Nelspruit) in the Mpumalanga region. The following early to mid-maturing selections were evaluated: Clarke, Dream, Fischer, Fukumoto 2, Hutton, M7 and Newhall.

When the ratio between sugar and acid is 10:1, the navel fruit are considered to be at peak maturity. A ratio of 9:1 is considered to be the build-up towards peak maturity of 10:1. After reaching the peak, the ratio increases to 11:1, after which 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.

Table 5.4.9.1. List of navel selections evaluated at Karino-koöp (Nelspruit) during the 2018 season.

Selection	Rootstock	Planted
Clarke	CC/SC	2011
Dream	SC	2011
Fischer	SC	2011
Fukumoto 2	SC	2011
Hutton	CC	2011

M7	CC/SC	2011
Newhall	SC	2011

Results and discussion

The trees at Karino-koöp were evaluated for the fourth time this season; this having an impact on the quality and quantity of the fruit. More information was available and future evaluations will be conducted.

Clarke

Clarke performed better this season and developed medium to large/very large (count 72 to count 48/40) fruit size on the trees at Karino and the external colour peaked at T2-3. The navel-end was fairly open and the fruit shape oblong; rind texture was fairly coarse. Clarke improved on the juice levels (up to 58%), better Brix 10 and acids 0.8% from the second evaluation. Based on the internal quality results in Table 5.4.9.2, estimated maturity will be the end of May to the middle of June.

Dream

The juice levels of the Dream fruit were similar this season compared to Clarke, averaging 54%, good Brix of up to 10 and similar acids (0.8%). Dream fruit developed a fairly smooth rind, small navel-end and fairly round fruit shape. The external colour development improved (older trees) and peaking between T1 and 3 with the last evaluation. Maturity seems to be end of May to middle June according to Table 5.4.9.2.

Fischer (control)

The acid levels on Fischer navel decreased, averaging 0.8% for the season. Juice and Brix improved up to 58% and 9 (lower) respectively. Externally the fruit colour development peaked from T1 to T4. Fruit size was medium to large for navel production, medium fruit size (count 72 to 56). To estimate maturity was difficult due to the low acid levels early in the season, based on the internal quality results in Table 5.4.9.2, estimated maturity will be the end of April to the middle of May.

Fukumoto 2

Fukumoto 2 was selected from Spain for compatibility to citrange and citrumelo rootstock in comparison to the incompatibility problems of the normal Fukumoto selection. All the fruit characteristics remain similar between the two Fukumoto selections. Fukumoto 2 was planted on Swingle citrumelo to test the scenario. The fruit was fairly round with a flat fruit-end and open navel-end, similar to the normal selection. Fruit size was similar this season and varied from medium to large (count 88 to count 64). Juice levels were lower (48%), lower Brix of 9.2 and still very low acids (0.5%) with a delayed external colour of T5. To estimate maturity was difficult due to the low acid levels early in the season, future evaluations will provide more information.

Hutton

The size range on the Hutton trees went to erratic again for the season and peaked from medium to large/extra-large fruit (count 72 to count 40). Juice levels increased compared to the 2017 season and averaged at 52%, better for good quality navel production. Rind texture remained fairly coarse due to the young tree age and navel-ends were open on 60% of the fruit. Colour developed between T2 and T5 with the final evaluation. To estimate maturity was difficult due to the low acid levels early in the season, future evaluations will provide more information.

M7

M7 produced a good (CC) to very good (SC) yield on the trees for the season, as well as early external colour development (Carrizo T3, Swingle T3). There were chimeras and mutations on a number of the fruit in the trial block. The juice levels (up to 52%) was good for the season compared to the other navel selections at the trial site. Fruit size on CC was bigger (count 88 to count 56) compared to SC (count 105 to count 64) due to the crop load on the trees. Brix and acid content on both rootstocks was good to very good, up to 10.5 and 0.9% respectively. Based on the internal quality results in Table 5.4.9.2, estimated maturity will be the end of March to the middle of April.

Newhall

Newhall produced medium fruit (count 88) on the trees with low acids (0.6%) taking into consideration the Swingle rootstock combination. In general, Swingle increases acid levels in the fruit, as well as delaying external colour development. Brix average decreased to 9.0 and juice levels peaked at a very low 42%. The Newhall fruit remained fairly green (T6) when the final evaluation was completed. Maturity seems to be middle of April to end of April.

Conclusion

This was the fourth evaluation of all the navel selections at this new trial site in Karino, so information is limited and future evaluations will improve recommendations on these varieties. The juice levels on most of the combinations improved from low to average/good; above the minimum export requirement of 48% with the exception of Fukumoto and Newhall. Acids remained low from the beginning of the season up to peak maturity with the exception of M7. The external colour development on all the selections was delayed for the season, not the ideal situation with the low internal quality and more specifically the low acid levels.

Future evaluations will be crucial to determine the performance of these early to mid-navel selections for the Karino area.

Table 5.4.9.2. Internal fruit quality data for Navel selections at Karino- koöp (Nelspruit) during the 2018 season.

Cultivar	Root stock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix °	Acid (%)	Ratio	Fruit external colour
Clarke	CC	28/05/2018	76-85	72-56	58.2	10.9	0.80	13.6	T2-3
Clarke	CC	15/06/2018	82-91	56-40	50.1	8.9	0.78	11.4	T2-4
Dream	SC	28/05/2018	71-83	88-56	56.6	11.0	0.70	15.7	T2-3
Dream	SC	15/06/2018	81-95	64-40	51.5	7.0	0.50	14.0	T1-3
Fisher	SC	28/05/2018	74-83	72-56	56.3	10.8	0.50	21.6	T2-3
Fisher	SC	15/06/2018	83-97	56	59.8	10.1	0.50	20.2	T1-4
Fukumoto 2	SC	6/4/2018	71-85	88-56	48.7	8.1	0.50	16.2	T7
Fukumoto 2	SC	30/04/2018	74-78	72-64	46.3	10.3	0.50	20.6	T5
Hutton	CC	28/05/2018	74-86	72-48	56.0	10.0	0.70	14.3	T3-4
Hutton	CC	15/06/2018	81-93	56-40	48.9	10.0	0.50	20.0	T2-5
M7	CC	8/3/2018	69-76	88-72	54.2	8.2	1.15	7.1	T8
M7	CC	6/4/2018	79-90	64-40	52.3	13.5	0.65	20.8	T3
M7	CC	30/04/2018	70-82	88-56	54.6	14.1	0.65	21.7	T3
M7	SC	8/3/2018	66-72	105-88	47.1	6.9	1.00	6.9	T8
M7	SC	6/4/2018	65-77	105-72	46.1	14.1	0.60	23.5	T3
M7	SC	30/04/2018	67-78	105-64	69.9	14.2	0.55	25.8	T3
Newhall	SC	30/04/2018	69-70	88	41.6	8.9	0.55	16.2	T6

5.4.10 PROGRESS REPORT: Evaluation of Lemon selections in the intermediate production areas (Marble Hall)

Project 941B by J. Joubert (CRI)

Summary

This was the second year to evaluate the lemon cultivars at the Moosrivier trial site in Marble Hall. Plaat B bore their second fruit at this trial site for the northern areas due to high temperatures and the typical lemon growth rate (aggressive). Eureka, Lisbon en Genoa had a better fruit set compared to the rest of the lemon selections at the trial site; Genoa setting one main crop on the trees per season. Lisbon Yen Ben developed better fruit on the trees (count 113-110) compared to the other selections and was more tolerant to cold conditions. All the selections were planted at the end of the 2013 season on X639 rootstock, due to the higher pH soils in the production area as well as compatibility with the Eureka-type lemons.

Opsomming

Hierdie was die derde jaar wat die suurlemoen kultivars by Moorsivier in Marble Hall geëvalueer word. Dit was Plaat B se tweede oes by hierdie proef blok vir die noordelike areas as gevolg van die hoë temperature en die tipiese suurlemoen groeitempo (groeikragtig). Eureka, Lisbon en Genoa het 'n beter vrugset gehad as die res van die suurlemoen seleksies op hierdie proef perseel; Genoa set slegs een hoofset per seisoen. Lisbon Yen Ben ontwikkel beter vruggrootte op die bome (telling 113-110) in vergelyking met die ander seleksies en was meer bestand teen koue toestande. Al die seleksies was aangeplant aan die einde van die 2013 seisoen op X639, vir die aanpasbaarheid by die hoër pH gronde en verenigbaarheid met Eureka-tipe suurlemoene.

Objectives

- To find Lemon selections suitable for the intermediate production area.
- To produce lemon selections with Eureka like fruit shape (elongated), high juice content, everbearing characteristics, low seed content and high rind oil for processing purposes.

Materials and methods

Field evaluations were conducted at Moorsivier (Marble Hall) on Eureka, Feminello, Genoa, Limoneira, Lisbon, Lisbon Yen Ben, Plaat B and Willowtree Long.

Table 5.4.10.1. List of lemon selections evaluated at Moorsivier (Marble Hall) during the 2018 season.

Selection	Rootstock	Topworked
Eureka	X639	2013
Feminello	X639	2013
Genoa	X639	2013
Limoneira	X639	2013
Lisbon	X639	2013
Lisbon Yen Ben	X639	2013
Plaat B	X639	2013
Willowtree Long	X639	2013

Results and discussion

Limoneira (32.3%), Plaat B (38.5%) and Lisbon (39.7%) developed the lowest juice percentages for the season, but Eureka had the highest juice percentage of 55.9% and Limoneira produced the biggest fruit size and still peaked from count 100 to count 64 through the season. The highest seed content per fruit was on Willowtree Long (13.3 seeds per fruit), followed by Genoa (12.4 seeds per fruit), Eureka (11.8 seeds per fruit) and Lisbon (10.8 seeds per fruit). The external colour was delayed and ranged from T4 to T6 at the trial site for all the selections.

Conclusion

Eureka produced elongated fruit; Willowtree Long and Plaat B was the only other selection with a more elongated type fruit on the trees, the rest were fairly round.

For the third season an average to good crop was produced on the trees. The lemon selections were not that vigorous and tree canopy was less dense on the X639 rootstocks. High temperatures can affect the fruit set and as well as the juice percentages. The four commercial Lemon selections; Eureka, Lisbon, Limoneira and Genoa performed well and were more suitable for the intermediate production areas compared to the experimental selections, but Feminello developed the second highest juice of 53.3%. Lisbon Yen Ben

producing a bigger fruit size (count 113-100), although the cultivar remains more tolerant to low temperatures.

Table 5.4.10.2. Internal fruit quality data for Lemon selections at Moosrivier (Marble Hall) during the 2018 season.

Cultivar	Rootstock	Date harvested	Fruit size (mm)	Count	Juice (%)	Avg seed	Fruit external colour
Eureka	X639	8/5/2018	54-65	162-100	55.9	11.8	T4
Femminello	X639	8/5/2018	58-67	138-88	53.3	2.6	T4-5
Genoa	X639	8/5/2018	63-72	100-64	49.8	12.4	T5
Lim 8A	X639	8/5/2018	65-73	100-64	32.3	8.3	T5-6
Lisbon	X639	8/5/2018	61-75	113-64	39.7	10.8	T5-6
Lisbon Yen Ben	X639	8/5/2018	59-63	113-100	50.7	0.7	T6
Plaat B	X639	8/5/2018	65-72	100-64	38.8	8.1	T5
Willowtree Long	X639	8/5/2018	65-70	100-75	45.5	13.3	T4

5.4.11 PROGRESS REPORT: Evaluation of Lemon selections in the intermediate production areas (Letsitele)

Project 75D by J. Joubert (CRI)

Summary

The 2018 season produced the first crop on the trees for the Lemon trial at Letsitele. Willowtree Long bore their second fruit at this trial site for the northern areas due to high temperatures and the typical lemon growth rate (aggressive). Lisbon and Eureka had a better fruit set compared to the rest of the lemon selections at the trial site. Lisbon Yen Ben developed the smallest fruit on the trees (count < 216) compared to the other selections. High temperatures during the flowering periods induced poor fruit set on some of the selections. All the selections were planted in November 2013 on X639 rootstock, due to the compatibility with the Eureka-type lemons.

Opsomming

Die 2018 seisoen was die tweede drag op die bome gewees vir die suurlemoen proef by Letsitele. Dit was Willowtree Long se tweede vrugset by hierdie proef blok vir die noordelike areas as gevolg van die hoë temperature en die tipiese suurlemoen groeitempo (groeikragtig). Lisbon en Eureka het 'n beter vrugset gehad as die res van die suurlemoen seleksies op hierdie proef perseel. Lisbon Yen Ben ontwikkel die kleinste vruggrootte op die bome (telling < 216) in vergelyking met die ander seleksies. Hoë temperature gedurende blom periodes het swak vrugset tot gevolg gehad vir sekere van die seleksies. Al die seleksies was geplant aan die einde van November 2012 op X639, vir die verenigbaarheid met Eureka-tipe suurlemoene.

Objectives

- To find Lemon selections suitable for the intermediate production area.
- To produce lemon selections with Eureka like fruit shape (elongated), high juice content, everbearing characteristics, low seed content and high rind oil for processing purposes.

Materials and methods

Field evaluations were conducted at Bosveld Citrus (Letsitele) on Eureka, Femminello, Genoa, Limoneira, Lisbon and Willowtree Long.

Table 5.4.11.1. List of lemon selections evaluated at Bosveld Citrus (Letsitele) during the 2018 season.

Selection	Rootstock	Planted
Eureka	X639	2013
Feminello	X639	2013
Genoa	X639	2013
Limoneira	X639	2013
Lisbon	X639	2013
Lisbon Yen Ben	X639	2013
Plaat B	X639	2013
Willowtree Long	X639	2013

Results and discussion

Plaat B (36.6%), Lisbon (38.9%) and Willowtree Long (39.3%) developed the lowest juice percentages for the season, but Femminello had the highest juice percentage of 55.7%. Lisbon produced the biggest fruit size and peaked from count 113 to count 75 through the season. The highest seed content per fruit was also on Lisbon (17.1 seeds per fruit), followed by Willowtree Long (14.0 seeds per fruit). The external colour ranged from T2 to T4 at the trial site.

Conclusion

Eureka produced elongated fruit; Willow Tree Long and Plaat B was the only other selections with a more elongated type fruit on the trees, the rest were fairly round.

For the second season a good to very good crop was produced on the trees. The lemon selections were not that vigorous and tree canopy was less dense. High temperatures can affect the fruit set and as well as the juice percentages. The four commercial Lemon selections; Eureka, Lisbon, Limoneira and Genoa performed well and seems to be more suitable for the hot production areas compared to the other experimental selections.

Table 5.4.11.2. Internal fruit quality data for Lemon selections at Bosveld Citrus (Letsitele) during the 2018 season.

Cultivar	Rootstock	Date harvested	Fruit size (mm)	Count	Juice (%)	Avg seed	Fruit external colour
Eureka	X639	28/03/2018	58-65	138-100	47.5	9.8	T2-4
Femminello	X639	28/03/2018	49-60	216-113	55.7	2.5	T3-4
Genoa	X639	28/03/2018	55-65	162-100	45.3	13.3	T2-4
Lim 8A	X639	28/03/2018	57-65	138-100	41.0	9.9	T2-4
Lisbon	X639	28/03/2018	59-69	113-75	38.9	17.1	T2-4
Lisbon Yen Ben	X639	28/03/2018	30-55	<216	43.6	2.3	T2-4
Plaat B	X639	28/03/2018	57-62	138-113	36.6	4.5	T2-4
Willow Tree Long	X639	28/03/2018	52-63	189-100	39.3	14.0	T2-4

5.4.12 PROGRESS REPORT: Evaluation of Grapefruit on different rootstocks in a semi-desert production area (Kakamas)

Project 922 by J. Joubert and W. Swiegers (CRI)

Summary

Visual evaluations of Star Ruby and Nelruby bud-unions, indicated that the unions were in good condition and the combinations compatible (need to confirm with follow-up inspections). Sunki 812 is a hybrid rootstock cross

between a Sunki mandarin and Beneke trifoliolate (Sunki 812). The tree size of this combination is described as medium (similar to Carrizo tree size and growth rate), although Sunki 812 rootstock as a tree on its own is aggressive and develops into a fairly large tree. In combination with Star Ruby, the tree was a little bigger compared to Citrange 35 and Benton citrange (dwarfing rootstocks). Yield production was down this season due to pruning techniques to improve fruit size. Star Ruby outperformed Nelruby during the season, with improved fruit size for both selections, Star Ruby peaking at count 32 - 27 (big fruit) and Nelruby peaked count 36 followed by counts 27 and 48.

Seed counts on the Nelruby fruit were higher compared to Star Ruby being virtually seedless. Colour development on both selections and all the rootstock combinations was good with the best being Star Ruby on Citrange 35, Carrizo Citrange, Swingle Citrumelo and X639. The dwarfing rootstocks, Citrange 35 and Benton citrange performed very well, bearing in mind the impact of the high pH of the soil. Future evaluations will determine the adaptability of these rootstocks.

Evaluations to date show that these rootstocks could be of value to citrus producers, particularly Sunki 812, should high pH levels and calcareous soils be a problem. Sunki 812 was selected for its high tolerance to Phytophthora, citrus nematodes and tristeza, as well as better tolerance of high pH and calcareous soils.

Opsomming

Visuele evaluasies van die Star Ruby en Nelruby entlas, met 'n gesonde entlas verbinding, het bewys dit is verenigbaar met die kombinasies (moet bevestig met opvolg inspeksies). Sunki 812 is 'n hibried onderstam kruising tussen Sunki mandaryn en Beneke trifoliaat (Sunki 812). Die boomgrootte van hierdie kombinasie word as medium beskou (vergelyk met Carrizo boomgrootte en groeikragtigheid), alhoewel Sunki 812 onderstam as boom op sy eie baie groeikragtig is en 'n groot boom oplewer. In kombinasie met Star Ruby was die boomgrootte bietjie groter as Citrange 35 en Benton citrange (verdwergde onderstamme). Die oes produksie het baie afgeneem hierdie seisoen a.g.v. snoeipraktyke om vrug grootte te verbeter, (Star Ruby het beter presteer hierdie seisoen as Nelruby in hierdie proef) vruggrootte vir Star Ruby het gepeik by 27 – 32 (groot vrugte) en Nelruby het gepeik by grootte 36 gevolg deur 27 en 48 vrug grootte.

Saad tellings op die Nelruby vrugte was hoër in vergelyking met Star Ruby wat feitlik saadloos toets. Kleur ontwikkeling op albei seleksies en al die onderstam kombinasies was bietjie vertraag met die beste vir Star Ruby op Citrange 35, Carrizo Citrange, Swingle Citrumelo en X639. Beide die verdwergde onderstamme, Citrange 35 en Benton citrange het baie goed presteer wanneer die impak van die hoë pH van die grond in ag geneem word. Verdere evaluasies sal die aanpasbaarheid van hierdie onderstamme bevestig.

Evaluasies tot op datum toon aan dat hierdie onderstamme waardevol kan wees vir die sitrus produsente; meer spesifiek Sunki 812, waar hoë pH vlakke en kalkagtige gronde voorkom. Sunki 812 was vir sy hoë verdraagsaamheid teen Phytophthora, sitrus aalwurms en tristeza, asook beter weerstand vir hoër pH en kalkagtige gronde, geselekteer.

Objectives

- To investigate the performance of Star Ruby and Nelruby Grapefruit on suitable rootstocks in a hot citrus growing area on replant soils.
- To improve production, internal quality, rind colour and fruit size count distributions.

Materials and methods

Seeds of Benton Citrange, Citrange 35, Carrizo Citrange, Rough Lemon, Sunki 812, Swingle Citrumelo, Terrabella and X639 were propagated by Cedarberg Nursery, a CIS accredited nursery in the Citrusdal region of the Western Cape.

Star Ruby and Nelruby grapefruit was budded onto the following rootstocks at Cederberg nursery in 2010: Benton Citrange, Citrange 35, Carrizo Citrange, Rough Lemon, Sunki 812, Swingle Citumelo, Terra Bella and X639. The trees were planted at Karsten in March 2012.

Table 5.4.12.1. Number of trees per rootstock in the Star Ruby and Nelruby Grapefruit trial at Kakamas.

Selection	Rootstock	No. of trees
Star Ruby	BC	6
Star Ruby	C35	6
Star Ruby	CC	6
Star Ruby	RL	3
Star Ruby	Sunki 812	4
Star Ruby	SC	6
Star Ruby	TB	6
Star Ruby	X639	5
Nelruby	BC	6
Nelruby	C35	6
Nelruby	Sunki 812	5
Nelruby	SC	6
Nelruby	TB	6
Nelruby	X639	6

Results and discussion

The Grapefruit trial was harvested for the third time this season with a low to fair crop on the trees.

Star Ruby

The lowest crop production for the 2018 season was in combination with TB yielding 20.9 kg/tree (2017; BC – 49.9 kg/tree) and the best on RL yielding 66.5 kg/tree (2017; SC – 67.8 kg/tree), selected for high pH soil conditions (Table 5.4.12.4). The second highest crop was produced on C35 with 45.2 kg/tree, and the average yield for the Star Ruby trial was 38.8 KG (2017 – 59.4 kg). Internally fruit quality was good with Brix ranging from 8.1 up to 9.4 (average 8.8) and juice levels above 52% (Table 5.4.12.2).

The acid content remained fairly high this season above 1.3% (slightly higher 2017), decreasing the Brix:acid ratio to 6.0 (lower compare to 2017 ratio 8). Fruit size was bigger compared to 2017 (count 48) and peaked at count 27, followed by count 32 and count 36, producing a bigger fruit size on the trees for this season, due to the light crop.

Nelruby

Nelruby on TB produced the best juice content (58.1%), as well as the third highest Brix:acid ratio of 7.39, followed by BC, C35 and Sunki 812 with the highest Brix level (9.3) and C35 highest acid of 1.32% (Table 5.4.12.2). The external colour development on all 6 rootstock combinations peaking at T1 to T3/4. Most of the combinations peaked at count 36 (2017 peaked at count 48), followed by counts 27 and 48. The best crop on the Nelruby trees was in combination with C35 (74.4 kg/tree), followed by SC (48.7 kg/tree) and X639 (35.1 kg/tree). Both C35 and BC develop smaller trees size due to their dwarfing characteristics and Sunki 812 had the smallest tree size.

Conclusions

Star Ruby in combination with Sunki 812 developed very small tree size compared to the other rootstocks (slightly bigger than the two dwarfing rootstocks C35 and BC). The seed content in the Star Ruby fruit remained significantly lower in comparison with the Nelruby fruit. Fruit size distribution from Star Ruby was more even compared to the fruit sizes on Nelruby (count 27, 32 and 36). The bigger fruit size was due to the lower crop on the trees. Star Ruby cropped a better yield on the trees (average 38.8 kg/tree versus 37.7 kg) compared to

Nelruby this season. Star Ruby had improved colour development (deeper red blush on rind) where Nelruby was more yellowish.

Star Ruby and Nelruby was evaluated on eight and six rootstocks respectively, the most important combination of the above mentioned was Sunki 812 (Sunki mandarin x Beneke trifoliolate). Sunki 812 was selected for replant conditions, very specific high pH and calcareous soils. The first and second evaluation and harvest indicated that the other rootstocks outperformed Sunki 812, but Sunki 812 is starting to perform better and future evaluations will be crucial to determine the best combination in these semi-desert conditions.

Table 5.4.12.2. Internal fruit quality of Star Ruby and Nelruby Grapefruit on different rootstocks at Karsten Boerdery (Kakamas) on 23th May 2018.

Cultivar	Root-stock	Juice (%)	Brix °	Acid (%)	Ratio	Avg. seed	Colour
Nelruby	BC	52,2	9,3	1,14	8,16	1,0	T1-3
Nelruby	C35	56,9	9,3	1,32	7,05	1,3	T1-4
Nelruby	Sunki 812	56,6	9,3	1,22	7,65	2,0	T1-3
Nelruby	SC	57,1	8,8	1,23	7,15	1,8	T1-3
Nelruby	TB	58,1	9,2	1,25	7,39	0,7	T1-3
Nelruby	X639	55,5	9,2	1,29	7,13	2,8	T1-3
Star Ruby	BC	58,8	9,4	1,38	6,84	0,0	T1-3
Star Ruby	C35	56,4	8,6	1,36	6,32	0,0	T1-2
Star Ruby	CC	56,4	8,1	1,37	5,93	0,0	T1-2
Star Ruby	RL	56,7	8,5	1,34	6,37	0,0	T1-3
Star Ruby	Sunki 812	58,2	9,3	1,36	6,84	0,0	T1-3
Star Ruby	SC	56,7	8,5	1,34	6,37	0,0	T1-2
Star Ruby	TB	52,4	8,9	1,38	6,45	0,0	T1-3
Star Ruby	X639	56,0	8,7	1,34	6,49	0,0	T1-2

Table 5.4.12.3. Fruit size distribution at Karsten Boerdery during the 2018 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	BC	27	42,02	Nelruby	BC	27	25,17
Star Ruby	BC	32	19,96	Nelruby	BC	32	13,76
Star Ruby	BC	36	10,31	Nelruby	BC	36	21,48
Star Ruby	BC	40	3,24	Nelruby	BC	40	14,09
Star Ruby	BC	48	2,96	Nelruby	BC	48	14,09
Star Ruby	BC	64		Nelruby	BC	64	11,41
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	C35	27	49,21	Nelruby	C35	27	10,72
Star Ruby	C35	32	18,70	Nelruby	C35	32	7,82
Star Ruby	C35	36	13,19	Nelruby	C35	36	38,05
Star Ruby	C35	40	6,50	Nelruby	C35	40	16,61
Star Ruby	C35	48	6,89	Nelruby	C35	48	15,43
Star Ruby	C35	64	5,51	Nelruby	C35	64	11,36
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	CC	27	48,02	Nelruby	Sunki 812	27	20,06
Star Ruby	CC	32	18,07	Nelruby	Sunki 812	32	10,36
Star Ruby	CC	36	10,89	Nelruby	Sunki 812	36	23,30
Star Ruby	CC	40	6,44	Nelruby	Sunki 812	40	15,21
Star Ruby	CC	48	8,66	Nelruby	Sunki 812	48	14,56

Star Ruby	CC	64	7,92	Nelruby	Sunki 812	64	16,50
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	RL	27	46,93	Nelruby	SC	27	10,64
Star Ruby	RL	32	23,83	Nelruby	SC	32	9,33
Star Ruby	RL	36	15,23	Nelruby	SC	36	34,04
Star Ruby	RL	40	7,13	Nelruby	SC	40	13,75
Star Ruby	RL	48	3,93	Nelruby	SC	48	20,29
Star Ruby	RL	64	2,95	Nelruby	SC	64	11,95
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	Sunki 812	27	28,62	Nelruby	TB	27	24,44
Star Ruby	Sunki 812	32	10,06	Nelruby	TB	32	11,11
Star Ruby	Sunki 812	36	44,97	Nelruby	TB	36	27,78
Star Ruby	Sunki 812	40	5,97	Nelruby	TB	40	12,22
Star Ruby	Sunki 812	48	5,97	Nelruby	TB	48	13,33
Star Ruby	Sunki 812	64	4,40	Nelruby	TB	64	11,11
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	SC	27	49,18	Nelruby	X639	27	9,22
Star Ruby	SC	32	15,03	Nelruby	X639	32	7,58
Star Ruby	SC	36	12,57	Nelruby	X639	36	32,17
Star Ruby	SC	40	8,74	Nelruby	X639	40	12,30
Star Ruby	SC	48	8,47	Nelruby	X639	48	20,29
Star Ruby	SC	64	6,01	Nelruby	X639	64	18,44
Cultivar	Rootstock	Size	% Fruit				
Star Ruby	TB	27	41,89				
Star Ruby	TB	32	17,12				
Star Ruby	TB	36	13,51				
Star Ruby	TB	40	9,46				
Star Ruby	TB	48	9,01				
Star Ruby	TB	64	9,01				
Cultivar	Rootstock	Size	% Fruit				
Star Ruby	X639	27	46,23				
Star Ruby	X639	32	17,92				
Star Ruby	X639	36	15,58				
Star Ruby	X639	40	9,87				
Star Ruby	X639	48	6,49				
Star Ruby	X639	64	3,90				

Table 5.4.12.4. Production per tree of Star Ruby and Nelruby Grapefruit trees on different rootstocks at Karsten Boerdery (Kakamas) during the 2017 season.

Cultivar	Rootstock	2017 Kg/tree	2018 Kg/tree
Nelruby	BC	75.3	28,1
Nelruby	C35	80.2	74,4
Nelruby	Sunki 812	51.8	31,7
Nelruby	SC	83.9	48,7
Nelruby	TB	62.3	7,9
Nelruby	X639	62.1	35,1
Star Ruby	BC	49.9	34,3
Star Ruby	C35	58.4	45,2
Star Ruby	CC	61.7	30,7
Star Ruby	RL	62.0	66,5

Star Ruby	Sunki 812	58.5	40,8
Star Ruby	SC	67.8	32,7
Star Ruby	TB	58.2	20,9
Star Ruby	X639	58.6	39,4

5.4.13 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 due to enough cold units for colour break. The orchards were planted between 1991 and 2000. The trees are mature with large tree canopies. Some of the selections were planted on different rootstocks. The order of ripening was as follows; Miho Wase, Miyagawa Wase, Okitsu, Dobasi Beni and Ohtsu. 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 deel van n kommersiële aanplanting. Die proef se ligging is goed geskik vir Satsuma produksie a.g.v. die hoë koue eenhede wat nodig is vir kleurontwikkleng. Die boorde is op verskillende tye aangeplant vanaf 1991 tot 2000 en die bome is volwasse met genoegsame boom volume. Van die seleksies is ook op verskillende onderstamme geplant. Die volgorde van rypwording is: Miho Wase, Miyagawa Wase, Okitsu, Dobasi Beni en Ohtsu. Die oesvenster 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 East Cape Midlands part of the Eastern Cape. The following selections were evaluated: Miho Wase, Okitsu, Miyagawa Wase, Dobashi Beni and Ohtsu.

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.

Table 5.4.13.1 List of Satsuma selections evaluated at Saxfold Park (Adelaide) during 2018.

Selection	Rootstock	Planted
Ohtsu	Carrizo Citrange	Unknown

Dobashi Beni	Unknown	Unknown
Miyagawa Wase	Swingle Citrumelo	1997
Miho Wase	Swingle Citrumelo	Unknown
Okitsu	Carrizo Citrange	1997

Results and discussion

Miho Wase

Miho Wase was the first selection to mature this season. The rind was smooth, and the fruit peeled easily. It developed a good fruit size (count 2). Miho Wase is known to have fruit size counts of 2 - 3. The fruit was seedless. The fruit colour on the colour plate was T5. The ratio was already at 10.6 close to over maturity. Fruit matured internally prior to good colour development. 57% was a very good juice percentage for the Miho Wase fruit it was also the highest. Miho Wase had the highest Brix° with maturity at 10° as well as a good Acid %, 0.94%.

Dobashi Beni

Dobashi Beni was the mid - late selection Satsuma for this trial site, with peak maturity being late April beginning of May. Peak maturity was about a week earlier. The fruit size was the same than last year with a 2 count. The juice percentage of the Dobashi Beni were 50 % lower compared to last season 56.2%. Dobashi Beni had the third highest Brix° 9.7° and Acid % of 0.99% close to peak maturity. Dobashi Beni had a seed count of 0.0 - 0.5 and the fruit colour on the colour plate was T6 - T7. The fruit of the Dobashi Beni was flat with smooth rind that peeled easily.

Miyagawa Wase

The fruit size of Miyagawa Wase at peak maturity was count 1, bigger than last year count of 2. The juice % lowered towards peak maturity to a juice % of 51.2% and it was lower than last year high juice % of 59.4%. The Brix° and acid percentage were very good towards peak maturity, being 9.7° and 1.04% respectively. Colour development was delayed compared to the internal maturity. There were no seeds in Miyagawa Wase. The colour on the colour plate towards peak maturity was T5 – T6. The delayed rind colour could be due to the Swingle citrumelo rootstock. Fruit had better taste than Miho Wase, and the fruit was smooth and flat. Internal colour was a deep orange.

Ohtsu

This selection is the late maturing selection for the Satsumas trial site. Ohtsu was the last selection to reach peak maturity. Ohtsu had a good fruit size count with a 1 - 2 count. Ohtsu juice % increased with maturity and it also had the third highest juice % of the 5 selections with 51.5% towards peak maturity. The Brix° of Ohtsu was the highest at build up towards peak maturity being 10.6° with a very good acid percentage of 1.10%. The acid percentage of Ohtsu was also the highest. The selection was basically seedless 0.0 – 0.2 seeds. Ohtsu colour on the colour plate towards build up to peak maturity was T7 the lowest. Peelability was found to be easy and fruit had a flat shape.

Okitsu

Okitsu fruit size count was a count 1 towards peak maturity. Okitsu juice percentage was the second highest percentage of the selections with 51.9% towards peak maturity. The Brix° of Okitsu towards peak maturity was 9.8° with a very good acid percentage of 1.12%. There were no seeds and Okitsu colour on the colour plate towards peak maturity was T6. Okitsu also had a delayed colour development compared to internal maturity. The fruit peelability was easy.

Conclusion

All of the selections had a good fruit size count with a count 2 - 1. Miho Wase and Ohtsu had the best juice percentages: 57% and 51.5% respectively. Ohtsu had the highest Brix° of all the Satsuma selections (10.6°). Ohtsu also had the highest acid percentage of all the Satsuma selections (1.10%). Dobashi Beni had the

highest seed count with 0.0 – 0.5 seed per fruit. All the selections rind colour development was delayed compared to their internal maturity development.

Table 5.4.13.2. Internal fruit quality data for Satsuma selections in the Adelaide region (Saxfold) of the East Cape Midlands during the 2018 season.

Date	Cultivar	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2018-04-04	Dobashi Beni	Unknown	1	43,4	9,5	1,25	7,6	0,0	T7
2018-04-13	Dobashi Beni	Unknown	2	50,0	9,7	0,99	9,8	0,5	T6
2018-03-20	Miho Wase	SC	2	57,0	10,0	0,94	10,6	0,0	T5
2018-03-20	Miyagawa Wase	SC	1	56,5	9,6	1,15	8,3	0,0	T6
2018-04-04	Miyagawa Wase	SC	1	51,2	9,7	1,04	9,3	0,0	T5
2018-04-04	Ohtsu	CC	1	48,4	9,9	1,29	7,7	0,0	T7
2018-04-13	Ohtsu	CC	2	51,5	10,6	1,10	9,6	0,2	T7
2018-03-20	Okitsu	CC	3	56,9	10,0	1,20	8,3	0,0	T6
2018-04-04	Okitsu	CC	1	51,9	9,8	1,12	8,8	0,0	T6

5.4.14 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 a relatively new trial and 2018 was the third season with fruit on the trees. The trees were topworked in 2012 to the following selections which was also the order of ripening: Miho Wase, Aoshima, Ueno, Immamura, and the season was finished off with Dobashi Beni. The order of ripening might change a bit due to the young trees. 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 'n relatiewe nuwe proef en 2018 was die bome se derde drag. Die bome was in 2012 getopwerk na die volgende seleksies toe, wat ook dien as die volgorde van rypwording; Miho Wase, Aoshima, Ueno, Immamura en die seisoen was afgesluit met Dobashi Beni. Die rypwordings volgorde gaan bietjie verskil a.g.v. jong bome. 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, Ueno, Aoshima, Imamura, and Dobashi Beni.

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 is approximately three weeks long. Fruit harvested before and after this period would result in higher instances of quality and rind issues.

Table 5.4.14.1. List of Satsuma selections evaluated at Invercloy (Kirkwood) during 2018.

Selection	Rootstock	Topworked
Aoshima	Carrizo	2012
Miho Wase	Carrizo	2012
Ueno	Carrizo	2012
Dobashi Beni	Carrizo	2012
Imamura	Carrizo	2012

Results and discussion

Aoshima

Aoshima was the second selection to reach peak maturity. The fruit size count for Aoshima was count 1xxx (big fruit). Aoshima had low juice percentage below 50%. All 3 seasons Aoshima had a low juice %. Brix was 8.4°, second lowest Brix for the season compare to some of the other selections. The fruit was seedless. The external colour development of the Aoshima was not very good, with a T6 - T7 on the colour plate. T 6 on the colour plate while the fruit was over mature. Fruit is very pebbly.

Miho Wase

Miho Wase was again the first selection to mature to peak maturity this year. Miho Wase are also used as the control for the early maturing selections for this site. The selection had a bigger fruit size count of 1xxx this season compared to the previous season's 1x. Juice percentage for Miho Wase was the highest this season with 54.6% juice. Miho Wase had a Brix° of 9.4° and acid percentage of 0.84% with an 11.1 ratio. The colour was T6 on the colour plate. The fruit had no seeds and the external colour development was once again behind the internal development. The internal colour was a deep orange.

Ueno

Ueno is a mid to late maturing selection. At this Satsuma trial site, it reached peak maturity more towards the mid maturing selection range. Ueno's fruit size count was big ranging from count 1xx to 1xxx. Count 1xx was towards build up to peak maturity and count 1xxx was when the fruit was over mature. The juice percentage was lower this season at 50.5%, compared to last season's 54.5%. Ueno Brix° was 8.5° and the acid percentage was 0.75% at a ratio of 11.3. Ueno had no seeds and the colour of Ueno on the colour plate was a T6 towards peak maturity and a T5 when it was over mature. The fruit was flat and peelability was easy.

Imamura

Imamura is one of the late maturing selections for this Satsuma trial site. Due to the age of the trees the selection matured a bit earlier than if it was an older tree. Imamura normally reach peak maturity beginning to end of May in cool production regions. The juice percentage for Imamura was on the lower side with a juice percentage of 47%. Although Imamura was over mature the Brix° was 11.2° the highest of all the selections and a very good acid of 1.02%. Seed count was seedless. The colour development was T4 on the colour plate, one of the best compared to the other selections, but still delayed. Internal colour was deep orange and the fruit rind varied from smooth to coarse.

Dobashi Beni

Dobashi Beni are the control selection for the mid to late maturing Satsuma selections. Dobashi Beni was the last selection to reach peak maturity. Fruit size count was very good with count 1. The juice percentage towards peak maturity, was 43.8%. Brix° was 8.1° towards peak maturity. The acid percentage was low to start off with, 0.89%. Internal colour is deep orange, rind is smooth and peelability is easy. Dobashi Beni were seedless. This selection showed the best external colour development going being T4 on the colour plate during build up towards peak maturity.

Conclusion

Aoshima, Miho Wase and Ueno had a big fruit size i.e. count 1xxx. Dobashi Beni and Imamura had a good fruit size count ranging from 1 to 1xx. Miho Wase had the best juice percentage being 54.6%. All the other selections had a juice percentage below 50%. Imamura had the highest Brix° at peak maturity being 11.2° and Sugiyama had the lowest Brix° being 8.5° of all the Satsuma selections. All the selections were seedless.

Table 5.4.1.13. Internal fruit quality data for Satsuma selections in the Addo and Kirkwood region of the Eastern Cape during the 2017 season.

Date	Cultivar	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2018-04-03	Aoshima	CC	1xxx	47,5	8,4	0,95	8,6	0,0	T7
2018-04-19	Aoshima	CC	1xxx	41,1	8,7	0,63	13,8	0,0	T6
2018-05-02	Dobashi Beni	CC	1	43,8	8,1	0,89	9,1	0,0	T4
2018-04-03	Imamamura	CC	1x	52,2	11,5	1,82	6,3	0,0	T7
2018-05-02	Imamamura	CC	1xx	47,0	11,2	1,02	11,0	0,0	T4
2018-03-20	Miho Wase	CC	1xxx	54,6	9,4	0,84	11,1	0,0	T6
2018-04-03	Ueno	CC	1xx	47,9	7,8	1,01	7,7	0,0	T6
2018-04-19	Ueno	CC	1xxx	50,5	8,5	0,75	11,3	0,0	T5

5.4.15 **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 2 different trial sites. Two of the sites are in the Kirkwood region of the Sundays River Valley. The selections in that trial site is as follow and it is also the order of ripening: RHM, Tasty 1, St Andre, Edit x Nova, Leanri, Samba, Tango, Gold Nugget and Tanor Late. At the other site in the Kirkwood region, we evaluated the following selections in their order of ripening: Etna, Sirio and Tasty 1 and they were all directly topworked onto the rootstock.

Opsomming

Die mandaryn proef is opgedeel in 2 verskillende proef persele. Die twee persele is in Sondagsrivier Vallei n.l. Kirkwood. By die een proef perseel in die Kirkwood area is van die seleksies op 'n tussenstam getopwerk. Die volgorde van rypwording by die perseel was as volg: RHM, Tasty 1, St Andre, Edit x Nova, Leanri, Samba, Tango, Gold Nugget en Tanor Late. By die ander perseel in Kirkwood is al die seleksies direk op die onderstam en was die volgende seleksies geëvalueer in hulle orde van rypwording: Sirio, Etna en Tasty 1.

Objectives

- To select mandarin hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new mandarin hybrid cultivars and to determine the climatic suitability of these cultivars in cold production regions

Materials and methods

Field evaluations and laboratory analyses were conducted on mandarin hybrid selections from the Sundays River Valley. A range of new mandarin hybrids have been added to this area. The following varieties were evaluated: Edit x Nova, Etna, Sirio, Tanor Late, Saint Andre, Tango, Gold Nugget, Leanri, RHM, Samba and Tasty 1.

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 5.4.15.1. List of Mandarin hybrid selections from Kirkwood region of the Sundays River Valley (Dunbrody) during the 2018 season.

Selection	Rootstock	Topwork
Saint Andre	Carrizo	2013
Edit x Nova	Carrizo with Midnight interstock	Unknown
Leanri	Carrizo with Midnight interstock	Unknown
Gold Nugget	Carrizo with Midnight interstock	Unknown
RHM	Carrizo with Midnight interstock	Unknown
Samba	Carrizo with Midnight interstock	Unknown
Tanor Late	Carrizo with Midnight interstock	Unknown
Tango	Carrizo	2013
Tasty 1	Carrizo with Midnight interstock	Unknown

Table 5.4.15.2 List of Mandarin hybrid selections from Kirkwood region of the Sundays River Valley (Invercloy) during the 2018 season.

Selection	Rootstock	Topwork
Etna	Carrizo	2012
Sirio	Carrizo	2012
Tasty 1	Carrizo	2012

Results and discussion

RHM

RHM kicked the season off for this Kirkwood trial site. It had a good fruit size count of 1x. Acid drop quickly but stay stable around 0.80% at peak maturity and with the good sugars contributing to good flavour and eating

quality. The external colour development was delayed with T6 on the colour plate when the fruit was over mature. The selection has good Brix° at 10° and acid % just around 0.80%. Juice percentage for RHM was very high between 58 - 59%. There were seed for this selection during evaluations with a count of 0.4 seeds per fruit (fruit is virtually seedless). The fruit is firm with a smooth rind and a deep orange internal colour.

Edit x Nova

Edit x Nova were the fourth selection to reach peak maturity. It is an early to mid-maturing mandarin hybrid. It had a good fruit size count ranging from 1x - 1xx. Edit x Nova hang very well on the tree. The sugars and acid percentage don't change much close to and at peak maturity, while the external colour development improves. This could be due to the good internal quality. The selection has good Brix° close to 11.2° and acid % just above 1.00%. This internal quality contributes to the good fruit flavour. Juice percentage for Edit x Nova was lower this season at 53.6% compared to last season 63%. The selection was seedless. Towards peak maturity the colour was a T1 on the colour plate. The rind colour was deep orange as well as the internal colour.

Saint Andre

At peak maturity the Saint Andre has a very good juice percentage peaking at 55.2%. Fruit size of the Saint Andre was good having ranged from 1 to 1x fruit size count. The sugar had a slight increase towards peak maturity as expected with the acid % stabilizing around 1.00%. Brix° was 11.9° towards build up to peak maturity and 12.5° at peak maturity. During the evaluations there were no seeds. The Saint Andre had an external colour of T5 on the colour plate towards peak maturity and at peak maturity it was T1 on the colour plate. Rind is slightly pebbly and flesh is deep orange. Fruit is flat to round and peelability is easy and the flavour very good. The trees had a good crop on them.

Gold Nugget

Gold Nugget fruit size count was good with 1x count. The juice percentage of Gold Nugget was low below 50 %. At peak maturity the Gold Nugget had a T1 colour on the colour plate. Brix° stayed constant just above 11.5° during the ripening period. Acid percentage did decrease a bit, but the acid percentage was still good at 0.96% at peak maturity. Gold Nugget's good sugars and acid contribute to this tasty fruit. There were no seeds in the Gold Nugget. Peelability of the fruit is easy and the rind oil does not bother. The fruit is round and pebbly.

Tango

Tango is mid to late maturing, seedless selection. The previous season the selection was a T6 on the colour plate before peak maturity, this season it was T1 on the colour plate just before peak maturity. Juice percentages (around 50%) towards peak maturity. The fruit size for Tango ranged between counts 1 - 2. Tango had the highest Brix° and acid percentage this season at peak maturity. Brix° was 13.4° and acid percentage was 1.16% at peak maturity. The rind is shiny and smooth and the flesh have a deep orange colour.

Etna

Etna are an early maturing mandarin hybrid. Etna reached peak maturity second on this trial site. External colour development was good with a T3 – T1 on the colour plate at peak maturity. Etna will be able to degreen. Fruit size count are 1xxx big fruit. Juice percentage for Etna was good, just below 55% at peak maturity. Etna were seedless during all the evaluations. Etna was one of selections with the lowest sugar and acid percentage towards peak maturity. Brix° 9.2° and acid percentage was 0.82%. Etna have a deep orange internal colour.

Sirio

Sirio is also early maturing mandarin hybrid. It was the first selection to reach peak maturity at this trial site. Fruit size was big for Sirio with a count of 1xxx. Juice percentage was above 50% for the selection at peak maturity. Seed count was low 0.4 – 0.9 seeds per fruit. Rind colour development was good with a T1 at the colour plate (peak maturity). Sirio's rind colour is a deep orange as well as the flesh. Sirio have a good internal quality at peak maturity and it give the fruit a good flavour. Peelability not easy.

Tanor Late

Tanor Late is a late maturing mandarin hybrid. Fruit size count for the selection was big with a 1xxx count. Tanor Late had a good juice percentage, just below 55 % (peak maturity). Tanor Late were seedless. Brix:

Acid ratio towards peak maturity was very good 12.7° and 1.16% acid. The good internal quality contributes to better shelf life for the fruit as well as the good flavour of the fruit. External colour development was very good being T1 on the colour plate towards peak maturity. Tanor Late rind colour is a beautiful dark orange colour and internally the colour is an excellent deep orange colour. Tanor Late had a very good crop on the trees. Peelability for Tanor Late is easy. There are small thorns on the bearing branches.

Samba

Samba is an early to mid-maturing mandarin hybrid selection. Samba on Carizzo rootstock with Midnight interstock produced an average first crop with good internal quality. Trees are fast growing and thornless. Colour development was early in the season (deep orange) with a smooth rind texture on the fruit. T1 on the colour plate towards peak maturity. Fruit were completely seedless this season in the combined trial block and fruit size peaked (count 2 to 1) very good fruit size. Juice % increased towards peak maturity to very good juice % above 55%. Brix was already at 10.7° towards peak maturity and acid 1.09%.

Leanri

Leanri is also an early to mid-maturing mandarin hybrid. It had a big fruit size count ranging from 1xx - 1xxx. Leanri have a very good juice percentage that increased towards peak maturity. Juice % was just below 60% towards peak maturity. The sugars and acid percentage was one of the highest towards peak maturity. The Brix° was 12.8° and acid % of 1.18% when the ratio was 10.8. This internal quality contributes to the good fruit flavour. The selection was seedless during the evaluations. Towards peak maturity the colour was already a T1 on the colour plate. The rind colour was deep orange as well as the internal colour.

Tasty 1

Tasty 1 is a mid-maturing selection. Tasty 1 on the interstock reached peak maturity before Tasty 1 on the rootstock. Tasty 1 on CC fruit size count was 1x and the fruit size count on the interstock was slightly bigger and the count range between 1xx – 1xxx. Juice percentage was below 50% on the rootstock as well as with the interstock. Keeping in mind that Tasty 1 on the interstock was its first crop. Internal quality was better directly onto the rootstock. Brix and acid % was higher. Colour development was also better for Tasty 1 on the rootstock reaching T1 on the colour plate compared to T3 with the interstock. Seed count was between 0.0 – 1.5 seeds per fruit

Conclusion

The following selections had the largest fruit size count with a 1xxx count: Leanri, Tasty 1, Etna, Sirio and Tanor Late. The selections with the highest juice percentage above 55% was: Leanri, St Andre, RHM and Samba. The selections with the highest °Brix was Leanri, Tango, St Andre, Tanor Late and Tasty 1. Tasty 1 and Sirio was the selections that had the most seeds per fruit. Most of the selections had a colour T1 on the colour plate.

Table 5.4.15.5 Internal fruit quality data for Mandarin hybrid selections from Kirkwood region of the Sundays River Valley (Dunbrody) during the 2018 season.

Date	Selection	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2018-05-16	Edit x Nova	CC with Interstock	1x	54,4	10,0	0,97	10,3	0,0	T6
2018-06-14	Edit x Nova	CC with Interstock	1xx	53,6	11,2	1,08	10,4	0,0	T1
2018-05-30	Leanri	CC with Interstock	1xxx	56,6	10,4	1,13	9,2	0,0	T5
2018-06-13	Leanri	CC with Interstock	1xx	59,8	12,8	1,18	10,8	0,0	T1
2018-08-07	Gold Nugget	CC with Interstock	1x	49,4	11,6	1,04	11,2	0,0	T1

2018-08-21	Gold Nugget	CC with Interstock	1x	48,4	11,7	0,96	12,2	0,0	T1
2018-05-11	RHM	CC with Interstock	4	58,3	8,2	0,86	9,5	0,4	T7
2018-05-16	RHM	CC with Interstock	1x	59,7	10,3	0,66	15,6	0,0	T6
2018-05-02	Saint Andre	CC	1	56,9	11,9	1,08	11,0	0,0	T5
2018-05-30	Saint Andre	CC	1x	55,2	12,5	1,07	11,7	0,0	T1
2018-06-28	Saint Andre	CC	1x	54,6	13,0	1,01	12,9	0,0	T1
2018-05-30	Samba	CC with Interstock	2	50,0	9,9	1,06	9,3	0,0	T5
2018-06-14	Samba	CC with Interstock	1	55,7	10,7	1,09	9,8	0,0	T1
2018-06-28	Tango	CC	1	50,5	13,8	1,34	10,3	0,0	T1
2018-07-18	Tango	CC	2	50,4	13,4	1,16	11,6	0,0	T1
2018-07-25	Tango	CC	1	51,7	14,9	1,14	13,1	0,0	T1
2018-07-18	Tanor Late	CC with Interstock	1xxx	53,0	13,0	1,48	8,8	0,0	T4
2018-08-07	Tanor Late	CC with Interstock	1xxx	54,3	12,9	1,23	10,5	0,0	T3
2018-08-21	Tanor Late	CC with Interstock	1xxx	52,4	12,7	1,16	10,9	0,0	T1
2018-05-16	Tasty 1	CC with Interstock	1xx	48,0	8,8	0,86	10,2	0,3	T6
2018-05-30	Tasty 1	CC with Interstock	1xxx	46,3	8,9	0,78	11,4	1,3	T5
2018-06-14	Tasty 1	CC with Interstock	1xx	44,8	9,8	0,77	12,7	1,5	T3

Table 5.4.15.6 Internal fruit quality data for Mandarin hybrid selections from Kirkwood region of the Sundays River Valley (Invercloy) during the 2018 season.

Date	Selection	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2018-05-02	Etna	CC	1xxx	54,4	9,2	0,82	11,2	0,0	T3
2018-05-30	Etna	CC	1xxx	54,2	9,6	0,71	13,5	0,0	T1
2018-04-19	Sirio	CC	1xxx	53,2	10,5	1,02	10,3	0,4	T3
2018-05-02	Sirio	CC	1xxx	51,3	10,7	0,88	12,2	0,5	T1
2018-05-30	Sirio	CC	1xxx	51,2	11,8	0,88	13,4	0,9	T1
2018-05-30	Tasty 1	CC	1x	45,7	11,2	1,21	9,3	0,5	T4
2018-06-28	Tasty 1	CC	1x	48,8	12,8	1,05	12,2	0,0	T1

5.4.16 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

Loerie is the main trial site in the Gamtoos River Valley area, but there is a new site on the way with all of the latest selections in Patensie. Trees have been planted in 2017. Both trial sites will form part of the Gamtoos

River Valley. At Loerie the season started off with Nova and was followed up by St Andre, Sirio, Etna, Tasty 1, Nadorcott, Gold Nugget, and the season ended with Tango.

Opsomming

Loerie is die hoof perseel in die Gamtoos Rivier Vallei, maar daar is 'n nuwe perseel in Patensie wat al die nuwe seleksies gaan bevat. Die bome was in 2017 geplant. Albei persele maak deel uit van die Gamtoos Rivier Vallei. By Loerie het die seisoen begin met Nova gevolg deur St Andre, Sirio, Etna, Tasty 1, Nadorcott, Gold Nugget, en die seisoen het geëindig met Tango.

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: Saint Andre, Etna, Sirio, Nova, Tasty 1, Tango, Gold Nugget, and Nadorcott.

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 5.4.16.1 List of experimental mandarin hybrid selections evaluated in the Loerie (N. Ferreira) region of the Gamtoos River Valley during the 2018 season.

Selection	Rootstock	Topwork
Etna	Carrizo	2012
Sirio	Carrizo	2012
Saint Andre	Carrizo	2012
Tasty 1	Carrizo	2012
Gold Nugget	Carrizo	2012
Tango	Carrizo	2012
Nova	Carrizo	2012
Nadorcott	Carrizo	2012

Results and discussion

Gold Nugget

The fruit size for Gold Nugget was count 1x. Fruit for this selection is pebbly and peelability is easy with low levels of rind oil. Before peak maturity was reached the external colour reach T4 on the colour plate. For this selection it is a yellow orange colour. There a thorns on the bearing branches, but does get smaller as the tree gets older. Gold Nugget is a seedless variety. At peak maturity the internal juice percentages were just below 50%. Internal quality for the selection is good with high sugars and good acids around 1%, this also contribute to the good flavour of Gold Nugget.

Tango

Tango had a very good fruit size (count 1) and were seedless, as it is a seedless variety. The selection had good external colour development T1 on the colour plate towards peak maturity. Tango rind and internal colour is a deep orange. The rind also has a natural shine and peels very easy, and the rind oil don't bother. Tango juice percentage was high about 60%. Tango's Brix and acid ratios were good, indicating that the fruit will have a good shelf life and give Tango a good flavour.

Nova

Nova was the first selection to reach peak maturity. Nova had the same fruit size count (1) this season compared to last years (1) count. Seed count for Nova was 0.0 seeds per fruit during the evaluations (Nova is not a seedless variety). Nova external colour development was delayed, being T5 on the colour plate range when the Brix: Acid ratio was 13.2. The juice % for Nova at peak maturity was very good, and one of the highest, above 60%. Internal quality was good for Nova at peak maturity Brix above 11° and acid above 1%.

Saint Andre

Saint Andre was the second selection to reach peak maturity in this production region. The fruit size count was between 1 and 1xx. The juice percentage this season was a little bit lower compared to the previous season. This season's percentage was still good above 55% compared to last season's 67.2%. At peak maturity the colour was T5 on the colour plate. The acids and sugars remained stable during the production season for Saint Andre. Acid % was still above 0.80% when the fruit was over mature. Internal quality was very good and it contribute to the good flavour. The selection was seedless during the evaluations.

Etna

The fruit size count for the Etna this season was bigger, count 1xx – 1xxx count, compared to last season 2 to 1. The juice percentage for Etna was good above 55 % juice at peak maturity and better compared to Sirio. The Brix and Acid levels of Etna were very slightly lower than Sirio towards peak maturity. Sirio had a slightly higher Brix and acid. Etna reached peak maturity after Sirio in the mandarin range of new experimental cultivars. Etna had a T1 on the colour plate range at peak maturity. On all three evaluations there was no seed, Etna is not a seedless selection.

Sirio

Sirio also had a big fruit size count that ranged between 1xx – 1xxx. Sirio developed a lower juice percentage compared to Etna. The juice percentage increased towards peak maturity to just below 55%. 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 at peak maturity. Sirio seed count ranged between 0.0 – 0.3 seeds per fruit.

Tasty 1

Tasty 1 developed a large/extra-large fruit size and peaked at count 1xxx. The external colour development was good with a T2 on the colour plate before peak maturity. The juice percentages were not good for Tasty 1 being below 50%. On two of the evaluations there were no seeds in the fruit. At peak maturity the Brix was 10.5 with acid % of 0.85%.

Nadorcott

Nadorcott was one of the selections that developed the smallest fruit size (count 1) for this trial site. The selection was seedless during the 3 evaluations. There was a good colour development (T1) at peak maturity. Rind texture was very smooth with a natural shine. Selection developed good internal quality with high juice levels (up to 60%), Brix averaging 11 and acceptable acids (0.9%).

Conclusion

Half of the selections (Etna, Nadorcott, Sirio and Tango) had a very good external colour development (T1) at peak maturity. The following selections had the largest fruit size (count 1xxx); Etna, Sirio and Tasty 1. Nadorcott, Nova, St Andre and Tango cropped the smallest fruit size (count 1). Nadorcott, Nova and Tango developed juice percentages above 60%. Tasty 1 had juice percentages below 50%. Nadorcott, Nova, St Andre, Sirio, Tango and Tasty 1 had the highest Brix level above 11°. The selection with the highest seed count was Sirio.

Table 5.4.16.2 Internal fruit quality data for experimental mandarin hybrid selections from the Loerie (N. Ferreira) region of the Gamtoos River Valley region during the 2018 season.

Date	Selection	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg-Seed	Colour
2018-05-15	Etna	CC	1xxx	55,2	8,7	0,80	10,9	0,0	T4
2018-05-29	Etna	CC	1xx	55,6	9,7	0,86	11,3	0,0	T5
2018-06-11	Etna	CC	1xxx	57,6	9,9	0,83	11,9	0,0	T1
2018-05-29	Nadorcott	CC	5	57,6	9,5	1,05	9,0	0,0	T5
2018-06-26	Nadorcott	CC	1	60,6	11,1	0,97	11,4	0,0	T1
2018-08-06	Nadorcott	CC	1	55,2	12,0	0,89	13,5	0,0	T1
2018-05-15	Nova	CC	1	62,4	11,6	0,88	13,2	0,0	T5
2018-05-29	Nova	CC	1	60,3	11,6	0,82	14,1	0,0	T1
2018-06-26	Gold Nugget	CC	1x	47,2	10,5	1,12	9,4	0,0	T6
2018-07-11	Gold Nugget	CC	1x	50,0	10,8	0,95	11,4	0,0	T4
2018-05-07	Saint Andre	CC	1	56,4	10,8	0,96	11,3	0,0	T6
2018-05-15	Saint Andre	CC	1	57,7	11,7	0,93	12,6	0,0	T5
2018-05-29	Saint Andre	CC	1xx	59,0	11,1	0,83	13,4	0,0	T2
2018-05-07	Sirio	CC	1xx	48,9	10,0	0,97	10,3	0,3	T5
2018-06-11	Sirio	CC	1xxx	50,8	11,4	0,90	12,7	0,0	T1
2018-06-26	Sirio	CC	1xxx	54,2	11,7	0,91	12,9	0,0	T1
2018-06-26	Tango	CC	1	60,2	11,6	1,23	9,4	0,0	T1
2018-07-11	Tango	CC	1	56,0	11,9	1,18	10,1	0,0	T1
2018-07-24	Tango	CC	1	60,4	12,6	1,06	11,9	0,0	T1
2018-05-29	Tasty 1	CC	1x	44,7	9,9	1,10	9,0	0,0	T5
2018-06-11	Tasty 1	CC	1xxx	46,9	11,3	1,03	11,0	0,0	T2
2018-06-26	Tasty 1	CC	1xxx	46,6	10,5	0,85	12,4	0,0	T2

5.4.17 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. The season started with Tami 2/65 and then RHM followed, Etna, Samba, Mor 26, Or 4, IRM 1, IRM 2, Furr, Tango, Gold Nugget, Shani SL, Nadorcott ARC, Nadorcott LS, Nadorcott and Winola. At the Paarl site most of the new experimental selections were topworked. Cross pollination is also high in this site. During the season Sirio was evaluated.

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 Tami 2/65, RHM gevolg deur Etna, Samba, Mor 26, Or 4, IRM 1, IRM 2, Furr, Tango, Gold Nugget, Shani SL, Nadorcott ARC, Nadorcott LS, Nadorcott en Winola. By die Paarl perseel is die meeste van die nuwe seleksies nou oorgewerk. Kruisbestuiwing is ook baie hoog in die perseel. Sirio was tydens die seisoen geëvalueer.

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 and Paarl region of the Western Cape. The following selections were evaluated: Tami 2/65, Sirio, RHM, Etna, Samba, Mor 26, Or 4, IRM 1, IRM 2, Furr, Tango, Gold Nugget, Shani SL, Nadorcott ARC, Nadorcott LS, Nadorcott and Winola.

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 5.4.17.1. List of experimental mandarin hybrid selections evaluated in the Citrusdal region of the Western Cape during the 2018 season.

Selection	Rootstock	Topwork	Planted
Furr (Clemcott)	CC	2011	
Gold Nugget	CC	2010	
ARC Nadorcott	CC	2010	
Nadorcott LS	CC	2010	
Nadorcott	CC		2009
RHM	CC		2013
Tango	CC	2010	
Shani SL	CC	2010	
Etna	CC	2012	
Samba	CC	2012	
Winola	CC		2009
IRM 1	CC		2009
IRM 2	CC	2010	
Mor 26	CC		Unknown
Or 4	CC		Unknown
Tami 2/65	CC	2010	

Table 5.4.17.2. List of experimental mandarin hybrid selections evaluated in the Paarl region (Babylonstoren) of the Western Cape during the 2018 season.

Selection	Rootstock	Planted
Sirio	CC	2013

Results and discussion

Tami 2/65

Tami 2/65 was the first selection to reach peak maturity at the Citrusdal site. The fruit size for Tami 2/65 was good with fruit size count range between 2 – 1xx. Internal juice percentage was good above (55%). Internal colour is a deep orange. The fruit peels easily. The selection was seedless. Rind colour development was not

good with T6 on the colour plate at peak maturity. The selection doesn't have high acid and it tends to drop quickly leaving you with a short harvesting period. Crop on the trees looked good.

Furr (Clemcott)

Furr is used as a control for the mid-maturing mandarin selections. The juice content was low, below 50% (peak maturity). The fruit size count was range from 1 - 1xxx. Furr peels easily and has a very good eating quality. Due to the high cross pollination in the mixed trial block, Furr produced a number of seeds per fruit; up to 3.4. Furr's external colour development was very good this season; T1 on the colour plate range at peak maturity. Brix: Acid ratio for Furr was very good. High sugars and good acid and this give Furr its good flavour.

Etna

Etna is an experimental early – mid maturing mandarin hybrid. Etna reached peak maturity more to the mid maturing range. The fruit size count for the Etna this season ranged from count 1 – 1xx. Etna had a juice percentage below 55%. Juice % reduced towards peak maturity. Internal quality at peak maturity for Etna was not good. Brix was below 10 and acid % below 0.8 %. Etna had a T1 on the colour plate at peak maturity. Etna have a deep orange internal colour. The seed count for Etna ranged between 0.8 – 1.3 seeds per fruit.

Sirio

Sirio is also an early – mid maturing experimental mandarin hybrid. Fruit size count for Sirio was good it ranged between 1 - 1xxx. Internal quality for Sirio was not good, with juice % around 40% at peak maturity. The Brix and acid % was better at peak maturity with Brix 10.7 and acid % 0.86%. This gives Sirio its good flavour. Sirio seed count was 0.0 – 0.9 seeds per fruit. Sirio had no external colour development problems, being fully coloured at peak maturity. Sirio have internally as well externally deep orange colour and peelability is not easy.

Mor 26

Size count for the selection was very good with count 2 – 1x. The juice percentage for Mor 26 was very low, below 45% juice percentage at peak maturity. Rind colour development was good to reach T2 on the colour plate at peak maturity. The seed count was 0.5 – 0.7 seeds per fruit. Internal quality was good at peak maturity Brix° above 12.5° and acid % were above 1.01%. This good Brix: Acid ratio will contribute to good eating fruit with good flavour and shelf life.

Or 4

Or is a late maturing mandarin hybrid. The size count for this selection was very good count 1x. Fruit is round to oblate. Juice percentage at peak maturity to just above 45%, lowered compared to last season. Internal quality for Or is very good. Brix is high above 13° and the acids was still above 1.00% even when the fruit was over mature. This good Brix: Acid ratio will contribute to excellent eating fruit with great flavour and shelf life. Peelability is easy and oily. Or reached T1 – T2 on the colour plate at peak maturity. Average seed count was 0.5 seeds per fruit.

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 12.4°. 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 was at count 1x – 1xx at peak maturity and nearly fully coloured (T2). The juice percentage for this selection was below 50%. Gold Nugget is seedless.

Nadorcott, Nadorcott ARC & Nadorcott LS

Nadorcott ARC and Nadorcott LS are induced Nadorcott selections to minimise the average seeds per fruit. Both selections have the same growth habit and characteristics as the Nadorcott. Fruit size for these selections ranged between counts 2 – 1x. The internal juice percentages were below 50% for all 3 selections towards peak maturity. The three Nadorcott selections developed good Brix above 12.5° with acids around 1.00%, ensuring a good balance and eating quality. The fruit was fully coloured (T1) before peak maturity. The highest seed count during the three evaluations was 0.3 seeds per fruit for Nadorcott. This is in a high cross pollination

trial site. Fruit have a natural shine on them. Nadorcott ARC reached peaked maturity first, then Nadorcott LS followed by Nadorcott.

Samba

Samba is an early – mid maturing experimental low seeded mandarin hybrid, with seed count that peaked at 1.9 seeds per fruit. Samba have a favourable fruit size count that range between counts 4 – 2. Fruit is round to oblate with halo. Peelability is easy for Samba and can be oily. Long before peak maturity was achieved, Samba was a T 1 on the colour plate. Samba have an exceptional deep orange external colour and a very deep internal colour. At peak maturity; Samba have high Brix and good acids and this give Samba its unique and excellent flavour. The juice percentage at peak maturity for this selection was 50.6%.

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 was seedless. The fruit size peaked at count 1 in Citrusdal. Internally the juice percentage for Tango was just above 50%. At the trial site the Brix: acid ratio was good, with (Brix 12°) and (acid 0.91%).

IRM 1 & IRM 2

The IRM 1 is a late maturing experimental mandarin hybrid. IRM 2 is a mid to late maturing experimental mandarin hybrid. During this season IRM 1 was very early as it normally reached peak maturity 2 weeks after IRM 2. The fruit size count for both selections ranged between 2 – 1x at peak maturity. IRM 1 had the higher juice content between the 2 selections. Internal quality for IRM 1 was better than that of IRM 2. IRM 1, Brix was 13.5° and acid was 1.11% at peak maturity. IRM 2 close to peak maturity was (Brix 12.6° and acid 1.12%). IRM 2 had the higher seed count peaked at 0.5 seeds per fruit. External colour development was good for both selections IRM 1 (T 2 on the colour plate) at peak maturity and IRM 2 (T1 on the colour plate). IRM 1 & 2 is prone to ribbing.

Shani SL

In this high pollination site Shani SL seed count peaked at 0.6 seeds per fruit. The fruit size was on the bigger side peaking at count 1xxx this season compared to last season count that peaked at count 1. Brix and acid part of internal quality was good, Shani SL had a high Brix: acid ratio; Brix of 12.7° and acid of 1.11% towards peak maturity at an 11.5 ratio. Juice percentage 50.6% was not good. The fruit was fully coloured before peak maturity.

Winola

Winola is a late maturing mandarin selection. It was the last selection to reach peak maturity. The fruit had a very good colour development (T1) before peak maturity. Winola had good Brix (13.7°) with acids (1.15%). The selection had 0.1 seeds per fruit on average. Fruit size count ranged 1 – 1xxx.

RHM

RHM was second to reach peak maturity at the trial site. The fruit size count range from small – medium (count 4 – 1). Acid drop quickly but stay stable around 0.80% at peak maturity and with the good sugars it contributed to good flavour and eating quality. The external colour development was delayed with T6 on the colour plate when the fruit was at peak maturity. The selection has good Brix° at 11.3° and acid % of 0.88%. Juice percentage for RHM was low 45.9%. There were seed for this selection during evaluations with a count of 1.9 seeds per fruit. The fruit is firm with a smooth rind and a deep orange internal colour. Some of the fruit tends to split.

Conclusion

Gold Nugget, Furr, Nadorcott LS, Shani SL, Tami 2/65, Sirio, Winola and Etna had the largest fruit size (1xx - 1xxx). Samba, Tango and RHM had the smallest fruit size with a count 4 – 3. Furr had the most seeds per fruit on average (3.4 seeds per fruit) followed by RHM & Samba (1.9 seeds per fruit). Gold Nugget, Nadorcott ARC & LS, Tango and Tami 2/65 were the only selections which were completely seedless. None of the selections had a juice percentage over 55% at peak maturity. All the selections that were a T1 on the colour plate range

(good colour development) before or at peak maturity was IRM 2, Or 4, Furr, Nadorcott, ARC Nadorcott, Nadorcott LS, Shani SL, Etna, Samba, Sirio and Winola.

Table 5.4.17.3. Internal fruit quality data for experimental mandarin hybrid selections from the Citrusdal region of the Western Cape during the 2018 season.

Date	Cultivar	Rootstock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2018-07-11	Gold Nugget	CC	1x - 1xx	49,9	12,4	1,06	11,7	0,0	T 2
2018-08-01	Gold Nugget	CC	1x - 1xx	44,5	13,3	0,94	14,1	0,0	T 2
2018-06-22	IRM 1	CC	1x	54,1	13,5	1,11	12,2	0,4	T 2
2018-07-11	IRM 1	CC	2 - 1x	55,0	14,1	1,01	14,0	0,3	T 2
2018-06-06	IRM 2	CC	2 - 1x	32,6	11,4	1,15	9,9	0,5	T2 - T3
2018-06-22	IRM 2	CC	1x	39,7	12,6	1,12	11,2	0,5	T 1
2018-07-11	IRM 2	CC	1 - 1xx	49,5	12,6	0,91	13,9	0,3	T 2
2018-06-22	Mor 26	CC	1	42,0	12,5	1,01	12,4	0,7	T 2
2018-07-11	Mor 26	CC	2 - 1x	43,9	14,0	0,90	15,6	0,5	T 2
2018-06-06	Furr	CC	1 - 1xx	45,5	12,7	1,20	10,6	3,4	T1
2018-07-11	Furr	CC	1xx - 1xxx	51,6	13,1	0,90	14,6	3,0	T 1
2018-07-11	Nadorcott	CC	2 - 1x	36,4	12,8	1,49	8,6	0,0	T 1
2018-08-01	Nadorcott	CC	1 - 1x	43,3	13,1	1,25	10,5	0,3	T 1
2018-09-03	Nadorcott	CC	2 - 1x	46,9	13,2	1,03	12,8	0,0	T 1
2018-07-11	Nadorcott ARC	CC	2 - 1x	49,7	12,0	1,08	11,2	0,0	T 1
2018-08-01	Nadorcott ARC	CC	2 - 1x	46,8	13,0	1,04	12,5	0,0	T 1
2018-09-03	Nadorcott ARC	CC	3 - 1	47,8	12,5	0,81	15,4	0,0	T 1
2018-06-22	Nadorcott SL	CC	2	43,2	12,7	1,50	8,5	0,0	T2 - T3
2018-07-11	Nadorcott SL	CC	2 - 1xx	50,5	12,8	1,09	11,8	0,0	T 1
2018-08-01	Nadorcott SL	CC	2 - 1x	46,1	13,0	1,06	12,2	0,1	T 1
2018-06-22	Or 4	CC	1x	46,9	13,0	1,05	12,4	0,5	T1 - T2
2018-04-04	RHM	CC	3 - 4	40,9	10,8	1,02	10,5	1,9	T 8
2018-04-30	RHM	CC	4 - 1	45,9	11,3	0,88	12,8	1,0	T 6
2018-05-25	RHM	CC	2 - 1	56,6	12,3	0,76	16,3	1,5	T 3
2018-06-22	Shani SL	CC	1x	52,6	13,1	1,54	8,5	0,0	T1 - T2
2018-07-11	Shani SL	CC	1xx - 1xxx	50,6	12,7	1,11	11,5	0,3	T 1
2018-08-01	Shani SL	CC	1x - 1xxx	40,4	13,5	1,06	12,8	0,6	T 1
2018-04-04	Tami 2/65	CC	1xx - 2	54,2	9,3	1,04	9,0	0,0	T 6
2018-04-24	Tami 2/65	CC	1 - 1x	56,9	10,1	0,75	13,5	0,0	T5 - T6
2018-06-22	Tango	CC	3 - 1	46,3	11,7	1,08	10,9	0,0	T2 - T4
2018-07-11	Tango	CC	2 - 1	52,5	12,0	0,91	13,2	0,0	T 1
2018-04-23	Etna	CC	1 - 1xx	56,6	8,5	1,04	8,2	0,8	T6 - T7
2018-06-06	Etna	CC	1 - 1xx	53,4	9,3	0,76	12,3	1,3	T 1
2018-04-23	Samba	CC	4 - 3	54,1	10,2	1,22	8,4	1,9	T 6
2018-06-06	Samba	CC	3 - 2	50,6	11,9	1,00	11,9	0,2	T 1
2018-06-21	Samba	CC	4 - 2	39,3	13,1	1,05	12,5	0,3	T 1

2018-07-11	Samba	CC	4 - 2	36,8	13,9	0,89	15,6	0,5	T 1
2018-07-11	Winola	CC	1x - 1xx	51,1	13,0	1,46	8,9	0,1	T 1
2018-08-01	Winola	CC	1x - 1xxx	44,9	13,2	1,16	11,4	0,0	T 1
2018-09-03	Winola	CC	1 - 1xxx	48,6	13,7	1,15	11,9	0,0	T 1

Table 5.4.17.4. Internal fruit quality data for experimental mandarin hybrid selections from the Paarl region (Babylonstoren) of the Western Cape during the 2018 season.

Date	Cultivar	Rootstock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2018-05-02	Sirio	CC	1 - 1xx	51,2	9,6	1,06	9,0	0,3	T2 - T4
2018-06-05	Sirio	CC	1x - 1xxx	38,4	10,7	0,86	12,4	0,9	T 1
2018-06-20	Sirio	CC	1xx - 1xxx	41,6	11,0	0,85	13,0	0,0	T 1

5.4.18 PROGRESS REPORT: Cultivar characteristics and climatic suitability of mandarin hybrids in a cold production region (South West Cape)
Project 997E by W. Swiegers (CRI)

Summary

This is a new trial site in South West Cape. The trial trees had their second crop during the 2018 season. It's a variety block with a selection of all the new experimental cultivars from early maturing to late maturing selections. South West Cape is well suited for soft citrus. There is cross pollination in this block due to all the different selections that are present. A new site is going to be added to this site to cover more of the new selections. The order of ripening was as follow Goldup, RHM, Tami 2/65, Edit x Nova, Or 4, Taylor Lee LS, Shani SL, Mor 26, IRM 2 and the season ended with IRM 1.

Opsomming

Dit is 'n nuwe proef perseël in die Suid Wes Kaap. 2018 was die 2de seisoen met vrugte op die bome. Die meeste van die nuwe eksperimentele seleksies van vroeg tot laat rywordend kom in die perseël voor. Die Suid Wes Kaap is goed geskik vir sagte sitrus verbouing. Die kruisbestuiwing in hierdie proef perseël is hoog weens al die verskillende seleksies teenwoordig. Daar gaan nog 'n perseël bykom wat ook van die nuutste seleksies sal bevat. Die orde van rywording was as volg gewees: Goldup het die seisoen begin, gevolg deur RHM, Tami 2/65, Edit x Nova, Or 4, Taylor Lee LS, Shani SL, Mor 26, IRM 2 en IRM 1.

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 Buffeljagsrivier region of the South West Cape. The following selections were evaluated: Goldup, RHM, Tami 2/65, Edit x Nova, Or 4, Taylor Lee LS, Shani SL, Mor 26, IRM 2 and IRM 1.

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 5.4.18.1 List of experimental mandarin hybrid selections evaluated in the Buffeljagsrivier region of the South West Cape during the 2018 season.

Selection	Rootstock	Topwork
Edit x Nova	CC	2014
IRM 1	CC	2014
IRM 2	CC	2014
Mor 26	CC	2014
Shani SL	CC	2014
Tami 2/65	CC	2014
Taylor Lee LS	CC	2014
Goldup	CC	2014
Or 4	CC	2014
RHM	CC	2014

Results and discussion

Tami 2/65

Tami 2/65 is an early maturing experimental mandarin. Fruit size for Tami 2/65 was large – extra-large with fruit size count 1 – 1xxx. Internal juice percentage at peak maturity was just below (55%) lowered compared to last season. Rind is smooth and the colour is a deep orange. The fruit peels easy. The selection was seedless during the evaluations. Rind colour development was delayed with T4 on the colour plate at peak maturity. The selection doesn't have a high acid to start with and it drops quickly.

Edit x Nova

Edit x Nova is an early to mid-maturing experimental mandarin hybrid. Edit x Nova had very good internal quality. At peak maturity: Juice percentage was 58%; Brix° was 12° and acid percentage was 1.03%. This gave Edit x Nova its good flavour. The seed count for Edit x Nova was 0 seeds per fruit. Edit x Nova colour ranged between T2 – T1 on the colour plate at peak maturity. Fruit size for Edit x Nova was also favourable with 2 – 1xx count.

IRM 1

The IRM 1 is a late maturing experimental mandarin. The fruit size count for IRM 1 was very good ranged (count 2 – 1xx). Internally the juice content decrease towards peak maturity to below 45%. Brix: Acid ratio was very good at ratio 11.4; Brix was high 14.5° and acid was good 1.27%. Seed count peaked at 0.3 seeds per fruit. IRM 1 was a T1 on the colour plate at peak maturity. The rind was smooth and peelability easy.

IRM 2

IRM 2 is a mid to late maturing experimental mandarin hybrid. The fruit on the trees peaked at count 1xx. IRM 2 have firm fruit and the juice percentage was 52.5% at peak maturity. IRM 2 had a great taste and flavour at peak maturity with very high (Brix 15.5° and acid 1.34%). Seed for IRM 2 was 0.9 – 2.7 seeds per fruit. Long before peak maturity was reached IRM 2 already had a T1 colour on the colour plate.

Mor 26

Mor 26 fruit had a small - large size count, with (count 4 - 1). Towards peak maturity Mor 26 juice percentage

was 53.2%. Mor 26 internal quality was one of the best between all the selections. Very high sugars and good acids. Towards peak maturity with ratio 11.9 the Brix was already at 16.7° and acid 1.41%. Average seed count for this selection peaked at 1.1 seeds per fruit. Rind colour development was good to reach T2 – T1 colour on the colour plate towards peak maturity.

Taylor Lee LS

Taylor Lee LS is a mid to late maturing experimental mandarin hybrid. The trees bore large fruit on the trees with count 1 – 1x, and towards peak maturity the juice content was 41.4%. Brix: Acid ratio at 11.4 the selection had Brix 12.3° and acid of 1.08%. The selection seed counts were 0.3 seeds per fruit. Taylor Lee LS reached T2 colour on the colour plate before peak maturity.

Shani SL

Average seed count for Shani SL ranged between 0.0 – 0.8 seeds per fruit. Shani SL had a good Brix° and acid ratio towards peak maturity (ratio 10.3), the Brix was 12.1° and acid was 1.17%. The juice percentage for Shani SL was low towards peak maturity 39.8%, and the fruit was fully coloured up before peak maturity.

RHM

The fruit size count range from small – large (count 4 – 1). Acid drop quickly but stay stable around 0.80% at peak maturity. The external colour development was delayed with T5 - T6 on the colour plate when the fruit was at peak maturity. The selection had low Brix° at 9.7° and acid % of 0.85%. Juice percentage for RHM was low 46.3%. There were seeds in this selection during evaluations with a count of 1 seed per fruit. The fruit is firm with a smooth rind and a deep orange internal colour.

Or 4

Or is a late maturing mandarin hybrid. It reached peak maturity early this season. The size count for this selection was on the smaller side count 4 – 2. Fruit is round to oblate. Juice percentage increase towards peak maturity to just above 55%. Internal quality for Or is very good. Brix is high above 12.8° and the acids was still above 1.00% even when the fruit was over mature. This good Brix: Acid ratio will contribute to excellent eating fruit with great flavour and shelf life. Peelability is easy and oily. Or reached T2 on the colour plate towards peak maturity. Average seed count was 0.0 seeds per fruit.

Goldup

Goldup is an early maturing experimental mandarin hybrid. It reached peak maturity first in the trial block. Ratio of 12.7 was reached on 26 April. Internal quality was not good. Juice percentage was 43.8%, with 8.7 Brix and acid % of 0.69%. The trees are still very young and the internal quality may improve as the trees get older. Colour development was delayed at peak maturity of T4 – T6 on the colour plate. Fruit size ranged between 2 – 1x. Goldup was seedless during evaluations. Fruit shape are flat to oblate, fruit have a natural shine on them. Peelability is easy and the rind have a prominent aroma when it gets peeled. Rind oil is high.

Conclusion

Tami 2/65 had the largest fruit size peaking count (1xxx) and Mor 26, Or 4 had the smallest fruit size count (4). IRM 2 had the most seeds per fruit on average (2.7). Edit x Nova, Goldup, Or 4 and Tami 2/65 were the only selections that were seedless. Edit x Nova had the highest juice percentage of 58% at peak maturity. Most of the selections had a low juice % at peak maturity. Edit x Nova, IRM 1, IRM 2 and Shani SL were a T 1 on the colour plate before or at peak maturity. Selections that also had Brix above 14° towards peak maturity and at peak maturity were IRM 1 & 2, Mor 26, and Or 4.

Table 5.4.18.2. Internal fruit quality data for experimental mandarin hybrid selections from the Buffeljagsrivier region of the Western Cape during the 2018 season.

Date	Cultivar	Rootstock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2018-04-26	Edit x Nova	CC	2 - 1	51,3	10,6	1,17	9,1	0,0	T5 - T6

2018-05-21	Edit x Nova	CC	2 - 1x	58,0	12,0	1,03	11,7	0,0	T2
2018-06-19	Edit x Nova	CC	1x - 1xx	47,1	12,4	0,99	12,5	0,0	T 1
2018-04-06	Gold Up	CC	2 - 4	50,4	8,5	0,85	10,0	0,0	T6 - T7
2018-04-26	Gold Up	CC	2 - 1x	43,8	8,7	0,69	12,7	0,0	T4 - T6
2018-05-21	Gold Up	CC	2 - 1x	48,0	9,0	0,56	16,0	0,0	T1
2018-08-03	IRM 1	CC	1 - 1xx	53,0	14,2	1,33	10,7	0,3	T 2
2018-09-04	IRM 1	CC	2 - 1xx	42,4	14,5	1,27	11,4	0,0	T 1
2018-07-09	IRM 2	CC	2 - 1xx	41,8	13,6	1,52	8,9	0,9	T 1
2018-08-03	IRM 2	CC	2 - 1	52,5	15,5	1,34	11,5	2,7	T1
2018-07-09	Mor 26	CC	2 - 1	43,2	14,6	1,52	9,6	0,6	T 3
2018-08-03	Mor 26	CC	3 - 1	53,2	16,7	1,41	11,9	1,1	T 2
2018-09-04	Mor 26	CC	4 - 1	42,7	16,4	1,25	13,1	0,4	T 1
2018-06-19	Or 4	CC	4 - 2	46,6	12,8	1,08	11,9	0,0	T 2
2018-07-09	Or 4	CC	3 - 2	57,7	14,1	1,10	12,8	0,0	T 3
2018-04-06	RHM	CC	3 - 4	56,5	9,2	1,04	8,8	1,0	T 7
2018-04-26	RHM	CC	3 - 1	46,3	9,7	0,85	11,5	0,3	T5 - T6
2018-06-19	Shani SL	CC	2	36,5	11,3	1,16	9,7	0,0	T 4
2018-07-09	Shani SL	CC	2 - 1x	39,8	12,1	1,17	10,3	0,0	T1 - T2
2018-08-03	Shani SL	CC	3 - 1	49,6	13,4	1,02	13,1	0,8	T 1
2018-04-26	Tami 2/65	CC	1 - 1xx	52,1	9,0	0,96	9,3	0,0	T5 - T8
2018-05-21	Tami 2/65	CC	1x - 1xxx	54,5	9,3	0,73	12,8	0,0	T4
2018-06-19	Taylor Lee	CC	1 - 1x	41,4	12,3	1,08	11,4	0,3	T 2

5.4.19 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

There are 3 trial sites in the Sundays River Valley. They are all between Kirkwood and Addo. The trial site at Endulini is a new site with early to late maturing navel selections that had their first crop on them. For this site the season started with: LF Early, Lina, Trosky Early, Cara Cara, Glen Ora Late, Painter Early, Washington, Letaba Early, Kirkwood Red, Gloudi, Suitangi, Clark, Lane Late, HE Late, Autumn Gold, Navelina, Hutton, KS Late, and the season ended with Witkrans and Carninka Late. The other trial site at Arundel is a late maturing trial and the season kicked off with Hutton, followed with Clark, Chislett Summer, Cambria, Autumn Gold, Witkrans, Summer Gold, Rhode and the season finished with Barnfield Summer. The third site at Invercloy also have an early to late maturing selection. The season began with Fischer, DeWet 1, Fukumoto, Palmer, Lazyboy and the last selection to reach peak maturity was Newhall.

Opsomming

Daar is 3 proef persele in die Sondagsrivier vallei. Hulle is tussen Kirkwood and Addo gelee. Die nuwe proef perseel by Endulini het hulle eerste drag opgehad in die (2018) seisoen. Die proef bevat die meeste vroeë tot laat rypwordende nawel seleksies. Die seisoen het begin met: LF Early, Lina, Trosky Early, Cara, Glen Ora Late, Painter Early, Washington, Letaba Early, Kirkwood Red, Gloudi, Suitangi, Clark, Lane Late, HE Late, Autumn Gold, Navelina, Hutton, KS Late, en klaar gemaak met Witkrans en Carninka Late. Die ander proef perseel is by Arundel en dit is net laat rypwordende seleksies. Daar het die seisoen begin met Hutton, gevolg deur Clark, Chislett Summer, Cambria, Autumn Gold, Witkrans, Summer Gold, Rhode en Barnfield Summer het die seisoen afgesluit. Die derde proef perseel is by Invercloy en die perseel bevat ook vroeë tot laat

rypwordende seleksies. Die seisoen het daar afgeskop met Fischer, DeWet 1, Fukumoto, Palmer, Lazyboy en klaargemaak met Newhall.

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: LF Early, Lina, Trosky Early, Cara Cara, Glen Ora Late, Painter Early, Washington, Letaba Early, Kirkwood Red, Gloudi, Suitangi, Clark, Lane Late, HE Late, Navelina, Hutton, KS Late, Witkrans, Hutton, Chislett Summer, Cambria, Autumn Gold, Summer Gold, Rhode, Barnfield Summer, Fischer, DeWet 1, Fukumoto, Palmer, Lazyboy, Newhall and Carninka Late.

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 5.4.19.1. List of navel selections evaluated at Sundays River Valley (Endulini) during 2018.

Selection	Rootstock	Planted
Autumn Gold	CC	2015
Lane Late	CC	2015
Cara Cara	CC	2015
Carninka Late	CC	2015
Clark	CC	2015
Glen Ora Late	CC	2015
Gloudi	CC	2015
HE Late	CC	2015
Hutton	CC	2015
Kirkwood Red	CC	2015
KS Late	CC	2015
Letaba Early	CC	2015
LF Early	CC	2015
Lina	CC	2015
Navelina	CC	2015
Painter Early	CC	2015
Suitangi	CC	2015
Trosky Early	CC	2015
Washington	CC	2015
Witkrans	CC	2015

Table 5.4.19.2. List of navel selections evaluated at Sundays River Valley (Arundel) during 2018.

Selection	Rootstock	Planted
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Hutton	CC	1997
Clark	CC	1997
Chislett Summer	CC	1997
Cambria	CC	1997
Autumn Gold	CC	1997
Witkrans	CC	1997
Summer Gold	CC	1997
Rhode	CC	1997
Barnfield Summer	CC	1997

Table 5.4.19.3. List of navel selections evaluated at Sundays River Valley (Invercloy) during 2018.

Selection	Rootstock	Topworked
De Wet 1	CC	2012
Fischer	CC	2012
Fukumoto	CC	2012
Lazyboy	CC	2012
Newhall	CC	2012
Palmer	CC	2012

Results and discussion

Fukumoto

Fukumoto was one of the selection with the highest Brix for this trial site (above 11°). Fukumoto also had very good acid levels at peak maturity. The good Brix: Acid ratio give Fukumoto its good flavour. The colour development was very good, with colour plate T1 at peak maturity. The selection acids were stable and the fruit hanged well. Fukumoto produced a good fruit size which peaked at count 56 a favorable size for navel production and export. The navel-end on the fruit was fairly open and protruding, one of the characteristics of the selection. The juice percentage of Fukumoto was below 50% at peak maturity. Fukumoto was third selection to reach peak maturity at this trial site.

Lina

Lina was the second to reach peak maturity at this trial site. The selection had a delayed colour development with a colour plate T6 when it was over mature. The selection had a very good fruit size and peaked at count 56. The fruit shape was more elongated with a large navel-end (fairly open). Lina developed a juice content of (52.5%). Lina had good flavour.

Newhall

Newhall was the last selection to reach peak maturity in this trial site. The fruit size peaked ranged between 48 - 56. Newhall had a very good colour development (colour plate T1) before peak maturity. The selection's juice percentage was good around 55% at peak maturity. Internal quality was good.

Autumn Gold

The external colour development of the selection was good (colour plate T1) at the Arundel. At the Endulini site the colour development was slightly delayed, it could be that the trees had their first crop. The selection bore favorable fruit size fruit and peaked at count 56. Autumn Gold had a high juice percentage of 55.2% at Endulini site. Internal quality was very good on the older Autumn Gold trees. The navel ends were small.

Barnfield Summer

Barnfield Summer is a late maturing navel, and it was the last selection to reach peak maturity at Arundel. Barnfield Summer had a very good and preferred fruit size (count 56) for export. The juice percentages were just below 55% for this selection. Barnfield Summer have a very good external colour development, being a T1 at ratio 9.7. The acid remained good until peak maturity and it was supported with high Brix of 12.8. The navel end was small.

Glen Ora Late

The fruit size count of Glen Ora Late was good with a 48 count. The juice percentage for this selection was 53.7%. The external colour development of Glen Ora late was delayed with a T6 on the colour plate. When the selection reached peak maturity (ratio 10.4) the Brix levels were 10.3° with high acids (0.99%), indicating that the fruit can hang slightly longer on the trees. The rind is smooth to slightly coarse with small to closed navel end.

Lane Late

Lane Late is the control for the late maturing selections. Lane Late fruit size count were 56, the preferred size for export. Lane Late had very good juice percentage above (55%) at peak maturity. The external colour development was also delayed on Lane Late, (T5) by peak maturity. Lane Late also kept its acids quite well (good shelf life). The flavour was good. Lane Late had small protruding navel-end on the fruit.

Chislett

Chislett fruit size peaked at count 56. Chislett were the third selection from the late navel selections in this trial to reach peak maturity with a delayed external colour development (T3). The juice percentage of Chislett was a low 44.1% at peak maturity. The low juice % was followed up by good Brix 11.8 and acids of 1.13% at peak maturity.

Clarke

Clarke's fruit size count was 56. The young trees at Endulini had a slightly higher juice % at peak maturity 56.8%, but the Brix and acid % was lower. Internal quality was good on the older trees having a Brix of 12.8 and acid of 1,29%. The external colour development of Clarke at Endulini was delayed compared to the internal quality. The external colour was T6 on the colour plate. On the older trees external colour development had no problem reaching T1 at peak maturity.

Gloudi

The juice percentage of Gloudi was still good when the fruit was over mature, being 54.7%. Gloudi had a large fruit size count peaking at 48 count. With a ratio of 11.5 which is considered over mature, internal quality was still good Brix 11 and acid % 0,96%. Gloudi's acid levels remained high (good shelf life). Colour development was delayed being T5 on the colour plate. Gloudi had a close to small navel end.

Witkrans 3

Witkrans is a very promising late maturing navel. It was the second last selection to reach peak maturity in the Endulini trial site. The external colour development was good with a T1 on the colour plate at peak maturity. The fruit size count for Witkrans was good peaking at a count of 56 great for packing and export. The juice percentage for Witkrans was high and very good, above 55% and acids were still around 1% at peak maturity. Brix was also high and along with the acid give Witkrans great flavour. It has a close to small navel end.

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 Sundays 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 slight delayed in colour development being at colour plate T3 at peak maturity. The juice was low just below 50% at peak maturity and Brix was 12.4° with acids above 1.19%.

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 fair juice content of just over 50%. At peak maturity, the external colour peaked at colour plate T6. The Brix remained around 10° and acid % around 1 %.

Caloma

Caloma bore fruit with a large fruit size and peaked at count 56. The external colour development of Caloma was slightly delayed with a T3 on the colour plate at peak maturity. Acids kept quite well unfortunately the Brix was low. Juice content was just below 55% at peak maturity. Flavour was good. Crop looked good.

Washington

The external colour development was behind the internal quality of the fruit (T4) on the colour plate standards. Washington fruit size count peaked at count 56. The juice content of Washington tested around 50.8%. High Brix levels above 10.7° with acids of 1.05% assured good tasting fruit with good flavour.

Fischer (control)

The acid levels on Fischer navel were good, averaging around 1.0% for the season. Juice and Brix at peak maturity were 50.2% and 10.4 respectively. Externally the fruit colour development was delayed, and peaked from T6 to T5. Fruit size was optimum for navel production, large fruit size (count 48 to 56). Very good crop on the trees.

Hutton

Hutton had the preferred fruit size for navel production and export (count 56). Again the juice % was lower on the older trees, below 50% compared to younger trees, above 50% at peak maturity. Navel ends were open and the rind was coarse. Peak maturity was reached in June. On both sites the Brix was around 10 and acid around 1%, with T4 colour on the colour plate at peak maturity.

Palmer

The external colour development of the selection was delayed (colour plate T6) towards peak maturity. When the ratio was 11.6, T1 on the colour plate was achieved. The selection had a good fruit size and peaked at count 56. The acids dropped slowly, indicating that the selection can hang on the trees for slightly longer periods. This selection's juice percentage towards peak maturity was just below 50% but it increased towards peak maturity, Brix was around 10 with acid of 1%.

Lazyboy

Lazyboy has round fruit on the trees with good internal quality. The tree had a round "bushy" shape and bore most of the fruit on the outside of the tree. The fruit remained firm and hung on the tree for an extended period. In the 2018 season the selection had low juice percentages, below 50%, Brix of 11.5° and good acids (1.01%). Lazyboy had slightly bigger fruit, fruit size count 48.

Suitangi

Suitangi was one of the late maturing navel selections evaluated, the external colour development was slightly delayed, T3 on the standard colour plate. The selection normally has deep orange rind colour and it had a fairly small navel end. There was a fair - good crop on the trees this season. Suitangi peaked at count 64. Internally, Suitangi produced good quality with high juice content; every sample tested was over 54% juice. High Brix levels of over 10.5 with acids of 0.95%, assured good tasting fruit with good flavour.

Cara Cara

Cara Cara is the control for pigmented navels. Internal quality was good with juice % of 51.2%, Brix 10.1 and acid % at 0.96% at a Brix: Acid ratio of 10.5 (peak maturity). The external appearance was delayed at T5 on the colour plate. Fruit size is uniform medium large, (count 56). Navel ends are small. Fruit shape is round and rinds are smooth. Internal flesh colour is an intermediate red, and flavour is very good.

Carninka Late

Carninka late is a late maturing experimental navel. It was also the last selection to reach peak maturity at the Endulini trial site. Peak maturity was reached early this season mid-July; the trees are still young. Carninka late had a good fruit size, count 56. It also had good internal fruit quality at peak maturity, high juice % (57%), good Brix 11.9 and good acid % (1.13%). Fruit is firm and has a smooth peel. Colour development was very good reaching T1 on the colour plate at peak maturity. Fruit can hang on the trees for longer periods. Navel

end is close to small and flavour very good.

HE Late

HE Late is an experimental late maturing navel. Peak maturity on the young trees was reached end of June. It reached the preferred fruit size peaking at count 56 (medium large fruit). HE Late internal quality was very good especially keeping in mind it was the first crop. Juice percentage was above 55% and the Brix around 11 with an acid % of around 1%. Future evaluations will determine the exact maturity period and if the internal quality will stay good. With the acid % that stayed stable around 1% the fruit can hang on the tree to colour up to a T1 on the colour plate.

Kirkwood Red

Kirkwood red is a red pigmented navel. The fruit sometimes has external blush and internal colour pigment dark red in. The colour development was delayed being T4 on colour plate at peak maturity. The variety mature slightly later than Cara Cara navel. Kirkwood Red fruit have relatively high juice content 56.7%. At peak maturity the Brix was 10.1 and acid % of 1.02%. The fruit is small with closed end navel and fruit sized peaked at count 48. The vascular bundles in the leaf and fruit stem are clearly red in colour and can also be found in bark and twigs. The tree is compact compared to Cara Cara tree. Fruit shape is round to slightly oval with small navel-end.

Letaba Early

It was the first crop on the trees for this selection; future evaluations will give us more info about this experimental variety. Peak maturity was reached mid-June. Fruit size was medium to medium large and the counts ranged between (count 64 – 56). At peak maturity (ratio 10) the internal quality was as follows: juice percentage (52.9%), Brix 10 and acid % (1%). Colour development was good reaching T2 on the colour plate at peak maturity.

LF Early

LF Early are an early maturing experimental navel. LF Early was the first selection to reach peak maturity at this new trial site. Future evaluations will give us a better indication how early the selections are. Fruit size was very good with count 56. On 08.05.18 the ratio was 12.8. Juice percentage was 50.1%, the Brix was low (9.2) and as expected when the fruit is over mature the acid % was a bit low. Colour development was delayed at T6 on the colour plate.

Navelina

Navelina is an earlier maturing navel than Washington with very good internal quality and external appearance. In this trial site Navelina reached peak maturity after Washington. It could be that the trees had their first crop and are still young. The tree is not as vigorous as the Washington. Fruit are medium to large (counts 56) with a very small to closed navel. The fruit colours earlier than Washington, with T1 at peak maturity. Fruit shape is slightly elongated, and rinds are smooth. The fruit rind develops a deep uniform orange colour at maturity. The flavour is very good with high sugars, acid levels and a ratio of 9.8:1

Painter Early 2

A slightly earlier maturing navel, a week or two earlier than Palmer and Washington with good internal quality and external appearance. Maturity was a bit out of order due to the age of the trees and colour development were also delayed at T4 on the colour plate. Fruit size is a uniform medium to large, count 56. Navel ends are small. Fruit shape is round and rinds are smooth. Internal flesh colour is orange. The flavour is good with moderate juice percentage.

Rhode

Rhode was the second last selection to reach peak maturity in this trial site. Rhode had a very good fruit size count 56. Colour break was early. Toward peak maturity external colour was T3 and at peak maturity colour was T1 on the colour plate. Juice percentage was a fair 50%. Brix and acid percentage at peak maturity was moderate.

Summer Gold

Summer Gold is a mid/late late maturing navel. Summer Gold has good internal quality and external appearance at peak maturity. The good internal quality tends to drop quickly after peak maturity is reached. At ratio 9.1 the internal quality was as follow: Juice 51.2%, Brix 11.5 and acid 1.27%, but at ratio 12.1: Juice was 48.8%, Brix 9.2% and acid 0.76%. The tree is similar in shape navel selections, but vigour is less. Fruit size is a uniform medium to large at count 56. Navel ends are small. Fruit shape is round. The fruit rind develops a deep orange colour at 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 Chislett Summer, Glen Ora Late, Gloudi and Lazyboy peaking at count 48. All of the selections had a T1 on the colour plate except the following selections: Chislett Summer, Hutton, Cara Cara, Gloudi, Kirkwood Red, Letaba Early, LF Early, Lina, Painter Early, Suitangi, De Wet 1 and Lazyboy. The following selections had juice percentage above 55%: Witkrans 3, Autumn Gold, Lane Late, Carninka Late, Clarke, Glen Ora Late, HE Late, Kirkwood Red, Navelina and Newhall. Autumn Gold, Barnfield Summer, Cambria, Clarke and Witkrans 3 had the highest Brix above 12.0° for this trial at peak maturity. All the navel selections were seedless.

Table 5.4.19.2. Internal fruit quality data for early to late Navel selections from the Addo (Arundel) region of the Sundays River Valley during the 2018 season.

Date	Selection	Root stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Colour	Avg Seed
2018-06-14	Autumn Gold	CC	56	52,2	12,0	1,40	8,6	T2	0,0
2018-06-27	Autumn Gold	CC	56	51,8	12,4	1,27	9,8	T1	0,0
2018-07-12	Barnfield Summer	CC	56	54,8	12,8	1,32	9,7	T1	0,0
2018-06-27	Cambria 3	CC	64	48,0	12,4	1,19	10,4	T3	0,0
2018-07-12	Cambria 3	CC	56	50,8	13,0	1,22	10,7	T1	0,0
2018-06-14	Chislett Summer	CC	48	47,7	11,0	1,26	8,7	T4	0,0
2018-06-27	Chislett Summer	CC	48	44,1	11,8	1,13	10,4	T3	0,0
2018-06-14	Clark	CC	56	53,3	12,8	1,29	9,9	T1	0,0
2018-07-12	Clark	CC	64	53,8	13,3	1,21	11,0	T1	0,0
2018-06-14	Hutton	CC	56	49,4	10,5	1,03	10,2	T4	0,0
2018-06-27	Rhode	CC	56	52,8	11,5	1,26	9,1	T3	0,0
2018-07-12	Rhode	CC	56	50,1	10,5	0,97	10,8	T1	0,0
2018-06-27	Summer Gold	CC	56	51,2	11,5	1,27	9,1	T2	0,0
2018-07-12	Summer Gold	CC	56	48,8	9,2	0,76	12,1	T1	0,0
2018-06-27	Witkrans 3	CC	56	52,6	13,1	1,33	9,8	T4	0,0
2018-07-12	Witkrans 3	CC	56	58,9	13,2	1,25	10,6	T1	0,0

Table 5.4.19.3. Internal fruit quality data for early to late Navel selections from the Addo/Kirkwood (Endulini) region of the Sundays River Valley during the 2018 season.

Date	Selection	Root stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Colour	Avg Seed
2018-06-14	Autumn Gold	CC	56	53,4	10,0	1,05	9,5	T6	0,0
2018-06-27	Autumn Gold	CC	56	55,2	9,3	0,95	9,8	T4	0,0
2018-07-12	Autumn Gold	CC	64	56,2	11,2	0,91	12,3	T1	0,0
2018-06-14	Lane Late	CC	56	53,5	10,1	1,03	9,8	T6	0,0
2018-06-27	Lane Late	CC	56	56,3	10,5	1,03	10,2	T5	0,0
2018-07-12	Lane Late	CC	56	55,4	10,7	0,86	12,4	T1	0,0

2018-05-08	Cara Cara	CC	56	50,4	8,7	1,05	8,3	T8	0,0
2018-06-14	Cara Cara	CC	56	51,2	10,1	0,96	10,5	T5	0,0
2018-06-27	Cara Cara	CC	56	51,9	10,4	0,88	11,8	T2	0,0
2018-06-14	Carninka Late	CC	56	56,0	11,0	1,16	9,5	T2	0,0
2018-07-12	Carninka Late	CC	56	57,0	11,9	1,13	10,5	T1	0,0
2018-06-14	Clark	CC	56	56,8	9,9	1,01	9,8	T6	0,0
2018-06-27	Clark	CC	56	56,9	10,3	1,00	10,3	T4	0,0
2018-07-12	Clark	CC	56	57,0	11,4	0,97	11,8	T1	0,0
2018-06-14	Glen Ora Late	CC	48	53,7	10,3	0,99	10,4	T6	0,0
2018-07-12	Glen Ora Late	CC	48	56,5	11,4	1,00	11,4	T1	0,0
2018-06-27	Gloudi	CC	48	54,7	11,0	0,96	11,5	T5	0,0
2018-06-27	HE Late	CC	56	56,7	10,8	1,08	10,0	T5	0,0
2018-07-12	HE Late	CC	56	52,1	11,5	1,11	10,4	T1	0,0
2018-07-25	HE Late	CC	56	57,5	11,9	1,02	11,7	T2	0,0
2018-06-14	Hutton	CC	56	54,7	10,0	1,06	9,4	T6	0,0
2018-06-27	Hutton	CC	56	53,6	10,3	1,06	9,7	T4	0,0
2018-07-12	Hutton	CC	56	55,1	11,3	0,93	12,2	T1	0,0
2018-05-08	Kirkwood Red	CC	56	52,5	9,2	0,98	9,4	T7	0,0
2018-06-14	Kirkwood Red	CC	48	56,7	10,1	1,02	9,9	T4	0,0
2018-06-27	Kirkwood Red	CC	56	56,0	10,6	0,99	10,7	T2	0,0
2018-06-14	Caloma	CC	56	53,8	9,9	1,13	8,8	T5	0,0
2018-06-27	Caloma	CC	56	54,8	9,7	1,01	9,6	T3	0,0
2018-07-12	Caloma	CC	64	53,6	11,5	0,98	11,7	T1	0,0
2018-05-08	Letaba Early	CC	56	50,3	9,0	1,09	8,3	T6	0,0
2018-06-14	Letaba Early	CC	64	52,9	10,0	1,00	10,0	T2	0,0
2018-05-08	LF Early	CC	56	50,1	9,2	0,72	12,8	T6	0,0
2018-05-08	Lina Navel	CC	56	52,5	10,0	0,80	12,5	T6	0,0
2018-05-08	Navelina	CC	56	53,8	9,2	1,05	8,8	T7	0,0
2018-06-27	Navelina	CC	56	56,4	11,1	1,13	9,8	T1	0,0
2018-05-08	Painter Early 2	CC	56	52,8	9,2	1,03	8,9	T7	0,0
2018-06-14	Painter Early 2	CC	56	49,7	11,2	1,08	10,4	T4	0,0
2018-06-27	Suitangi	CC	64	54,4	10,5	0,95	11,1	T3	0,0
2018-05-11	Trosky Early	CC	56	50,2	8,7	1,06	8,2	T7	0,0
2018-05-08	Trosky Early	CC	56	49,6	10,7	1,03	10,4	T5	0,0
2018-06-14	Trosky Early	CC	56	53,2	12,7	1,03	12,3	T1	0,0
2018-05-08	Washington	CC	56	53,0	9,1	0,97	9,4	T7	0,0
2018-06-14	Washington	CC	56	50,8	10,7	1,05	10,2	T4	0,0
2018-06-27	Washington	CC	56	53,4	11,5	0,90	12,8	T1	0,0
2018-08-07	Witkrans 3	CC	56	55,5	11,1	0,99	11,2	T1	0,0

Table 5.4.19.4. Internal fruit quality data for early to late Navel selections from the Kirkwood (Invercloy) region of the Sundays River Valley during the 2018 season.

Date	Selection	Root stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Colour	Avg Seed
2018-05-02	De Wet 1	CC	56	52,3	10,0	0,90	11,1	T6	0,0
2018-04-19	Fischer Navel	CC	48	50,2	10,4	1,01	10,3	T6	0,0

2018-05-02	Fischer Navel	CC	56	51,1	11,0	1,02	10,8	T5	0,0
2018-05-21	Fischer Navel	CC	56	54,8	11,6	0,99	11,7	T1	0,0
2018-05-02	Fukumoto	CC	56	48,2	11,7	1,08	10,8	T1	0,0
2018-06-07	Fukumoto	CC	56	50,5	12,4	0,89	13,9	T1	0,0
2018-06-07	Lazyboy	CC	48	47,3	11,5	1,01	11,4	T6	0,0
2018-05-21	Newhall	CC	56	57,8	10,9	1,19	9,2	T1	0,0
2018-06-07	Newhall	CC	48	55,7	10,8	1,06	10,2	T1	0,0
2018-05-02	Palmer Navel	CC	56	49,4	10,0	1,06	9,4	T6	0,0
2018-05-21	Palmer Navel	CC	56	51,4	11,3	0,97	11,6	T1	0,0

5.4.20 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. Washington was used as a control at the trial site. Painter Early 2 and Ryan 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 in order of ripening was as follows: Washington and De Wet 1. The late maturing selection evaluated and order of ripening consist of Lazyboy and Caloma.

Opsomming

Hierdie proef bestaan uit 'n paar eksperimentele vroeë-, middel- en laat navel seleksies. Washington is as kontrole gebruik in die proefperseël. Ryan en Painter Early 2 is die 2 vroeë seleksies wat geëvalueer was. De Wet 1 is 'n mid-rypwordende nawel met 'n ronde vrugvorm. Die vrugte het 'n toe nawel-ent. Die ander mid-rypwordende kultivar wat ge-evalueer was bestaan Washington. Die laat navel seleksie wat ge-evalueer was bestaan uit Lazyboy, Caloma en Suitangi. Die volgorde van rypwording was as volg: Ryan, Painter Early 2, Washington, De Wet 1, Lazyboy en Caloma het die seisoen afgesluit.

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: Ryan, Painter Early 2, Washington (control), De Wet 1, Lazyboy and Caloma.

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 5.4.20.1. List of navel selections evaluated at Loerie site in the Gamtoos River Valley, Eastern Cape during the 2018 season.

Selection	Rootstock	Topworked
De Wet 1	Carrizo	2012
Caloma	Carrizo	2012
Ryan	Carrizo	2013
Painter Early 2	Carrizo	2012
Washington	Carrizo	2012
Lazyboy	Carrizo	2013

Results and discussion

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 that is slightly pebbly, 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. Less sanitation is necessary due to less fruit drop. The selection had good fruit size and peaked at count 56; perfect for navel production and export. Fruit shape was round. The internal quality was moderate with juice content around 50%. At peak maturity, the external colour peaked at colour plate T6. The Brix remained around 9° and acid percentage around 0.9 %.

Caloma

Caloma bore fruit with a medium large fruit size and peaked at count 56. The fruit shape for Caloma is round and the fruit is firm. Caloma's internal quality was excellent and one of the best in the trial site. The selection had a high good percentage of 54.6%, high Brix 10.8° and a good acid around 1% at peak maturity. The external colour development of Caloma was delayed with a T5 on the colour plate at ratio 10.2. Caloma's rind is smooth and the navel ends are small to close. Flavour of Caloma is very good.

Ryan

Ryan was the first selection at peak maturity in this trial site. This selection had a good fruit size count that ranged at count 56 - 48 at peak maturity. The external colour development range was delayed T6 (colour plate). Internal quality was poor. Low juice %, low Brix and low acids.

Painter Early 2

Painter Early 2 was the second selection to mature for this navel trial. Medium size fruit were on the trees with count 64. Painter Early 2 were at T5 on the colour plate at peak maturity. The juice percentage of Painter Early 2 increase towards peak maturity to around 50%. Painter Early 2 had fair Brix and acid level at peak maturity. Fruit shape is round with smooth rind and small navel ends. The flavour for this selection is very good.

Washington

Washington was used as a control. Washington is a mid-maturing navel. The external colour development behind the internal quality of the fruit (T5) on the colour plate at peak maturity. Washington fruit size count peaked at count 56. The juice content of Washington tested around 50% juice. High Brix levels above 10.5° with acids of 1%, assured good tasting fruit with good flavour. Navel ends were medium for Washington.

Lazyboy

Lazyboy is a late maturing navel with good internal quality. Brix are 10.2 and fruit hangs well on the tree with acid percentage of 1%, and even when the fruit is over mature the acid is still above 0.8%. Fruit size is a uniform medium to medium large peaking at count 56. Navel ends are mainly small to closed with occasional fruit having more open navels. Fruit shape is round and rinds are smooth. Internal colour is orange. Externally the colour development was delayed T6 at peak maturity. The flavour is very good.

Conclusion

The fruit size of all the navel selections peaked at count 56 except Painter Early 2 that had a fruit size count of 64 at peak maturity. The Navel selection with the highest juice percentage was Caloma (54.6%). All of the selections had a delayed external colour development on the colour plate at peak maturity. Washington, Caloma and Lazyboy developed the highest Brix values for this trial.

Table 5.4.20.2. Internal fruit quality data for Experimental Navel selections from the Gamtoos River Valley region of the Eastern Cape during the 2018 season.

Date	Selection	Rootstock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Colour	Avg. Seed
2018-05-15	De Wet 1	CC	56	50,6	8,8	0,91	9,7	T6	0,0
2018-05-23	De Wet 1	CC	56	49,5	9,0	0,88	10,2	T6	0,0
2018-06-11	De Wet 1	CC	56	48,3	9,6	0,82	11,7	T1	0,0
2018-06-11	Caloma	CC	56	55,4	10,4	1,11	9,4	T5	0,0
2018-06-26	Caloma	CC	56	54,6	10,8	1,06	10,2	T5	0,0
2018-07-11	Caloma	CC	56	55,9	11,0	0,95	11,6	T1	0,0
2018-06-05	Lazyboy	CC	56	46,1	9,9	1,00	9,9	T6	0,0
2018-06-11	Lazyboy	CC	64	46,1	10,2	1,00	10,2	T6	0,0
2018-06-26	Lazyboy	CC	56	41,5	10,3	0,88	11,7	T6	0,0
2018-04-24	Painter Early 2	CC	64	48,4	9,0	0,93	9,7	T6	0,0
2018-05-07	Painter Early 2	CC	64	49,4	9,1	0,91	10,0	T5	0,0
2018-05-15	Painter Early 2	CC	64	51,4	9,2	0,80	11,5	T4	0,0
2018-04-24	Ryan	CC	56	47,5	8,3	0,95	8,3	T6	0,0
2018-05-07	Ryan	CC	56	48,6	8,8	0,74	11,9	T6	0,0
2018-05-15	Ryan	CC	48	49,6	9,6	0,78	12,3	T5	0,0
2018-05-15	Washington	CC	56	50,4	9,1	1,06	8,6	T6	0,0
2018-05-23	Washington	CC	56	50,0	10,5	1,00	10,5	T5	0,0
2018-05-30	Washington	CC	56	49,3	11,0	1,01	10,9	T2	0,0

5.4.21 PROGRESS REPORT: Cultivar characteristics and climatic suitability of experimental navel oranges in a cold production region (Western Cape)

Project 998D by W. Swiegers (CRI)

Summary

Citrusdal is probably one of the best regions to farm Navels in the country. The trial consists of most of the new selections and a few newer ones will be added. The trial consists of a few experimental early, mid and late navel selections in 2 trial sites. Fischer was used as control for the early selections, Washington was used as control for the mid maturing navel selections and Late late was the control for the late navel sections. Most of the trees are older and have big tree volumes. Rayno Early, Tibs Early, Fukumoto, Fischer and Gerhard Early started the season as the early navel selections for evaluation. The mid navel selections that were evaluated in order of ripening were as follows: Washington, Cara Cara, Kirkwood Red, Navelina and Palmer. The late selections that were evaluated and were last to reach peak maturity were Gloudi, Cambria, Glen Ora Late, Witkrans 3, Carninka and Lane Late.

Opsomming

Citrusdal is seker een van die beste streke in die land vir Navels. Die proef het die meeste van die nuwe seleksies. Daar gaan nog uitgebrei word op hulle. Hierdie spesifieke proef bestaan uit 'n paar eksperimentele vroe-, middel- en laat nawel seleksies in 2 proef persele. Fischer is as kontrole gebruik vir die vroe seleksies,

Washington word as kontrole gebruik vir die middel seleksies en Lane Late dien as kontrole vir die laat nawel seleksies. Die meeste van die bome is al ouer en die bome het 'n groot boom volume. Die orde van rypwording was as volg, beginnende met die vroeë seleksies Rayno Early, Tibs Early, Fukumoto, Fischer, Gerhard Early gevolg deur die middel seleksies se volgorde, Washington, Cara Cara, Kirkwood Red, Navelina, Palmer, en die seisoen was afgesluit met die laat seleksies se orde van rypwording Gloudi, Cambria, Glen Ora Late, Witkrans 3, Carninka en Lane Late.

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 Citrusdal Valley. The following selections were evaluated: Rayno Early, Tibs Early, Fukumoto, Fischer, Gerhard Early, Washington, Cara Cara, Kirkwood Red, Navelina, Palmer, Gloudi, Cambria, Glen Ora Late, Witkrans 3, Carninka and Lane Late.

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 5.4.21.1. List of navel selections evaluated at various sites in the Citrusdal, Western Cape during the 2018 season.

Selection	Rootstock	Planted	Topworked
Cara Cara	Carrizo	2009	
Fischer	Carrizo	2009	
Fukumoto	Carrizo	2009	
Gerhard Early	Carrizo	2009	
Glen Ora Late	Carrizo	2009	
Gloudi	Carrizo	2009	
Kirkwood Red	Carrizo	2009	
Lane Late	Carrizo	2009	
Washington	Carrizo	2009	
Carninka	Carrizo		2010
Navelina	Carrizo	2009	
Rayno Early	Carrizo	2009	
Tibs Early	Carrizo		2014
Witkrans 3	Carrizo		2011
Cambria	Carrizo		2011
Palmer	Carrizo		2011

Results and discussion

Fukumoto

Fukumoto was the selection with the highest Brix for this trial site (above 14.4°) last season, but this season Brix was lower at 11.6°. Fukumoto also had very good acid levels (1.03%) at over maturity. The good Brix: Acid ratio give Fukumoto its great flavour. It is also a great eating fruit. The colour development was delayed, with colour plate T3 – T2 at peak maturity. The acids remain stable even when the fruit was over mature (above 1% for 4 weeks) fruit will hang well and have a good shelf life. Fukumoto produced a small - medium size fruit which peaked at count 56. The navel-end on the fruit was medium open and protruding, one of the characteristics of the selection.

Gerhard Early

Gerhard Early is an experimental early maturing navel. Gerhard Early was the last of the early selections to reach peak maturity at the trial site. Gerhard Early tree bore small - medium size fruit that peaked at count 56. Gerhard Early had a delayed colour development (colour plate T3 – T5) when the fruit was at peak maturity. Sugars and acids were also good at peak maturity Brix 11.3° and acid 1.10%.

Fischer

Fischer (control) had a delayed colour development with colour plate T5 – T7 at peak maturity. Fischer had a good fruit size which peaked at count 48. Fischer internal quality was very good at peak maturity, Brix 10.2° and acid around 0.99%. The flavour was very good. The navels end for Fischer was small to closed and the fruit had a smooth rind. The crop was very good.

Glen Ora Late

Glen Ora Late is late maturing navel with a very good flavour. The fruit size count of Glen Ora Late peaked at 40 count (big fruit). The external colour development for Glen Ora Late was delayed (T5 on the plate) as peak maturity was reached. Glen Ora Late acids stayed stable and the fruit did manage to reach (T1 on the colour plate) just as the fruit reached (ratio 11.3). Along with the good and stable acids the Brix was also good above 10°. The rind is smooth to slightly coarse with small to close navel end.

Lane Late

Lane Late (control) was the last selection to reach peak maturity. Lane Late trees produced medium - large fruit, (fruit size counts 56 – 40) at peak maturity. The external colour development was delayed on Lane Late, (T4) by peak maturity. It reached T1 when the ratio was 11. Lane Late also kept its acids quite well (good shelf life). The flavour was good. Lane Late had small protruding navel-end on the fruit. The crop on the trees was good.

Gloudi

Gloudi is a promising late navel selection. The fruit shape was round and the fruit firm with a small navel end. Gloudi had a very good fruit size counts 56 the preferred count for navel production and exports. There was also bigger fruit count 48. The selection had a delayed colour development being at colour plate T4 at peak maturity, degreening would have to be done. Gloudi Brix was moderate 10.3° with acids around 1.00%. Even when the fruit was well over mature the acid was at 0.93 (good shelf life).

Washington

Washington was used as control for the mid maturing navel, and it was first to reach peak maturity of the mid maturing selections. The external colour development was behind the internal quality of the fruit (T4 – T6) on the colour plate at peak maturity, but when the fruit was over mature T1 was reach. Washington fruit size count peaked at count 56. High Brix levels above 10.5° with acids of 1.07%, assured good tasting fruit with good flavour. Navel ends were medium for Washington.

Cara Cara

Cara Cara is the control for pigmented navels. Cara Cara is a mid-maturing pigmented navel. Compared to last season delayed colour development T7 on the colour plate at over maturity was this season colour development much better T2 – T3 at peak maturity. The fruit size was ranged between count 64 - 48. Fruit shape was round with smooth rind. The Navel ends were small. The flavour was good due to the good internal quality. Internal colour was an intermediate red in the beginning of the season and as the season went on the red flesh became a bit deeper in red.

Kirkwood Red

Kirkwood Red is a mid-maturing pigmented navel. Peak maturity was reached later than Cara Cara navel. The colour development for Kirkwood Red was good T1 on the colour plate due to the good acids to keep the fruit longer on the trees. Internal quality for Kirkwood Red was good. The flavour was very excellent. Fruit size for Kirkwood Red was small-medium (count 88) to Large (count 48). Flesh colour was deep red, even the fruit stem was red.

Carninka Late

Carninka Late is a late maturing experimental navel. It was the second last selection to reach peak maturity at the trial site. Peak maturity was reached early this season in beginning of July. Carninka late had fruit size, count ranged between (count 72 – 48). The internal fruit quality at peak maturity, was fair, Brix 9.7 and good acid % (0.99%). Fruit is firm and have a smooth peel. Colour development was delayed reaching T5 on the colour plate at peak maturity. Fruit can hang on the trees for longer periods. Navel end is close to small and flavour very good.

Navelina

Navelina is an earlier maturing navel than Washington with very good internal quality and external appearance. The tree is not as vigorous as the Washington. Fruit size peaked at large fruit (counts 48) with a very small to closed navel. The fruit had a delayed colour (T5 – T6) at peak maturity. Fruit shape is slightly elongated, and rinds are smooth. The flavour is very good with good sugars, acid levels and a ratio of 10.5:1

Rayno Early

Rayno Early is a very early maturing experimental navel. It was the first selection to reach peak maturity at the trial site. Fruit size was small – medium with counts 105 – 64. It managed to get very good Brix at peak maturity with stable and good acids. T4 – T6 was the colour on the colour plate when the fruit was over mature.

Tibs Early

Tibs Early is also an experimental early maturing navel. It was the second selection to reach peak maturity at the trial site. Tibs Early fruit size count was peaked at count 40 (large fruit). At peak maturity the Brix is around 10 with acid percentage of 1. Tibs also keep its acid stable. Colour development was delayed with T3 – T4 at a ratio of 11.4:1.

Witkrans 3

Witkrans 3 is a very promising late maturing navel. The trees also had a good crop on them. It was the third last selection to reach peak maturity in the trial site. The external colour development was delayed with a T5 on the colour plate at peak maturity. The fruit size count for Witkrans was good, peaking at a count of 56 which is great for navel production and export. Witkrans acids were just below 1% at peak maturity. Brix was good and along with the good acid it will give Witkrans great flavour. It has a close to small navel end.

Cambria

Cambria is a well-known mid-late navel selection with very good internal quality. The fruit shape was more elongated compared to the other navel selections. Cambria had small - medium size fruit and peaked at count 72. The selection had a delayed in colour development being at colour plate T4 – T5 at peak maturity. The Brix was 9.9° with acids above 0.93% and Brix:acid ratio 10.7:1

Palmer

The external colour development of the selection was delayed (colour plate T7) towards peak maturity. When the ratio was 12.8:1, T2 – T3 on the colour plate were achieved. The selection had a good fruit size and peaked at count 48. The acids were low towards peak maturity and dropped quickly, indicating that the selection could not hang on the trees for a longer period. Brix was also low. It could just be this season.

Conclusion

The following selections (Fukumoto, Gerhard Early, Tibs Early, Washington and Witkrans 3) were the only selections that peaked at fruit size count 56. The selections were seedless. The best colour development were from Kirkwood Red and Cara Cara both the pigmented navels. The selections with the highest Brix were Washington and Gerhard Early respectively.

Table 5.4.21.2. Internal fruit quality data for Experimental Navel selections from the Citrusdal region of the Western Cape during the 2018 season.

Date	Selection	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Colour	Avg. Seed
2018-05-25	Cara Cara	CC	56 - 48	26,6	11,0	1,04	10,6	T2 - T3	0,0
2018-06-22	Cara Cara	CC	64 - 56	32,8	12,3	0,88	14,0	T1 - T3	0,0
2018-06-28	Carninka	CC	64 - 48	29,3	9,7	0,99	9,8	T 5	0,0
2018-07-11	Carninka	CC	72 - 56	24,9	10,0	0,91	11,0	T1 - T3	0,0
2018-04-04	Fischer	CC	56 - 72	30,2	9,3	1,25	7,4	T6 - T7	0,0
2018-04-30	Fischer	CC	72 - 56	36,8	10,2	0,99	10,3	T5 - T7	0,0
2018-05-25	Fischer	CC	56 - 48	28,1	11,7	0,95	12,3	T1 - T4	0,0
2018-04-04	Fukumoto	CC	72 - 105	31,7	10,4	1,33	7,8	T 5	0,0
2018-04-30	Fukumoto	CC	88 - 56	36,3	11,6	1,03	11,3	T3 - T4	0,0
2018-04-04	Gerhard Early	CC	64 - 88	33,8	10,6	1,23	8,6	T 6	0,3
2018-04-30	Gerhard Early	CC	88 - 64	42,7	11,3	1,10	10,2	T3 - T5	0,0
2018-05-24	Gerhard Early	CC	72 - 56	32,9	12,0	0,91	13,2	T1 - T3	0,0
2018-06-06	Glen Ora Late	CC	56 - 40	30,5	9,6	1,10	8,7	T4	0,0
2018-06-28	Glen Ora Late	CC	56 - 40	30,5	10,3	0,96	10,8	T 5	0,0
2018-07-11	Glen Ora Late	CC	56 - 40	65,1	10,5	0,93	11,3	T1 - T3	0,0
2018-06-06	Gloudi	CC	56 - 48	39,5	10,3	1,05	9,8	T 4	0,0
2018-06-28	Gloudi	CC	56 - 48	30,9	10,3	0,93	11,0	T 4	0,0
2018-04-30	Kirkwood Red	CC	56 - 48	36,1	9,3	1,11	8,4	T5 - T7	0,0
2018-05-25	Kirkwood Red	CC	64 - 40	32,0	10,2	0,97	10,5	T2 - T5	0,0
2018-06-22	Kirkwood Red	CC	64 - 48	35,6	10,4	0,99	10,5	T1 - T3	0,0
2018-07-11	Kirkwood Red	CC	88 - 56	28,4	11,0	0,94	11,6	T 1	0,0
2018-06-06	Lane Late	CC	56	43,2	9,6	1,10	8,7	T3 - T5	0,0
2018-06-28	Lane Late	CC	56 - 40	31,2	9,9	1,01	9,8	T 4	0,0
2018-07-11	Lane Late	CC	56 - 40	29,5	10,2	0,90	11,4	T1 - T3	0,0
2018-04-30	Navelina	CC	64 - 48	38,4	9,7	1,10	8,8	T6 - T7	0,0
2018-05-24	Navelina	CC	64 - 48	30,7	10,4	0,99	10,5	T5 - T6	0,0
2018-04-04	Rayno Early	CC	72 - 105	27,5	9,9	1,13	8,7	T 6	0,0
2018-04-30	Rayno Early	CC	105 - 64	46,6	16,5	0,99	16,6	T4 - T5	0,0
2018-04-04	Tibs Early	CC	40 - 56	26,4	9,7	1,15	8,5	T6 - T7	0,0
2018-04-30	Tibs Early	CC	64 - 56	39,2	10,8	0,95	11,4	T3 - T4	0,0
2018-04-04	Washington	CC	56 - 72	32,3	10,0	1,41	7,1	T 6	0,0
2018-04-30	Washington	CC	72 - 56	37,6	10,5	1,07	9,8	T4 - T6	0,0
2018-05-25	Washington	CC	64 - 56	28,0	11,5	1,05	11,0	T1 - T5	0,0
2018-06-28	Witkrans 3	CC	72 - 56	32,0	10,4	0,97	10,7	T5	0,0
2018-07-11	Witkrans 3	CC	105 - 72	47,8	11,2	0,77	14,6	T1 - T2	0,0

2018-06-21	Cambria 4	CC	88 - 72	35,3	9,9	0,93	10,7	T4 - T5	0,0
2018-07-11	Cambria 4	CC	88 - 72	49,9	10,5	0,82	12,9	T1 - T4	0,0
2018-04-23	Palmer	CC	56 - 72	42,5	8,7	0,93	9,3	T 7	0,0
2018-06-06	Palmer	CC	64 - 48	39,4	9,8	0,77	12,8	T2 - T3	0,0

5.4.22 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 start a new trial site. The early maturing selection for the trial site is Turkey with Midnight as control. The mid-maturing Valencia selections are Alpha, Gusocora, Benny 2 and Midnight 1. The late maturing Valencia selections will be McClean SL, Lavallo, Ruby Red. At this trial site the season started with Turkey, followed by Midnight 1, Midnight, McClean SL, Gusocora, Benny 2, Alpha, Ruby Valencia and the season ended off with Lavallo. 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 'n moontlikheid om 'n perseel te begin. Die vroeë seleksie vir die proef perseel bestaan uit Turkey en Midnight wat as kontrole dien. Die mid seleksies is Alpha, Gusocora, Benny 2 en Midnight 1. Die laat rypwordende Valencia seleksies was as volg; McClean SL, Lavallo, en Ruby Red. Die proef perseel se seisoen het begin met Turkey, gevolg deur Midnight 1, Midnight, McClean SL, Gusocora, Benny 2, Alpha, Ruby Valencia en die seisoen het afgeëindig met Lavallo. 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, Benny 2, Gusocora (G5), Lavallo, McClean SL, Midnight (control), Midnight 1, Turkey and Ruby Red.

Table 5.4.22.1. Internal fruit quality minimum export requirements for Valencia types.

Variety	Juice %	Brix	Min Acid	Max Acids	Ratio	Colour
Valencia EU	48	8.5	0.6	1.8%	7.5:1	Colour plate 3 of set no. 34
Midnight	52	9.5	0.85	1.8%	7.5:1	Colour plate 3 of set no. 34
*Turkey	50	10.0	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34

*Interim internal fruit quality standards.

Table 5.4.22.2. List of Valencia selections evaluated at Panzi (Kirkwood) during 2018 season.

Selection	Rootstock	Topwork
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Alpha	CC	2011
Bennie 2	CC	2011
Gusocora G5	CC	2011
Lavalle	CC	2011
McClellan SL	CC	2011
Midnight	CC	2011
Midnight 1	CC	2011
Ruby Red	CC	2011
Turkey	CC	2015

Results and discussion

Alpha

Alpha bore medium size fruit this season on the trees, counts 56. The tree condition on Carrizo rootstock were good. Alpha Valencia were completely seedless and the fruit shape remained fairly round with a slightly pebbly rind. The external colour development peaked at T1 with good internal quality, high Brix supported with good acids by the time of maturity. Juice content peaked at 59.8% at peak maturity.

Bennie 2

The fruit size peaked from count 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). The selection had a seed count 0.5 – 1.2. There was no delay in external colour development (T1) at peak maturity. The rind colour was deep orange with a smooth to coarse rind. The flesh was orange and the fibre strength was soft compared to the other Valencia selections. Bennie 2 developed an excellent juice content. Bennie 2 had good internal quality and gave the selection its good flavour.

Gusocora

There was a slight delay in external colour development on the fruit (T3) but due to the good acids the fruit was able to hang and reached T1 colour and the colour plate. When the selection reached a T1 the Brix was around 10 with an acid around 1%. Gusocora was completely seedless and will be regarded as a seedless selection. The juice content of Gusocora this season ranged between 58 - 59% and the fruit size ranged between counts 64 – 56 bigger compared to 2017 season. The fruit was firm with a round shape and a smooth rind.

Lavalle

Lavalle had a very good export fruit size at count 56. Lavalle was completely seedless and the juice content of this selection increase towards peak maturity to a high of (63%) with a Brix: acid ratio of 8.7. Brix was also good along with the acids. There was no problem with the external colour development when Lavalle developed a T1 on the colour plate range at peak maturity. The fruit was reasonably easy to peel and the internal colour was orange with a slightly softer flesh. The flavour was also very good.

Midnight 1

Midnight 1 evaluation seed count was seedless. The fruit size ranged from count 64 to 56, perfect for export. The juice content of Midnight 1 decreased towards peak maturity around 58.6% at peak maturity. Midnight 1 reached T1 on the colour plate before peak maturity. Brix and acid content was lower compared to Midnight (control)

McClellan SL

Fruit shape for McClellan SL is fairly round fruit with a soft fibre strength that peels easy, containing low rind oil levels. All the fruit evaluated remained completely seedless. The trees bore medium size fruit on the trees (count 72 to 56). The internal quality was good with high juice levels for this trial site (61.7%). Juice content decrease as the fruit hang but not with much. There was no delay in external colour development being a T1, before peak maturity.

Midnight

Midnight was used as control in this trial site. Midnight trees cropped medium fruit size with 56 count. The juice content of Midnight peaked at 58.7%. The external colour development of Midnight was very good with a T1 on the colour plate range at peak maturity. Midnight internal quality was good and it gave Midnight its good flavour. The selection was seedless.

Turkey

Fruit size for Turkey was perfect for export with fruit size count range (count 64 – 56). Turkey juice content decreased towards peak maturity. It was still above 50% for export. Brix was below the 10 for export. The external colour of this selection was T1 at peak maturity. Turkey were also seedless during the evaluations. Fruit shape was round with coarse rind. The rind colour was deep orange.

Ruby Red

Ruby Red bore small fruit that peaked at count 88 the previous season, this season the fruit was slightly bigger ranging between 72 - 56. The juice content of Ruby Red at peak maturity was above 60%. At peak maturity Ruby's external colour development was T1 on the colour plate range. Fruit seed count ranged between 0.0 – 0.6 seeds per fruit. At peak maturity the internal quality was very good. The colour of the flesh was red, and the selection have a unique taste.

Conclusions

Most of the Valencia selections had no problem with external colour development, all of them reached T1 on the colour plate at peak maturity. All of the selection's, except Turkey, internal and external qualities complied with the minimum export requirement for Valencia types. The following selections had a seed count; Benny 2 with the highest count of 1.2 seeds per fruit and Ruby Red with a seed count of 0.6 seeds per fruit. All the other selections were completely seedless. Most of the selections had a fruit size count of 64 - 56. Ruby Red and McClean SL fruit size count was 72 the smallest during one evaluation. The following selections developed a juice content above 60% at peak maturity; McClean SL, Lavalles and Ruby Red.

Table 5.4.22.3. Internal fruit quality data for Valencia selections at Panzi (Sundays River Valley) during the 2018 season.

Date	Cultivar	Root stock	Count	Juice %	Brix°	Acid %	Ratio	Colour	Avg. Seed
2018-08-28	Alpha	CC	56	58,3	10,3	1,16	8,9	T1	0,0
2018-09-14	Alpha	CC	56	59,8	11,4	1,30	8,8	T1	0,0
2018-09-25	Alpha	CC	56	58,7	11,3	1,21	9,3	T1	0,0
2018-08-15	Benny 2	CC	56	58,5	9,4	1,22	7,7	T1	1,2
2018-08-28	Benny 2	CC	56	59,6	9,9	1,16	8,5	T1	0,5
2018-09-14	Benny 2	CC	56	57,5	9,6	1,00	9,6	T1	0,8
2018-08-01	Gusocora	CC	64	57,9	10,1	1,20	8,4	T3	0,0
2018-08-15	Gusocora	CC	56	59,2	9,9	1,11	8,9	T1	0,0
2018-08-28	Gusocora	CC	56	58,4	10,8	1,11	9,7	T1	0,0
2018-09-25	Gusocora	CC	64	58,5	9,4	0,95	9,9	T1	0,0
2018-08-28	Lavalle	CC	56	58,7	10,8	1,38	7,8	T1	0,0
2018-09-14	Lavalle	CC	56	63,0	10,1	1,16	8,7	T1	0,0
2018-09-25	Lavalle	CC	56	60,9	10,6	1,13	9,3	T1	0,0
2018-07-18	Mc Clean SL	CC	64	61,7	9,6	1,21	7,9	T1	0,0
2018-08-01	Mc Clean SL	CC	56	59,7	10,2	1,22	8,4	T1	0,0
2018-08-28	Mc Clean SL	CC	72	58,9	10,0	1,08	9,3	T1	0,0
2018-07-18	Midnight	CC	56	58,7	10,4	1,21	8,6	T1	0,0

2018-08-15	Midnight	CC	56	58,0	10,8	1,32	8,2	T1	0,0
2018-08-28	Midnight	CC	56	55,8	9,6	1,05	9,1	T1	0,0
2018-08-01	Midnight 1	CC	64	59,2	9,2	1,01	9,1	T1	0,0
2018-08-15	Midnight 1	CC	56	58,6	9,8	0,96	10,2	T1	0,0
2018-08-28	Midnight 1	CC	64	57,3	9,9	0,97	10,2	T1	0,0
2018-08-15	Ruby Valencia	CC	72	61,1	9,4	1,33	7,1	T1	0,3
2018-08-28	Ruby Valencia	CC	56	60,2	10,4	1,25	8,3	T1	0,0
2018-09-14	Ruby Valencia	CC	64	60,7	10,5	1,15	9,1	T1	0,6
2018-06-21	Turkey	CC	56	57,5	9,3	1,19	7,8	T4	0,0
2018-07-03	Turkey	CC	64	51,6	9,4	1,13	8,3	T1	0,0

5.4.23 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Valencia oranges in a cold production region (Citrusdal)

Project 1097B by W. Swiegers (CRI)

Summary

The climate and the soil make this region a good region to farm Valencias. The Valencias tend to get high sugars to acid ratio in this region. It gives fruit with great flavours and it is also good eating fruit. Valencias also don't have a navel end, and this make sanitation easier. Most of the trees were planted in 2009 and consist of early-, mid- and late maturing selections. The order of ripening was not as it is on the maturity chart, and that is due to the acids that were slow to drop in the season. The order of ripening was as follow starting with Midnight H14 and Midnight F17, Turkey, Gusocora, McClean SL, Val Late, Henrietta, Midnight, Benny, Alpha, Louisa, Delta, Ruby Valencia and the season finished off with Lavalles and Lavalles 2.

Opsomming

Die klimaat en die grond maak die streek geskik vir die verbouing van Valencias wat hoë suikers tot suur verhouding het. Dit maak goeie eet vrugte met goeie geure. Valencia's het ook nie 'n Navel ent nie wat sanitasie makliker maak. Die meeste bome is in 2009 geplant en bestaan uit vroeë-, mid- en laat rypwordende seleksies. Die volgorde van rypwording was baie deurmekaar, dit is a.g.v. die sure wat baie stadig geval het. Die orde van rypwording was as volg, Midnight H14 was eerste en Midnight F17 het hom opgevolg, gevolg deur Turkey, Gusocora, McClean SL, Val Late, Henrietta, Midnight, Benny, Alpha, Louisa, Delta, Ruby Valencia, en die seisoen is afgesluit met Lavalles en Lavalles 2.

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 Midnight H14 and Midnight F17, Turkey, Gusocora, McClean SL, Val Late, Henrietta, Midnight, Benny, Alpha, Louisa, Delta, Ruby Valencia, Lavalles, Lavalles 2.

Table 5.4.23.1. Internal fruit quality minimum export requirements for Valencia types.

Variety	Juice %	Brix	Min Acid	Max Acids	Ratio	Colour
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Valencia EU	48	8.5	0.6	1.8%	7.5:1	Colour plate 3 of set no. 34
Midnight	52	9.5	0.85	1.8%	7.5:1	Colour plate 3 of set no. 34
*Turkey	50	10.0	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34

*Interim internal fruit quality standards.

Table 5.4.23.2. List of Valencia selections evaluated at Kweekkraal (Citrusdal) during 2018 season.

Selection	Rootstock	Topwork
Alpha	CC	2009
Bennie	CC	2009
Henrietta	CC	2010
Delta	CC	2009
Gusocora	CC	2009
Turkey	CC	2009
Valencia Late	CC	2009
McClellan SL	CC	2009
Midnight	CC	2009
Ruby Valencia	CC	2009
Lavalle	CC	2009
Lavalle 2	CC	2011
Midnight H14	CC	2011
Midnight F17	CC	2016
Louisa	CC	2011

Results and discussion

Alpha

The internal quality was good, Brix was between 11.1 – 12.0 and acids were below the max acids of 1.8% to export. Fruit size varied from count 88 to 56, slightly on the smaller size but still good for Valencia production and export. External colour peaked on T1. As the fruit hang on the trees the internal quality got better to export fruit of higher standards. During the 3 evaluations there was no seed count.

Bennie

The fruit size count peaked at count 72 to 56, medium fruit size and a good Valencia export size. Bennie had a soft fibre strength compared to the other Valencia selections. But due to the high acid the fruit were left to hang so that the acids can come down and Brix up and also get better colour development. This made that the selection matured much later. The selection had a much better internal quality. Seed count ranged between 0.0 – 0.6 seeds per fruit. There was no delay in external colour development (T1) before peak maturity. Bennie developed a juice content of 57.2% towards peak maturity.

Gusocora

The fruit were left on the trees to the beginning of August and the fruit coloured up completely to T1 – T2 on the colour plate. The Brix also went up and the acids also dropped to get a fruit that has very good export internal quality. The Brix was above 11 and acids were still good at 0.97% with good juice content above 55%. Gusocora was completely seedless and will be regarded as a seedless selection for future plantings. Fruit size peaked at count 88 - 56.

Henrietta

The peelability of Henrietta was 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 0.4 seeds per fruit. Fruit size count range from (count 88 – 56). Henrietta had no problem with external colour development (T1) at peak maturity. Henrietta produced a good juice content around 57.2%.

Lavalle and Lavalle 2

There was no major difference between Lavalle and Lavalle 2. Lavalle selections had a very good export fruit size count peaking at 56. Lavalle selections were completely seedless and the juice content of this selection increased towards peak maturity to a high of (57.9%) with a Brix: acid ratio of 8.1. Brix was also good along with the acids. There was a delay with the external colour development when Lavalle selections developed a T3 on the colour plate range at peak maturity. The fruit was reasonably easy to peel and the internal colour was orange with a slightly softer flesh. The flavour was also very good.

Louisa

Fruit size count for Louisa was very good ranging from count 88 – 56. The fruit have smooth rinds, but rind oil is high. Fruit shape is round to slightly elongated. Louisa internal quality comply with all the export standards. Juice content of 54.5%, Brix at 10.6 and acid 1.18%. Colour development was higher than the T3 for export being a T2 on the colour plate. Fruit rind colour is more of a yellow colour.

Valencia Late

The Valencia Late produced small – medium size fruit at count 105 - 64. Acid levels were slightly lower in 2018 season at peak maturity (1.10%) compared to (1.54%) in the 2017 season. The juice content was low again this season peaking at 50%, and Brix was also down from 12.5 to 11.6. The external colour development was good with T1 and seed counts were between 0.5 – 0.8 seeds per fruit.

Delta

Delta, as the control variety, produced completely seedless fruit and a good yield on the trees. Fruit size peaked between count 88 – 64 bigger compared to 2017 season count 125 and 88. Good internal quality (end of August), Brix of 10.5 and acid content of 1.19%. The external colour of the fruit was between T3 and T1. The fruit was round with a smooth rind and peeled fairly easy and also had a good flavour.

McClellan SL

McClellan SL tree bore fruit with fruit size count ranging from count 88 - 48. The selection was seedless. External colour development reached T1 on the colour plate before peak maturity. Brix was good (above 10) peaking at 11.1 and acids remain fairly high towards the end of the season around 1%, resulting a very good Brix: Acid ratio. The fruit is firm with a round to elongated fruit shape with a smooth rind. Externally as well as internally the colour is deep orange.

Turkey

Fruit size was a very favourable for Valencia production and export, count ranged between 72 – 56. Brix was around 11 and acids around 1.28% this will meet the export standards as well as the external colour that was at T1 on the colour plate. There were seeds during the evaluation and it peaked at 0.3 seed per fruit. Fruit characteristics for Turkey were round fruit shape, with a very good flavour, soft rag, fairly thin rind, easy fruit peeling. The internal colour was light yellow, and externally the fruit remained yellow. This selection has the qualities of a mid-season orange; for instance, the exceptionally soft fruit, and the soft rind that can result in rind problems if managed incorrectly.

Midnight & Midnight F17 and Midnight H14

Midnight was used as control for this trial site but also as control for the other two Midnight selections. The fruit size development for Midnight peaked at count 48 followed by Midnight H14 peaking at count 56 and the smallest peak was for Midnight F17 with count 64. The smallest fruit size count for all 3 selections was count 88. All the Midnight selections bore round fruit on the trees with a medium to coarse rind, fibre strength was fairly soft and the fruit peeled easy. The Midnight selections were seedless. The colour development towards peak maturity was between T1 - T2 on the colour plate range for all the selections, but at peak maturity all the selections were T1 on the colour plate. The trees had a good yield on them. Midnight and Midnight H14 had the highest Brix above 11 and Midnight F17 were above 10. Midnight H14 was first to reach peak maturity, followed by Midnight F17 and Midnight was the last selection to reach peak maturity between them.

Ruby Valencia

Ruby Red bore small - medium fruit that peaked at count 105 - 56. The juice content of Ruby Red at peak maturity was above 55%. At peak maturity Ruby's external colour development was T2 – T3 on the colour plate range. Fruit seed count ranged between 1.8 – 3.0 seeds per fruit. At peak maturity the internal quality was very good. Brix was just below 11. The colour of the flesh was red, and the selection have a unique taste.

Conclusions

None of the selections had problems with their external colour development, that is due to the high acids, so the fruit were able to hang longer on the trees to fully colour up to T1 – T3 on the colour plate. All the selections met the minimum export standards. Ruby Valencia, Valencia Late, Bennie, Turkey and Henrietta were the selections on average with the highest number of seeds per fruit. All the other selections were completely seedless. The fruit size varied quite a lot between selections, but all of them were good enough for export. All the selections produced a Brix above 10 and acids content around 1%.

Table 5.4.23.3. Internal fruit quality data for Valencia selections at Kweekkraal (Citrusdal) during the 2018 season.

Date	Cultivar	Root stock	Count	Juice %	Brix°	Acid %	Ratio	Avg. Seed	Colour
2018-08-01	Alpha	CC	72 - 64	39,5	11,1	1,48	7,5	0,0	T 1
2018-09-03	Alpha	CC	88 - 64	43,1	12,0	1,33	9,1	0,0	T 1
2018-09-27	Alpha	CC	72 - 56	42,9	11,7	1,23	9,5	0,0	T 1
2018-08-01	Benny	CC	72 - 56	42,9	10,7	1,46	7,3	0,0	T 1
2018-09-03	Benny	CC	72 - 56	43,0	10,9	1,31	8,3	0,3	T 1
2018-09-27	Benny	CC	72 - 56	57,2	10,7	1,13	9,5	0,6	T 1
2018-08-01	Delta	CC	88 - 64	41,7	10,3	1,42	7,2	0,0	T2 - T3
2018-09-03	Delta	CC	88 - 64	43,7	10,5	1,19	8,8	0,0	T1 - T3
2018-09-27	Delta	CC	88 - 64	46,4	10,9	1,22	8,9	0,0	T1 - T2
2018-08-01	Gusucora	CC	72 - 56	42,6	11,1	1,22	9,1	0,0	T1 - T2
2018-09-03	Gusucora	CC	88 - 64	39,7	10,8	1,10	9,8	0,0	T 1
2018-09-27	Gusocora	CC	72 - 64	55,8	11,0	0,97	11,4	0,0	T 1
2018-08-01	Henrietta	CC	72 - 56	43,9	11,8	1,45	8,1	0,0	T2 - T3
2018-09-03	Henrietta	CC	72 - 64	43,0	10,6	1,27	8,3	0,4	T1 - T3
2018-09-27	Henrietta	CC	88 - 56	57,2	10,6	1,07	9,9	0,1	T 1
2018-08-02	Lavalle	CC	64 - 56	52,1	10,6	1,59	6,6	0,0	T3 - T4
2018-09-03	Lavalle	CC	72 - 64	45,4	11,6	1,51	7,7	0,0	T3 - T4
2018-09-27	Lavalle	CC	72 - 48	57,9	11,6	1,42	8,1	0,0	T2 - T3
2018-08-02	Lavalle 2	CC	72 - 56	49,1	10,6	1,63	6,5	0,0	T3 - T4
2018-09-03	Lavalle 2	CC	72 - 56	42,8	11,5	1,52	7,6	0,0	T 4
2018-09-27	Lavalle 2	CC	72 - 48	47,5	11,5	1,42	8,1	0,0	T 3
2018-08-02	Louisa	CC	72 - 56	47,6	9,9	1,29	7,7	0,0	T3 - T4
2018-09-03	Louisa	CC	72 - 64	39,6	10,4	1,25	8,3	0,0	T1 - T4
2018-09-27	Louisa	CC	88 -64	54,5	10,6	1,18	9,0	0,0	T 2
2018-08-01	Mc Clean SL	CC	72 - 48	38,6	10,6	1,20	8,9	0,0	T 2
2018-09-03	McClean SL	CC	72 - 56	43,2	10,9	1,09	10,0	0,0	T 1
2018-09-27	Mc Clean SL	CC	88 - 56	48,9	11,2	1,02	10,9	0,0	T 1
2018-08-01	Midnight	CC	88 - 56	42,7	11,6	1,33	8,7	0,0	T 2

2018-09-03	Midnight	CC	72 - 56	40,4	11,4	1,21	9,4	0,0	T 1
2018-09-27	Midnight	CC	64 - 48	45,3	11,5	1,19	9,7	0,0	T 1
2018-09-03	Midnight F 17	CC	88 - 64	42,0	10,8	1,20	9,0	0,0	T 1
2018-07-11	Midnight F17	CC	88 - 72	52,7	10,6	1,31	8,1	0,0	T 2
2018-08-01	Midnight F17	CC	88 - 64	45,2	10,3	1,22	8,5	0,0	T2 - T3
2018-07-11	Midnight H 14	CC	88 - 56	26,1	10,3	1,24	8,3	0,0	T 2
2018-08-02	Midnight H 14	CC	88 - 64	46,5	11,0	1,16	9,5	0,0	T 2
2018-09-03	Midnight H 14	CC	72 - 56	36,8	11,8	0,98	12,0	0,0	T 1
2018-08-02	Ruby Valencia	CC	105 - 88	56,6	10,4	1,63	6,4	3,0	T3 - T4
2018-09-03	Ruby Valencia	CC	88 - 64	48,7	10,4	1,38	7,6	1,8	T2 - T4
2018-09-27	Ruby Valencia	CC	88 - 56	48,4	10,9	1,34	8,1	2,2	T2 - T3
2018-07-11	Turkey	CC	72 - 56	49,6	10,7	1,24	8,7	0,3	T 1
2018-08-01	Turkey	CC	64 - 56	38,2	11,1	1,35	8,2	0,0	T 1
2018-09-03	Turkey	CC	72 - 56	40,9	11,0	1,28	8,6	0,3	T 1
2018-08-02	Val Late	CC	105 - 64	50,2	11,2	1,33	8,4	0,8	T1 - T2
2018-09-03	Val Late	CC	88 - 64	46,0	11,2	1,24	9,0	0,5	T1 - T3
2018-09-27	Val Late	CC	88 - 64	41,9	11,6	1,10	10,5	0,7	T 1

5.4.24 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Clementine mandarins in a cold production region (Sundays River Valley)

Project 1000B by W. Swiegers and Z. Zondi (CRI)

Summary

The selections that were evaluated were just some of the open selections and one selection from an agent. The open selections will be used as controls for the new selections in the future. For the Sundays River Valley there are two Clementine sites with most of the selections. Some of the selections are on interstock. The season started with Clemenpons, followed by Nules and ended with Esbal.

Opsomming

Die seleksies wat geëvalueer was die seisoen was 2 oop seleksies en een van 'n agent. Die oop seleksies dien as kontroles vir die nuwe seleksies. Vir die Sondags Rivier Vallei is daar 2 Clementine persele wat die meeste seleksies bevat. Van die seleksies is op 'n tussen stam. Die seisoen het begin met Clemenpons, gevolg deur Nules en ge-eindig met Esbal.

Objectives

- To select Clementine cultivars with improved and consistent productivity, fruit size, rind colour, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the cultivar characteristics of new Clementine cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Clementine selections from the Sundays River Valley region of the Eastern Cape; planted 2012. The following varieties were evaluated: Clemenpons, Esbal and Nules.

A ratio of 11:1 Clementines 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 5.4.24.1. List of Clementine selections evaluated at Invercloy (Kirkwood) during 2018.

Selection	Rootstock	Planted
Esbal	Carrizo	2012
Nules	Carrizo	2012

Table 5.4.24.2. List of Clementine selections evaluated at Invercloy (Kirkwood) during 2018.

Selection	Rootstock	Topworked
Clemenpons	Carrizo with Midnight as interstock	

Results and discussion

Clemenpons

Clemenpons was the first selection to reach peak maturity. Clemenpons is an early maturing Clementine, but it is known to have galls above the bud union. In this trial site Clemenpons were topworked on a interstock, to see if it will help with the galls. Fruit shape is round and the flesh is pale. There were no seeds during evaluations. Fruit size ranged between count 2 – 3. Clemenpons had a very good internal quality with juices above 55% and Brix above 10 and acids around 1% at peak maturity. Colour development was delayed peaking on T5 on the colour plate.

Esbal

Esbal was the last selection to reach peak maturity. Esbal normally mature earlier than Nules. Esbal's fruit was slightly smaller than Nules, with a fruit size count for Esbal 3 – 1. Rind colour development for Esbal was the best, with T1 when the fruit was at peak maturity. Fruit was round to oblate with a smooth to pebbly rind. Seed count for Esbal was 0.0 – 0.6 seeds per fruit. Esbal juice percentage was just above 50% at peak maturity. Internal quality at peak maturity was Brix (11.8°) and acid (0.99%).

Nules

Nules were slightly earlier to reach peak maturity. The selection had a juice percentage that ranged between (50.2 – 53%) at peak maturity. Nules had the largest fruit size count 2 - 1. Internal quality for Nules at peak maturity was good; Brix (11.7°) and acid (0.95%). Nules also kept its acids well. This contribute to Nules good flavour. Nules had 0.0 seeds per fruit. Rind colour development was not good with a T5 –T6 on the colour plate at peak maturity. Peelability is easy and internal colour is orange.

Conclusion

Esbal had the highest seed count of all the selections that were evaluated, seed count of 0.6 seeds per fruit. Most of the selections had delayed colour development. Degreening practices will be essential after harvesting to ensure optimal colour development. Esbal had the best rind colour development T1 on the colour plate. Esbal had the highest Brix (11.8°) just above Nules (11.7). Clemenpons had the smallest fruit size and peaked

at count 4 - 2. All the selections had a good juice percentage: all over 50%, with Clemenpons the highest above (55%).

Table 5.4.24.2. Internal fruit quality data for Clementine selections in the Sundays River Valley region (Invercloy) of the Eastern Cape during the 2018 season.

Date	Cultivar	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2018-03-20	Clemenpons	CC	4	59,0	10,6	1,10	9,6	0,0	T7
2018-04-03	Clemenpons	CC	3	55,9	10,1	1,00	10,1	0,0	T7
2018-05-11	Clemenpons	CC	2	57,4	10,2	0,91	11,2	0,0	T6
2018-04-19	Esbal	CC	3	58,2	10,4	0,95	11,0	0,0	T6
2018-05-16	Esbal	CC	2	56,5	11,1	0,97	11,4	0,0	T3
2018-05-30	Esbal	CC	1	51,8	11,8	0,99	11,9	0,6	T1
2018-04-19	Nules	CC	2	50,2	11,3	0,96	11,8	0,0	T6
2018-05-02	Nules	CC	2	53,0	11,7	0,95	12,3	0,0	T5
2018-05-16	Nules	CC	1	50,8	12,3	0,92	13,4	0,0	T4

5.4.25 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Clementine mandarins in a cold production region (Western Cape)

Project 1000D by W. Swiegers (CRI)

Summary

There are 2 trial sites in Citrusdal where we evaluated Clementines this season. The open selections are used as controls in the trial sites. The selections that were evaluated were early-, mid- and late selections. The early selections will play an important role as they start to overlap the Satsumas. Basol on navel interstock started the season in Citrusdal followed by Basol, Clemenpons, Nules, Clemenpons new, Clemenluz, Esbal, and Saratoga finished the season.

Opsomming

Daar is 2 proef persele in Citrusdal waar Clementines geevalueer was die seisoen. Die oop seleksies dien as kontroles vir die proef persele. Die seleksies wat geevalueer word is vroe-, mid – en laat seleksies. Die vroe seleksies gaan nog n belangrike rol speel in die toekoms soos wat dit begin oorvleul met Satsumas. Basol met die nawel tussenstam het die seisoen in Citrusdal begin, gevolg deur Basol, Clemenpons, Nules, Clemenpons nuwe, Clemenluz, Esbal, en Saratoga het die seisoen afgesluit.

Objectives

- To select Clementine cultivars with improved and consistent productivity, fruit size, rind colour, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the cultivar characteristics of new Clementine cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Clementine selections from various regions in the Western Cape. The following varieties were evaluated: Nules, Esbal, Clemenpons, Clemenpons new, Clemenluz, Basol, Basol with interstock and Saratoga.

A ratio of 11:1 Clementines 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 5.4.25.2. List of Clementine selections evaluated at Kweekkraal and Stargrow (Citrusdal) during 2018.

Selection	Rootstock	Planted
Clemenpons	Carrizo	2010
Saratoga	Carrizo	2012
Nules	Carrizo	2009
Basol	Carrizo	2010
Basol interstock	Carrizo	2011
Esbal	Carrizo	2009
Clemenluz	Carrizo	2009
Clemenpons new	Carrizo	2010

Results and discussion

Clemenpons and Clemenpons new

Clemenpons is early maturing Clementine selection. The new Clemenpons selection reached peak maturity after Clemenpons. The new selection had slightly bigger fruit, with fruit size ranging 4 – 2 at peak maturity and Clemenpons fruit size ranged between 5 – 3. The juice percentage for the new selection peaked at 58% compared to Clemenpons 45.1%. Clemenpons had slightly higher Brix and acid % compared to Clemenpons new selection. Both selections had high Brix 13 and good acid % around 1%. The colour development was delayed for both at peak maturity but the new Clemenpons selection had better colour development. At over maturity the new Clemenpons were T1 – T2 on the colour plate compared to Clemenpons T5 – T6 on the colour plate. Clemenpons had a higher seed count peaking at 5.3 seeds per fruit compared to Clemenpons new selections peaking at 0.4 seeds per fruit.

Basol and Basol interstock

Basol is the earliest maturing Clementine selection. Basol trees tend to develop galls on the trunk. The Basol trees with the navel interstock do not develop galls on the trees. Fruit size on both Basol's was small to medium with count 3 – 4, with Basol peaking at count 2. There was not a big difference in the juice percentages between the two Basol selections at peak maturity, both are around 53%. Basol on CC had a slightly lower Brix than the one on the interstock at peak maturity. Acid percentage for both selections was good above 1.00% at peak maturity. The selection on CC had a lower seed count 0.3 seeds per fruit and Basol with interstock had 0.8 seeds per fruit. External colour break was the same for both selections T6 on the colour plate, at peak maturity. The fruit peels easily. Basol have a very short harvest period before the fruit is over mature and start to granulate.

Esbal

Esbal normally mature earlier than Nules, but they were more or less together to reach peak maturity with Nules being a little bit earlier. Fruit size count for Esbal 1 – 1x (count). Fruit is round to oblate and rind is smooth to pebbly. Peelability is easy but rind oil bother. Rind colour development was better than Nules. In Citrusdal the colour was (T3 – T4) on the colour plate at peak maturity. The sugars and acids were good at peak maturity Brix (11.6°) and acid (0.93%). Seed count peaked at 1.5 seeds per fruit.

Nules

Nules is used as the control at all the sites. At peak maturity fruit size count was 3 – 2. The internal quality was good with Brix (11.8°) and acid (0.93%) at peak maturity ratio 12.7:1. Internal colour development was delayed being T5 – T6 on colour plate at peak maturity. Those acids will give the fruit good shelf life and the high sugars with acids will give Nules its good flavour. The rind is smooth and thin and it peels easy. In Citrusdal the seed count was 0.0 – 0.2 seeds per fruit. Yields were good for Nules.

Saratoga

Saratoga reached peak maturity quite late this year. It could be that the riper fruit was stolen. Fruit size for Saratoga was medium – large with a fruit size count of 2 – 1x. The juice decreased towards peak maturity with a low juice percentage, it could be due to granulation. External colour development is very good for Saratoga T1 on the colour plate towards peak maturity. The good internal quality makes it possible for the fruit to hang to colour up completely to a deep orange colour. The selection did have seeds during the evaluations, 0.1 – 1.1 seeds per fruit.

Clemenluz

Clemenluz is an early maturing Clementine selection. Nules is used as a control for this section. The Clemenluz reached peak maturity just after Nules according to the ratio. Compared to Nules, Nules had better internal quality, higher juice %, higher Brix and better acids at peak maturity. Clemenluz had the highest seed count 6.1 seeds per fruit. Colour development was delayed at peak maturity. Rind colour is a yellow orange and the peelability of the fruit is easy.

Conclusion

None of the selections were seedless, because they were planted in mix trial blocks. Nules were the selection with the lowest seed count 0.0 – 0.2 seeds per fruit. Clemenluz had the highest seed count 0.8 – 6.1 seeds per fruit. Most of selections had delayed colour development. Degreening practices will be essential after harvesting to ensure optimal colour development. Saratoga and Clemenluz were the only selections to reach T1 on the colour plate at peak maturity. In Citrusdal Clemenpons had the highest Brix (12.5°) and acid (1.21%) towards peak maturity. Clemenpons had the smallest fruit size and peaked at count 5 - 3. Esbal had the largest fruit size count 1 - 1x in Citrusdal. The selections that had a very good juice percentage over 55% at peak maturity were Clemenpons new.

Table 5.4.25.2. Internal fruit quality data for Clementine selections in the Citrusdal region (Kweekkraal and Stargrow) of the Western Cape during the 2018 season.

Date	Cultivar	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2018-03-08	Basol	CC	4 - 5	53,1	12,1	1,04	11,6	0,1	T 6
2018-04-04	Basol	CC	2 - 4	41,4	13,2	0,96	13,7	0,3	T 3
2018-03-08	Basol M7	CC	4 - 5	52,5	12,5	1,06	11,8	0,2	T 6
2018-04-04	Basol M7	CC	3 - 4	52,9	13,1	1,05	12,5	0,8	T2 - T4
2018-04-04	Clemenpons	CC	To small - 4	43,0	12,5	1,21	10,3	5,3	T 7
2018-04-24	Clemenpons	CC	5 - 3	45,1	13,9	1,04	13,4	2,3	T5 - T6
2018-04-04	Clemenpons nuwe	CC	3 - 5	46,4	11,5	1,04	11,0	0,4	T 7
2018-04-24	Clemenpons nuwe	CC	4 - 3	56,0	11,9	0,98	12,2	0,1	T4 - T5
2018-05-24	Clemenpons nuwe	CC	4 - 2	58,0	13,0	0,98	13,3	0,0	T1 - T2
2018-04-24	Esbal	CC	4 - 2	47,6	10,7	1,12	9,6	0,7	T5 - T6
2018-05-24	Esbal	CC	1 - 1x	43,6	11,6	0,93	12,5	1,5	T3 - T4
2018-04-04	Nules	CC	3 - 4	44,4	11,0	1,09	10,1	0,0	T 7
2018-04-24	Nules	CC	3 - 2	48,2	11,8	0,93	12,7	0,2	T5 - T6
2018-05-24	Nules	CC	2 - 1x	45,9	12,8	0,88	14,6	0,1	T2 - T3
2018-04-05	Clemenluz	CC	3 - 4	44,9	9,9	0,99	10,0	4,3	T7 - T8
2018-04-23	Clemenluz	CC	2 - 1	35,9	10,5	0,87	12,1	6,1	T6 - T8

2018-06-06	Clemenluz	CC	2 - 1x	38,7	10,7	0,86	12,4	0,8	T1
2018-04-23	Saratoga	CC	4 - 2	46,8	8,6	1,06	8,1	0,1	T6 - T8
2018-06-06	Saratoga	CC	2 - 1x	34,4	8,3	0,96	8,6	0,8	T1

5.4.26 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Clementine mandarins in a cold production region (South West Cape)

Project 1000E by W. Swiegers (CRI)

Summary

The trial site doesn't have a wide variety of selections at the moment. Buffeljagsrivier region will be one of the biggest Clementine trial sites in the future. The season started with Basol, followed by Nules and ended with Esbal. There was a clear indication of a delay in external fruit colour with a colour plate T4 for Nules. Esbal and Basol had a more promising colour development and peaked at colour plate T1 on the colour plate.

Opsomming

Die proef perseel het nie op die oomblik 'n wye verskeidenheid van seleksies nie. Buffeljagsrivier area gaan in die toekoms een van die grootste Clementine proef persele word. Die seisoen het begin met Basol, gevolg deur Nules en ge-eindig met Esbal. Daar was 'n definitiewe vertraging in eksterne vrugkleur met 'n kleurplaat T4 vir Nules. Esbal en Basol het 'n beter eksterne kleurontwikkeling met 'n kleurplaat T1.

Objectives

- To select Clementine cultivars with improved and consistent productivity, fruit size, rind colour, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the cultivar characteristics of new Clementine cultivars and to determine the climatic suitability of these cultivars in cold production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Clementine selections from the Buffeljagsrivier region of the South West Cape; the planting date was 2014. The following varieties were evaluated: Basol, Esbal and Nules.

A ratio of 11:1 Clementines 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 5.4.26.1. List of Clementine selections evaluated at Olivedale (Buffeljagsrivier) during 2018 season.

Selection	Rootstock	Planted
Basol	Carrizo	2014
Esbal	Carrizo	2014
Nules	Carrizo	2014

Results and discussion

Basol

Basol is an early maturing Clementine selection. Fruit size count for Basol was 2 - 5. Basol had a very good juice percentage towards peak maturity 64.4%. There were barely no seeds in this selection. This selection reached T1 at peak maturity. Basol had the highest Brix (11.1°) and acid (1.02%) towards peak maturity. The fruit peels easily. Basol's rind colour is deep orange. Basol have a very short harvest period before the fruit is over mature and start to granulate.

Esbal

Esbal was the last selection to reach peak maturity. Esbal mature later than Nules. Fruit size count for Esbal is 2 – 1x. Rind colour development was good with T1 (colour plate) when the fruit was at peak maturity. Fruit is round to oblate. Rind is smooth to pebbly. Peelability is easy but rind oil a bother. Seed count for Esbal peaked 1.2 seeds per fruit. Internal quality was good for Esbal, with good juice percentage.

Nules

Nules was the second selection to reach peak maturity. The selection had a good juice percentage just above (50%). Internal quality for Nules at peak maturity was good. Brix (11.8°) and acid (0.93%). This contributed to Nules good flavour. Nules seed count ranging from 0.9 – 1.1 seeds per fruit. Rind colour development was not good for Nules with a T4 on the colour plate at peak maturity. Nules had a good fruit size at count 3 - 1 (peak maturity). Yields were good for Nules. Peelability is easy and internal colour is orange.

Conclusion

Basol had the lowest seed count and Nules had the highest seed count. Basol and Esbal were the only selections to reach T1 on the colour plates. Degreening practices will be essential after harvesting to ensure optimal colour development for Nules. Basol had the highest Brix (13.1°). Basol had the smallest fruit size and peaked at count 5 - 3. Basol had the best juice percentage.

Table 5.4.26.2. Internal fruit quality data for Clementine selections in the Buffeljagsrivier region (Olivedale) of the South West Cape during the 2018 season.

Date	Cultivar	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg-Seed	Colour
2018-03-12	Basol	CC	To small - 5	48,1	12,1	1,23	9,8	0,0	T 5
2018-04-06	Basol	CC	2 - 5	64,4	11,1	1,02	10,9	0,1	T3 - T5
2018-04-26	Basol	CC	5 - 3	57,9	13,1	1,01	13,0	0,0	T 1
2018-04-26	Esbal	CC	5 - 2	49,9	9,7	1,05	9,2	0,0	T5 - T6
2018-05-21	Esbal	CC	3 - 1	53,5	10,7	0,97	11,0	0,4	T3 - T4
2018-06-19	Esbal	CC	2 - 1x	47,8	11,2	0,88	12,8	1,2	T 1
2018-04-26	Nules	CC	3 - 2	50,1	10,5	1,07	9,8	0,9	T5 - T7
2018-05-21	Nules	CC	3 - 1	50,4	11,8	0,93	12,7	1,1	T4

5.4.27 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Satsuma mandarins in a cold production region (Western Cape)

Project 57D by W. Swiegers (CRI)

Summary

The trial location is in an area well suited for Satsuma production. Most of the trees were planted in 2012. The trees look good with large tree canopies. The order of ripening was as follows; Miyagawa Wase started the season, followed by Aoshima, Miho Wase, Immamura, Sugiyama, Ueno, and Bela were the selection to finish the season.

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

Die proef se ligging is goed geskik vir Satsuma produksie. Die meeste bome is geplant in 2012. Die bome lyk goed met goeie boom volume. Die orde van rypwording was as volg: Miyagawa Wase het die seisoen begin gevolg deur Aoshima, Miho Wase, Imamura, Sugiyama, Ueno, en Bela het die seisoen klaargemaak.

Pluk periodes vir Satsumas sal strek van 2-3 weke aangesien vrugte se sure vinnig daal en die skil powerig 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 Satsuma selections from the Citrusdal region of the Western Cape. The following selections were evaluated: Aoshima, Sonet 2, Imamura, Miho Wase, Miyagawa Wase, Sugiyama, Ueno, Bela Bela.

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.

Table 5.4.27.1. List of Satsuma selections evaluated at Kweekkraal and Stargrow (Citrusdal) during 2018.

Selection	Rootstock	Planted
Aoshima	Carrizo	2013
Imamura	Carrizo	2012
Miho Wase	Carrizo	2011
Miyagawa Wase	Carrizo	2012
Sugiyama	Carrizo	2012
Ueno	Carrizo	2012
Bela	Carrizo	2012

Results and discussion

Aoshima

Aoshima is a mid – late maturing Satsuma. The selection reached peak maturity early in the season. Fruit size count for Aoshima ranged between counts 1xx - 1xxx. Aoshima was one of the selections with the biggest fruit size count. Ratio 12.8:1 the Brix was good (9.4°) as well as the acid percentage (0.73%). There were some seeds in the fruit 0.0 -0.2 seeds per fruit. The external colour development of the Aoshima was not good at all, with a T5 – T6 on the colour plate. The fruit is not pleasant to look at and was pebbly with some damage due to sunburn.

Miho Wase

Miho Wase was the third selection to mature in this trial site. The rind was smooth, and the fruit peeled easily. Fruit size for Miho Wase was mostly count 1 – 1xxx. The selection was seedless. Fruit colour on the colour plate was T5 – T6 at peak maturity. Fruit matured internally, rind colour development was delayed. Sugar at peak maturity was around 8.4° with an acid percentage around 0.74%.

Imamura

Imamura is a late maturing Satsuma. In this cold production region, it reached peak maturity mid-May. For a Satsuma, Imamura had a good Brix: Acid ratio, 9.4° and 0.69 % respectively, (ratio 13.6 well over mature). Seed count was one of the highest (0.2 – 0.8) seeds per fruit. External colour development was T2 - T5 on the colour plate. Internal colour was deep orange.

Bela

Bela is a new late maturing Satsuma selection. This was also the last selection to reach peak maturity in June. The fruit is large with a fruit size count of 1 – 1xxx. The rind was smooth to pebbly and peelability was easy. Internal colour is an excellent deep orange. At peak maturity the internal quality was good for Bela with Brix above 8.4° and acid above 0.84%. External colour development was delayed compared to the internal maturity with T6 on the colour plate. Seed count for the selection was the highest 0.3 – 1.2 seeds per fruit.

Miyagawa Wase

Miyagawa Wase was the first selection to reach peak maturity. The fruit size of Miyagawa Wase at peak maturity was count 1 – 1xxx. Brix: acid ratio 15:1; Brix was 8.2° and acid was 0.55%. Colour development was not good and delayed compared to the internal maturity. The colour on the colour plate at peak maturity was T6 – T7. Seed count for the selection was 0.1 – 0.3 seeds per fruit. Fruit was smooth and flat and the internal colour was deep orange.

Ueno

This selection is a mid to late maturing selection for this trial site. It reached peak maturity in May. Ueno had a large fruit size count with a 1xx – 1xxx count. The Brix° and acid percentage for Ueno towards peak maturity were 8.6° and 1.10% respectively. There were 0.0 – 0.8 seeds per fruit and Ueno colour on the colour plate at over maturity was T3 – T4. Fruit peeled easy.

Sugiyama

Sugiyama are mid to late maturing Satsuma. At this trial site it reached peak maturity in end April – beginning May. Sugiyama had good fruit size count at 1x - 1xxx. The Brix° and acid percentage of Sugiyama were 8.4° and 0.91% respectively towards peak maturity. Seed count for this selection ranged count 0.3 – 0.9 seeds per fruit. There was also a delay in colour development with a T7 on the colour plate close to peak maturity.

Conclusion

All the selections peaked with a large fruit size (count 1xxx). Aoshima and Imamura had the highest Brix° of all the Satsuma selections above (9°). Bela had the highest seed count with 1.2 seeds per fruit. Rind colour development was not good at T5 – T6 on the colour plate at peak maturity. Bela had the best internal colour.

Table 5.4.27.2. Internal fruit quality data for Satsuma selections in the Citrusdal region of the Western Cape during the 2018 season.

Date	Cultivar	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2018-04-24	Aoshima	CC	1xx - 1xxx	43,6	9,3	1,20	7,7	0,0	T5 - T8
2018-05-24	Aoshima	CC	1xxx	37,9	9,4	0,73	12,8	0,2	T5 - T6

2018-06-06	Bela	CC	1xx - 1xxx	37,1	8,4	0,84	10,1	0,3	T 6
2018-06-21	Bela	CC	1x - 1xxx	39,9	9,3	0,78	11,9	1,2	T 6
2018-04-23	Imamura	CC	1 - 1xx	49,4	9,1	1,43	6,3	0,8	T6 - T8
2018-06-06	Imamura	CC	1xxx	42,4	9,4	0,69	13,6	0,8	T2 - T5
2018-04-05	Miho Wase	CC	1 - 1xx	47,2	8,1	0,89	9,1	0,0	T6 - T8
2018-04-23	Miho Wase	CC	1xx - 1xxx	45,2	8,4	0,74	11,4	0,0	T5 - T6
2018-04-05	Miyagawa Wase	CC	1 - 1xxx	45,4	7,5	0,96	7,8	0,1	T 8
2018-04-23	Miyagawa Wase	CC	1xx - 1xxx	47,5	8,2	0,55	15,0	0,1	T5 - T7
2018-04-23	Sugiyama	CC	1x - 1xxx	47,5	8,4	0,91	9,2	0,9	T 7
2018-06-06	Sugiyama	CC	1xxx	33,5	8,2	0,61	13,4	0,3	T2 - T4
2018-04-23	Ueno	CC	1xx - 1xxx	45,2	8,6	1,10	7,8	0,4	T 8
2018-06-06	Ueno	CC	1xxx	41,6	9,1	0,68	13,4	0,0	T3 - T4

6 CITRUS IMPROVEMENT SCHEME (CIS)

P.H. Fourie, J.B. Meyer, M.M.N. du Toit, M.C. Mlangeni, M. le Roux, M. Ferreira, J.H.J. Breytenbach, C. Steyn and G. Cook (CRI)

Summary

The South African Citrus Improvement Scheme (CIS) strives to ensure a profitable citrus industry that is established with high quality citrus trees that are free from diseases and horticulturally true to type. Certified rootstock seed and budwood are supplied from the Citrus Foundation Block (CFB) outside Uitenhage. Following a record budwood supply year in 2016/17, which was dominated by lemon and mandarin supply, certified budwood supply in 2017/18 and 2018/19 again exceeded all previous records. A total of 7.34 million buds were supplied by the CFB or authorized for cutting in certified nurseries. Lemon demand declined from 14.8% to 6.1%, whilst mandarin supply increased from 43.5% to 48.2%, and Valencia from 18.3% to 19.1%. For the latter, 'Midnight' Valencia demand rose from 381 thousand in 2016/17 to 700 thousand buds in 2017/18 and 705 thousand in 2018/19. A huge increase in 'Star Ruby' demand was also experienced, from 106 thousand in 2016/17, to 299 thousand in 2017/18, to almost 349 thousand buds in 2018/19. Amidst the rise in budwood and rootstock seed demands, CFB's ability for primary supply in 2018/19 decreased from 67.8% in 2017/18 to 61.8% in 2018/19. Budwood stock of the 412 cultivars at CFB must be constantly managed to meet demand of sought-after varieties. In 2018/19, 19-thousand new multiplication trees were produced, 10-thousand redundant trees were removed, and the rootstock seed orchards are being expanded by another 2.5 ha.

Opsomming

Die doelwit van die Suid-Afrikaanse Sitrus Verbeteringskema (SVS) is om die winsgewendheid van die suider-Afrikaanse sitrusbedryf te verbeter deur te verseker dat die industrie gevestig word met hoë kwaliteit, siektevrye kwekerybome wat tuinboukundig tipe-eg is. Gesertifiseerde okuleerhout en saad word voorsien vanaf die in Sitrus Grondvesblok buite Uitenhage. Die rekord jaar in 2016/17, wat hoofsaaklik oorheers is deur aanvraag na suurlemoene en mandaryne, is in 2017/18 en 2018/19 het weereens alle vorige rekords oortref. 'n Totaal van 7.34 miljoen ogies is deur die Grondvesblok in samewerking met gesertifiseerde kwekerye verskaf. Die aanvraag na suurlemoene het van 14.8% tot 6.1% gedaal terwyl mandaryne van 43.6% tot 48.2%,

en Valencias van 18.3% tot 19.1%, gestyg het. Die vraag na 'Midnight' Valencia het van 381-duisend in 2016/17 tot 700-duisend in 2017/18 en 705-duisend ogies in 2018/19 gestyg. Daar was ook 'n groot aanvraag na 'Star Ruby' waarvan die verskaffing van 106-duisend in 2016/17, tot 299-duisend in 2017/18 en tot byna 349-duisend ogies in 2018/19 gestyg het. Te midde van die verhoogde aanvraag na okuleerhout en saad het die Grondvesblok die primêre verskaffing van 67.8% in 2017/18 tot 61.8% in 2018/19 gedaal. Okuleerhout voorraad van 412 kultivars moet konstant bestuur word om soveel as moontlik van die hoë aanvraag kultivars te kan verskaf: in 2018/19 is 19-duisend nuwe vermeerderingsbome gemaak, 10-duisend onnodige bome verwyder en die vestiging van nog 2.5 ha onderstam boorde gaan voort.

6.1 Introduction

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 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.

6.2 Budwood

This report summarises the seasonal supply of budwood from 1 July 2018 to 30 June 2019. A record number of 7.34 million buds were supplied by the Citrus Foundation Block (CFB) and authorised for cutting in certified nurseries (BCIN). This is 2.6% more buds than in the same period of 2017/18 and 9.2% more buds than in the same period of 2016/17. During this period 32 489 buds were exported to neighbouring countries.

Budwood demand generally increased in volume and was mostly from Western Cape (37.5%), Limpopo (28.8%), followed by the Eastern Cape (15.4%), Mpumalanga (8.3%) and the other provinces ranging from % to 0.3%.

Mandarin (48.2%) was the most popular citrus type, followed by Valencia (19.1%), navel (12.2%), Clementine (7.4%), lemon (6.1%) and grapefruit (4.9%); in 2017/18 this proportion was 43.5%, 18.3%, 10.9%, 6.4%, 14.8% and 4.7%, respectively (Tables 6.2.1 and 6.2.2). Valencia demand was stable in recent years with a 3-year average of 560 thousand buds. In the past two seasons, we experienced an unexpected increase and 1.3 and 1.4 million buds were supplied in 2017/18 and 2018/19, consequently surpassing the lemon demand. Whilst supply of lemon budwood decreased by 57.5% from 2017/18 (and 79.8% from 2016/17), it was still significant at 401 thousand buds supplied during 2018/19, ranking Eureka lemon in the 6th position in the top 30. Navel demand also increased slightly with 12.9% to 895 thousand buds. In previous seasons, a huge increase in Clementine bud demand was observed (219.8%, 19.1% and 81.4% in 2014/15; 2015/16 and 2016/17, respectively), but a slight decrease of 19.8% from 2016/17 levels was observed in 2017/18 to a total of 459 thousand buds. In 2018/19 an increase of 18.2% was seen, increasing supply to 542 thousand buds. The Clementine demand is still significant considering that the 10-year average in 2013/14 was 62 thousand buds. Grapefruit demand has been low for a number of years and increased from a low base of 45-, 77-, and 144 thousand buds in 2014/15, 2015/16 and 2016/17, respectively, to 336 thousand buds supplied in 2017/18, followed by 358 thousand in 2018/19. This increase of 136.2% is significant, but recent supply figures are still low when compared to the 10-year average of 535 thousand buds during the 1990's.

The top 30 cultivars comprised 93.4% of total number of buds supplied. ARC Nadorcott LS (ARCCIT9) was the most popular cultivar, followed by Midnight, Tango, RHM, Leanri, Eureka, Nules, Star Ruby and Or 4

(Table 6.1.3). ARC Nadorcott LS supply levels have increased year on year from 29 160 to 267 422 to 513 582 to 1 080 328 to 1 191 497 in 2018/19, and together with the other protected Mandarin varieties in the top 10, such as Tango, RHM, Leanri and Or 4, contributed to 40.7% to the total budwood supply. The majority of these buds were BCIN supplied in 2018/19, the highest being RHM (84.9%), ARC Nadorcott LS (63.1%) and Tango (53.5%). CFB has successfully increased the supply of Or 4 which resulted in a decrease in required BCINs from 15.2% to 2.8%), as well as Leanri, where the BCINs decreased from 65.3% to 39.1% in 2018/19. The top 10 cultivars (5.05 million) comprised 68.9% of all of the budwood supplied, of which 54.5% were supplied from the CFB (Table 6.1.3).

The overall CFB supply decreased from 67.8% in 2017/18 to 61.8% in 2018/19. BCIN proportion per variety type: mandarins (62.0% of which ARC Nadorcott LS (ARCCIT9), RHM, Tango and Leanri comprised 57.3%, and Lea and Or4 a further 3.7%), Valencia's (18.3% of which Midnight comprised 11.3%), Clementine (7.4% of which Nules comprised 5.4%), grapefruit (5.5% comprising only of Star Ruby), navel (5.9%) and other (0.9%) (Figures 6.2.1-7).

Table 6.1.1. Buds supplied during the period July to June 2016/17-2018/19.

Area	2016/17	Dist %	2017/18	Dist %	2018/19	Dist %
Local	6 719 186	99.9%	7 131 525	99.7%	7 308 920	99.6%
Eastern Cape	1 535 698	22.8%	1 448 102	20.2%	1 132 631	15.4%
Gauteng	96 400	1.4%	120 267	1.7%	242 275	3.3%
KwaZulu Natal	101 300	1.5%	47 600	0.7%	19 320	0.3%
Limpopo	1 899 713	28.2%	2 491 852	34.8%	2 112 137	28.8%
Mpumalanga	267 210	4.0%	460 315	6.4%	611 229	8.3%
North West	99 280	1.5%	164 000	2.3%	138 910	1.9%
Northern Cape	488 866	7.3%	275 950	3.9%	299 629	4.1%
Western Cape	2 230 719	33.2%	2 123 439	29.7%	2 752 789	37.5%
International	6 400	0.1%	24 355	0.3%	32 489	0.4%
Botswana	6 000	0.1%	11 000	0.2%	500	0.0%
India		0.0%		0.0%	1 250	0.0%
Mozambique		0.0%	3 965	0.1%		0.0%
Netherlands	400	0.0%		0.0%		0.0%
Nigeria		0.0%	4 000	0.1%	1 000	0.0%
USA		0.0%	40	0.0%	139	0.0%
Zambia		0.0%		0.0%	900	0.0%
Zimbabwe		0.0%	5 350	0.1%	28 700	0.4%
Total	6 725 586	100.0%	7 155 880	100.0%	7 341 409	100.0%

Table 6.1.2. Buds supplied during the period July to June 2016/17-2018/19.

Variety Type	2016/17	Dist %	2017/18	Dist %	2018/19	Dist %
Local	6 719 186	99.9%	7 131 525	99.7%	7 308 920	99.6%
Mandarin Hybrid	2 524 448	37.5%	3 114 568	43.5%	3 542 094	48.2%
Valencia	679 739	10.1%	1 306 702	18.3%	1 405 315	19.1%
Navel	457 723	6.8%	782 608	10.9%	894 572	12.2%
Clementine	572 153	8.5%	458 217	6.4%	540 740	7.4%
Grapefruit	144 428	2.1%	335 450	4.7%	358 214	4.9%
Lemon	2 229 113	33.1%	1 056 903	14.8%	447 657	6.1%
Lime	13 335	0.2%	29 830	0.4%	25 385	0.3%
Satsuma	66 583	1.0%	29 840	0.4%	35 145	0.5%
Midseason	8 844	0.1%	1 480	0.0%	34 860	0.5%
Kumquat	4 040	0.1%	8 300	0.1%	12 355	0.2%
Diverse	15 630	0.2%	2 107	0.0%	5 248	0.1%

Rootstock	3 150	0.0%	200	0.0%	2 410	0.0%
Pummelo		0.0%	5 320	0.1%	4 925	0.1%
International	6 400	0.1%	24 355	0.3%	32 489	0.4%
Total	6 725 586	100.0%	7 155 880	100.0%	7 341 409	100.0%

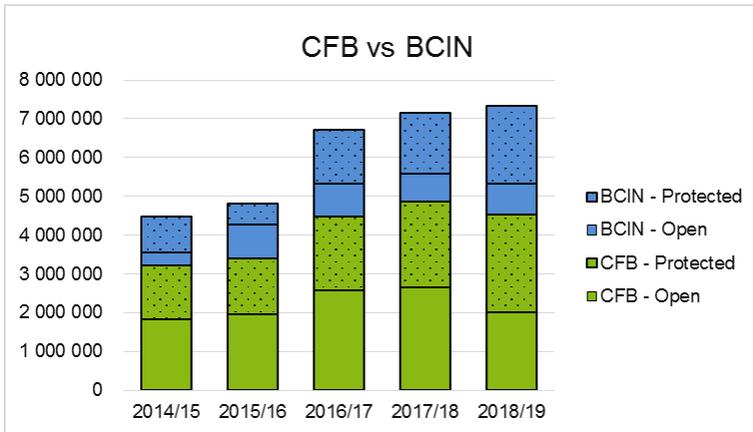


Figure 6.2.1. Budwood of open and protected cultivars (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2014/15-2018/19.

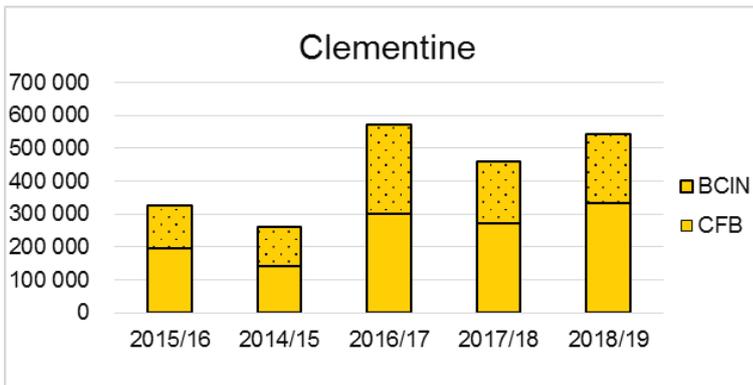


Figure 6.2.2. Clementine budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2015/16-2018/19.

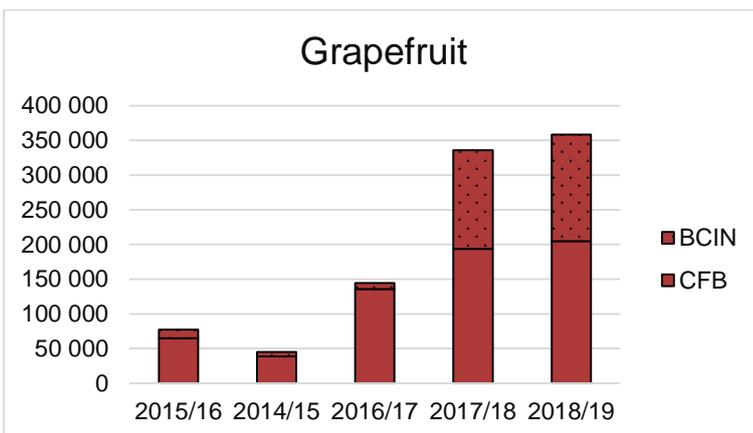


Figure 6.2.3. Grapefruit budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2015/16-2018/19.

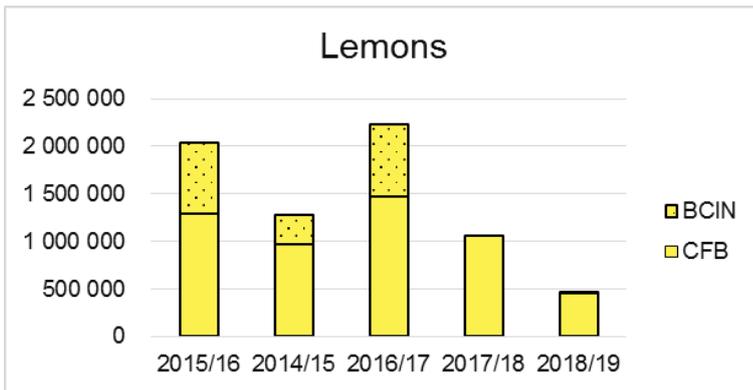


Figure 6.2.4. Lemon budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2015/16-2018/19.

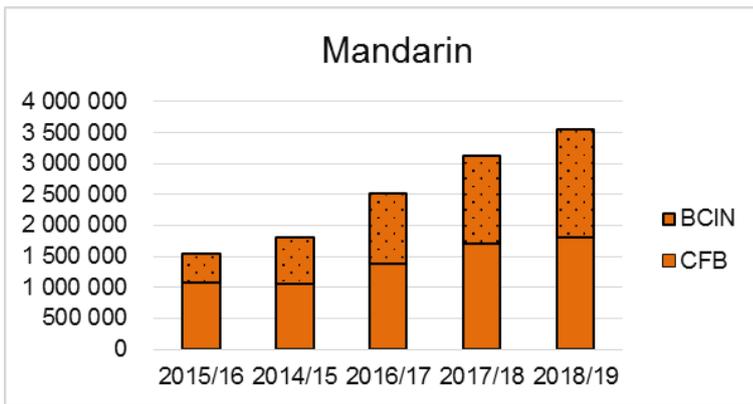


Figure 6.2.5. Mandarin hybrid budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2007/08-2017/18.

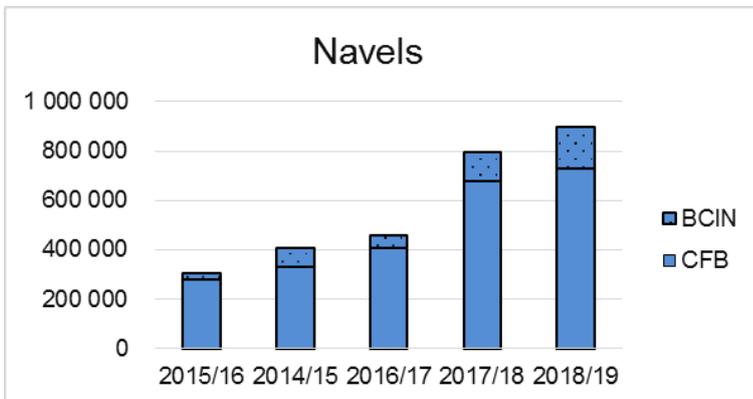


Figure 6.2.6. Navel budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2007/08-2017/18.

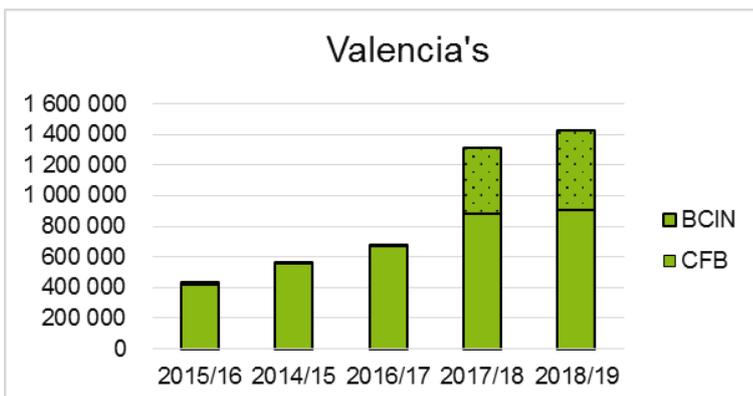


Figure 6.2.7. Valencia budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2007/08-2017/18.

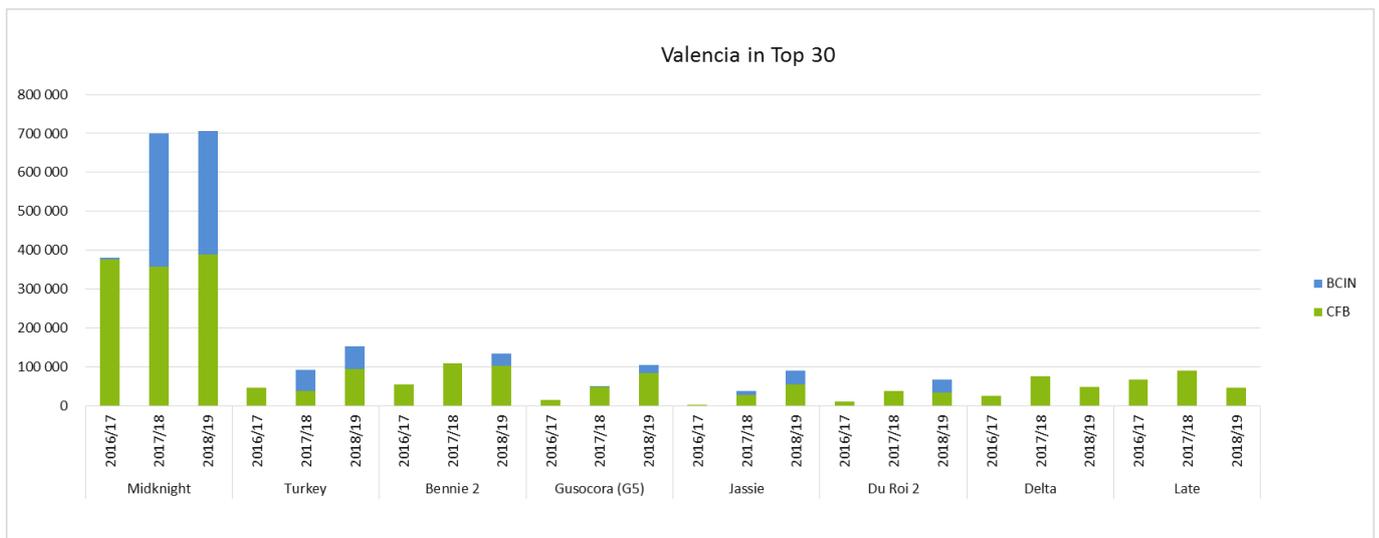


Figure 6.2.7. Budwood supply (BCIN/CFB) of the Valencia cultivars in the top 30 in 2018/19.

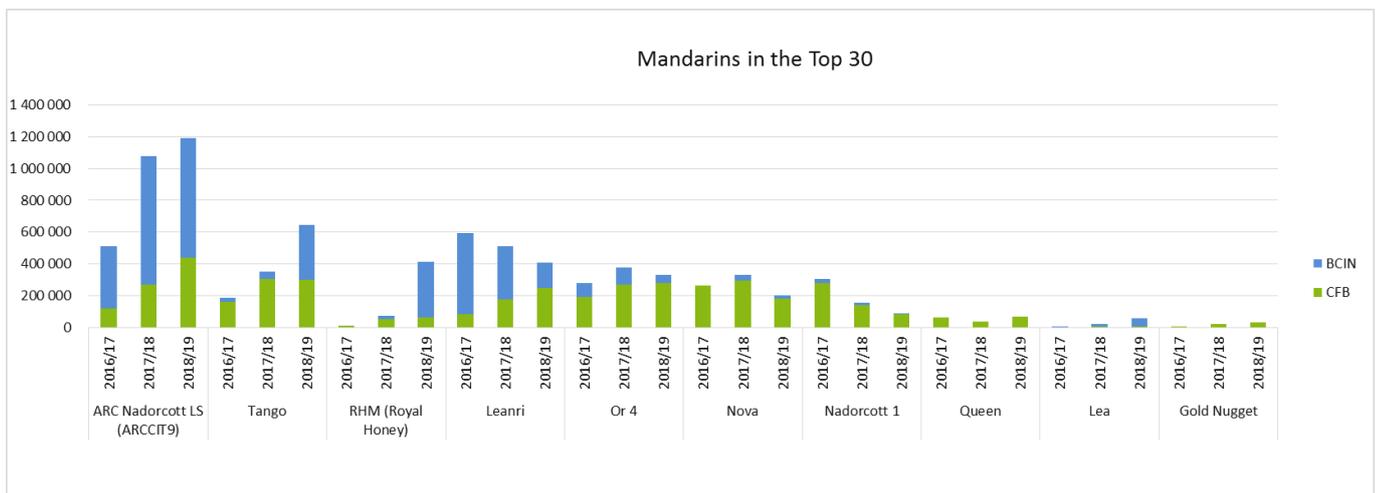


Figure 6.2.8 Budwood supply (BCIN/CFB) of the Mandarin cultivars in the top 30 in 2018/19.

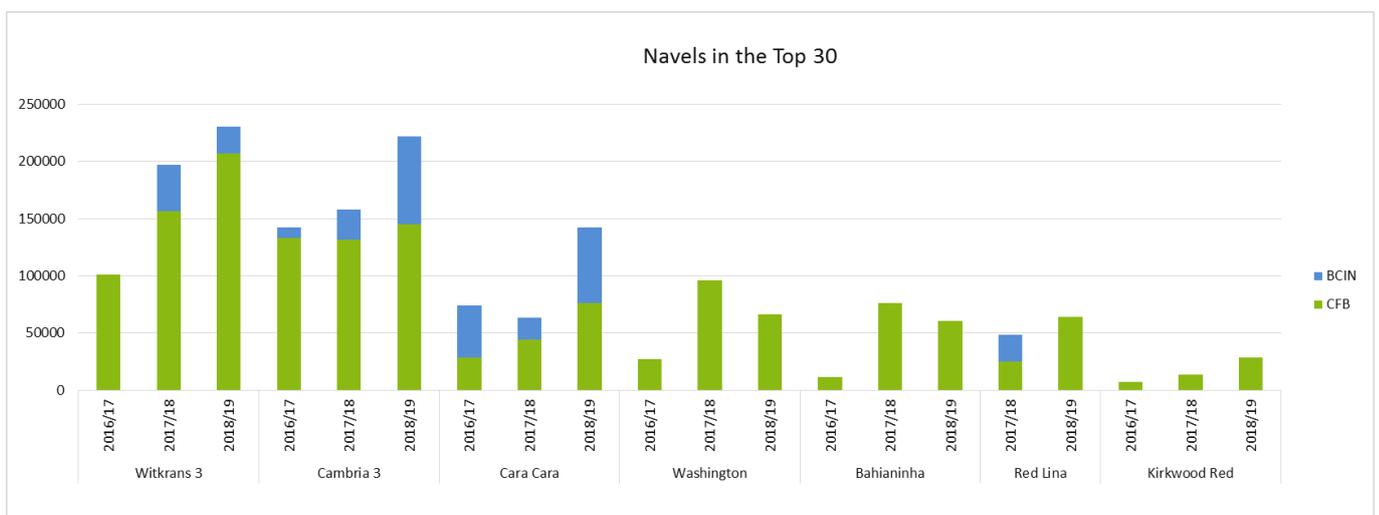


Figure 6.2.9 Budwood supply (BCIN/CFB) of the Navel cultivars in the top 30 in 2018/19.

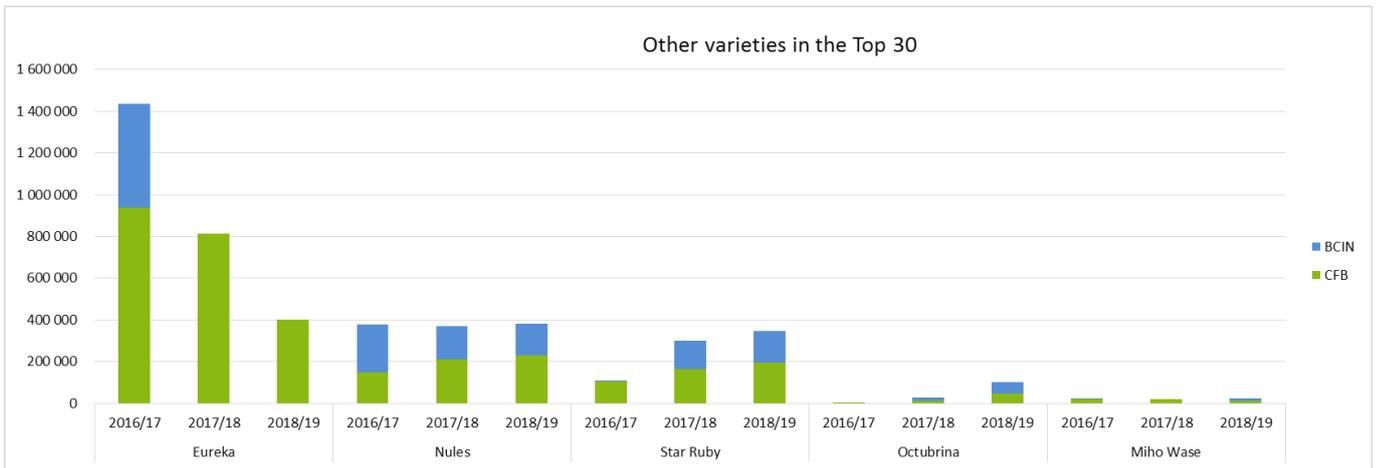


Figure 6.2.9 Budwood supply (BCIN/CFB) of the other variety types in the top 30 in 2018/19.

Table 6.2.2. Buds supplied per variety type per area (total number of buds per season) during the periods July to June from 2016/17-2018/19.

Variety Type	Year	EC	GP	KZN	LIM	MPU	NC	NW	WC	Local	International	Total
Clementine	2016/17	87 133	5 250		34 420	580	44 400	2 450	397 920	572 153		572 153
	2017/18	89 782	22 000		45 475	11 080	4 200	2 000	283 680	458 217	500	458 717
	2018/19	48 490	24 500		20 880	465	18 600	3 300	424 505	540 740	1 400	542 140
Diverse	2016/17	60		1 000	1 030	2 400		3 000	8 140	15 630	200	15 830
	2017/18		1 087		200			200	620	2 107		2 107
	2018/19	2 878	430		750				1 190	5 248		5 248
Grapefruit	2016/17	15 025	15 500	5 500	65 943	39 815			2 645	144 428		144 428
	2017/18	22 150	9 000	6 200	141 060	115 800	14 200	5 800	21 240	335 450	300	335 750
	2018/19	18 520	29 254		135 042	76 828	17 410	9 570	71 590	358 214		358 214
Kumquat	2016/17	70				1 020			2 950	4 040		4 040
	2017/18		880			1 200		2 000	4 220	8 300		8 300
	2018/19	1 000	1 285	2 000	1 700	600		5 000	770	12 355		12 355
Lemon	2016/17	778 248	100	44 000	571 694	89 750	202 000	22 700	520 621	2 229 113	200	2 229 313
	2017/18	189 239	22 500	18 400	515 050	68 265	35 800	40 700	166 949	1 056 903	3 000	1 059 903
	2018/19	213 150	7 960	9 320	44 850	25 152	39 000	15 000	93 225	447 657	2 350	450 007
Lime	2016/17	520		1 500	250	3 750			7 315	13 335		13 335
	2017/18	3 830	1 870		3 950	7 540	1 000	1 000	10 640	29 830		29 830
	2018/19	2 350	1 795		3 900	8 880		600	7 860	25 385	100	25 485
Mandarin Hybrid	2016/17	388 259			903 871	85 710	213 466	30 580	902 562	2 524 448	1 000	2 525 448
	2017/18	471 630	2 105	3 000	1 285 141	152 825	125 550	33 190	1 041 127	3 114 568	3 650	3 118 218
	2018/19	384 247	3 368		1 354 565	248 192	106 271	28 940	1 416 511	3 542 094	10 450	3 552 544
Midseason	2016/17	50							8 794	8 844		8 844
	2017/18					60	600		820	1 480		1 480
	2018/19	250				405			34 205	34 860		34 860
Navel	2016/17	141 022	37 050	8 500	75 805	13 455	17 800	20 650	143 441	457 723	2 000	459 723
	2017/18	287 817	9 800	4 500	123 180	39 575	33 700	32 260	251 776	782 608	10 640	793 248
	2018/19	295 195	7 840	3 500	97 650	54 197	30 900	34 710	370 580	894 572	939	895 511
Pummelo	2017/18	970			4 350					5 320		5 320
	2018/19				3 750	1 175				4 925	150	5 075
Rootstock	2016/17	150							3 000	3 150		3 150
	2017/18	100				100				200		200
	2018/19	1 550	200				450		210	2 410		2 410

Satsuma	2016/17	12 234			10 250	1 155	4 700	500	37 744	66 583	1 000	67 583
	2017/18	19 475		3 000	2 000	35			5 330	29 840		29 840
	2018/19	19 770		2 500	7 250	585			5 040	35 145		35 145
Valencia	2016/17	112 927	38 500	40 800	236 450	29 575	6 500	19 400	195 587	679 739	2 000	681 739
	2017/18	363 109	51 025	12 500	371 446	63 835	60 900	46 850	337 037	1 306 702	6 265	1 312 967
	2018/19	145 231	165 643	2 000	441 800	194 750	86 998	41 790	327 103	1 405 315	17 100	1 422 415

Table 6.1.3. Top 30 cultivars based on total number of buds supplied for seasons July to June from 2016/17-2018/19.

2016/17			2017/18			2018/19		
Cultivar	BCIN	CFB	Cultivar	BCIN	CFB	Cultivar	BCIN	CFB
Eureka LEM	499227	936185	ARC Nadorcott LS MAN**	813829	266499	ARC Nadorcott LS MAN	751 775	439 722
Leanri MAN	509643	82990	Eureka LEM		814031	Midnight VAL	316 785	388 445
ARC Nadorcott LS MAN **	394532	119050	Midnight VAL	343749	356781	Tango MAN	346 000	301 198
2PH Eureka SL LEM	250755	138482	Leanri MAN	334150	177509	RHM MAN #	351 738	62 535
Midnight VAL	5000	376247	Or 4 MAN	108360	266852	Leanri MAN	159 344	248 207
Nules CLE	229281	149535	Nules CLE	157661	212663	Eureka LEM		401 372
Nadorcott 1 MAN	26000	278555	Tango MAN	50000	303580	Nules CLE	150 000	231 433
Or 4 MAN	89610	191596	Nova MAN	37300	294375	Star Ruby GFT	153 685	195 049
Nova MAN		266245	Star Ruby GFT	136550	162610	Or 4 MAN	50 400	280 209
Lisbon LEM		209289	Witkrans 3 NAV	40950	156240	Witkrans 3 NAV	23 400	206 722
Tango	26791	160320	Cambria 3 NAV	26875	131252	Cambria 3 NAV	76 697	145 407
Andes 1 Clemenluz CLE	20244	127320	Nadorcott 1 MAN	17000	140700	Nova MAN	16 900	183 929
Cambria 3 NAV	9200	133291	Bennie 2 VAL		110148	Turkey VAL	58 143	94 670
ARCCIT1614 MAN *		109484	Washington NAV		96270	Cara Cara NAV	65 846	76 071
Star Ruby GFT	1100	105058	Turkey VAL	53600	38400	Bennie 2 VAL	31 433	103 700
Witkrans 3 NAV		101336	Late VAL		90681	Gusocora (G5) VAL	19 176	85 215
Limoneira 8A LEM		99219	Bahianinha NAV		75980	Octubrina CLE	53 570	48 353
IR M2 MAN	58610	21309	Delta VAL		75203	Nadorcott 1 MAN	8 500	82 484
Cara Cara NAV	45502	28680	RHM MAN #	20000	52359	Jassie VAL	34 603	55 842
Genoa LEM	4300	68281	Lisbon LEM		69065	Queen MAN		67 629
Late VAL		67365	2PH Eureka SL LEM		67700	Du Roi 2 VAL	33 050	33 526
Queen MAN		64240	Limoneira 8A LEM		63470	Washington NAV		65 870

Bennie 2 VAL		55650	Cara Cara NAV	19030	44151	Red Lina NAV		64 341
Turkey VAL		46612	IR M2 MAN	18300	33899	Bahianinha NAV		60 120
Tambor MAN	33116	5130	Gusocora (G5) VAL	2120	49635	Lea MAN	53 320	5 895
Lavalle VAL		36870	Red Lina NAV	23100	25070	Delta VAL		48 379
Belabela SAT	7415	27779	Alpha VAL		39520	Late VAL		46 880
Esbal CLE	20700	8362	Queen MAN		39010	Gold Nugget MAN		29 800
Mor 26 MAN		27248	Fischer NAV		37801	Kirkwood Red NAV		28 213
Washington NAV		27006	Du Roi 2 VAL		37780	Miho Wase SAT	12 950	11 310
Top 30	2 231 026	4 068 734	Top 30	2 202 574	4 329 234	Top 30	2 767 315	4 092 526
> Top	20 500	405 326	> Top	98 508	525 564	> Top	38 640	442 928
Total	2 251 526	4 474 060	Total	2 301 082	4 854 798	Total	2 805 955	4 535 454
	33.5%	66.5%		32.2%	67.8%		38.2%	61.8%

* ARCCIT1614 (B17) (Valley Gold) MAN

** ARCCIT9 (ARC Nadorcott LS) MAN

RHM (Royal Honey) MAN

6.3 Seed

A significant decrease in the fruit and seed per fruit yield were experience during 2018/19, leading to a reduction in supply of most rootstock varieties. The supply in Carrizo Citrange decreased with 63% from 2017/18, C35 Citrange (64% less) and Swingle Citrumelo (41% less). Rough Lemon were affected to a lesser extent and only a 9% reduction in the seed supply were seen. Even though the exact cause could not be identified, it is suspected that alternate bearing, affecting fruit set, as well as a cold spell during flowering, negatively affected pollination, could be contributing factors. Similar reductions in fruit and seed yields were experienced by nurseries with seed trees in the Eastern Cape. Yield estimate strategies were developed to improve communication with the nurseries.

During May to April 2019, 3412 litres of seed were supplied locally (Table 6.3.1) and 75 litres of seed were exported to SADC countries (Table 6.3.1). Carrizo citrange remains the most popular rootstock (31.7%), followed by Swingle Citrumelo (14.6%), Rough Lemon (8.1%), C35 Citrange (7.8%), X639 (6.8%) and Troyer citrange (6.1%).

The shift in demand towards Mandarins, Valencia's and navels has resulted in higher orders of trifoliolate hybrid cultivars and the demand exceeded the supply in most cases in 2018/19 (Figure 6.3.1). The supply data for 2018/19 were influenced by the reduced yield.

Because of the yield reduction as discussed above, as well as the higher demand when compare with previous seasons, supply did not meet the extremely high demand (Table 6.3.1) and in 2018/19 CRI imported Carrizo citrange (CC), Troyer citrange (TC), Rough Lemon (RL) and Volckameriana (VA) on behalf of nurseries from Australia and the USA.

Table 6.3.1. Seed (litres) supplied by the CFB during the periods May to April 2016/17-2018/19.

Area	2016/17	Dist %	2017/18	Dist %	2018/19	Dist %
Local	4 749	94.5%	6 737	99.0%	3 412	97.9%
Eastern Cape	613	12.2%	958	14.1%	322	9.2%
Gauteng	22	0.4%	131	1.9%	53	1.5%
KwaZulu Natal	27	0.5%	70	1.0%	25	0.7%
Limpopo	1 559	31.0%	2 473	36.3%	1 557	44.7%
Mpumalanga	338	6.7%	417	6.1%	228	6.5%
North West	179	3.6%	179	2.6%	81	2.3%
Northern Cape	352	7.0%	694	10.2%	248	7.1%
Western Cape	1 660	33.0%	1 817	26.7%	898	25.8%
International	277	5.5%	69	1.0%	75	2.1%
Botswana	2	0.0%	4	0.1%		0.0%
Chile	201	4.0%		0.0%		0.0%
Mauritius		0.0%	1	0.0%		0.0%
Mozambique	60	1.2%	6	0.1%	0	0.0%
South America		0.0%	12	0.2%		0.0%
Swaziland	7	0.1%	32	0.5%	14	0.4%
Zambia	7	0.1%	11	0.2%	3	0.1%
Zimbabwe		0.0%	3	0.0%	58	1.6%
Total	5 026	100.0%	6 806	100.0%	3 486	100.0%

Table 6.3.2. Seed (litres) supplied by the CFB during the periods May to April 2016/17-2018/19.

Rootstock cultivar	2016/17	Dist %	2017/18	Dist %	2018/19	Dist %
CFB	4841	96.3%	6168	90.6%	3113	84.2%
79AB*	6	0.1%		0.0%		0.0%
79AC*	6	0.1%	0	0.0%		0.0%

BC	20	0.4%	20	0.3%	15	0.4%
C35	644	12.8%	816	12.0%	290	7.8%
CC	2446	48.7%	3105	45.6%	1174	31.7%
CM		0.0%	0	0.0%		0.0%
MXT	222	4.4%	179	2.6%	151	4.1%
RL	475	9.5%	329	4.8%	299	8.1%
SC	670	13.3%	914	13.4%	541	14.6%
SXB	22	0.4%	26	0.4%	41	1.1%
TC		0.0%	347	5.1%	227	6.1%
VA	92	1.8%	108	1.6%	110	3.0%
X639	224	4.5%	307	4.5%	252	6.8%
YC	15	0.3%	19	0.3%	15	0.4%
Imported	139	2.8%		0.0%	155	4.2%
SPIN**	46	0.9%	638	9.4%	431	11.6%
Total	5026	100.0%	6806	100.0%	3698	100.0%

*Experimental **Seed produced in nurseries

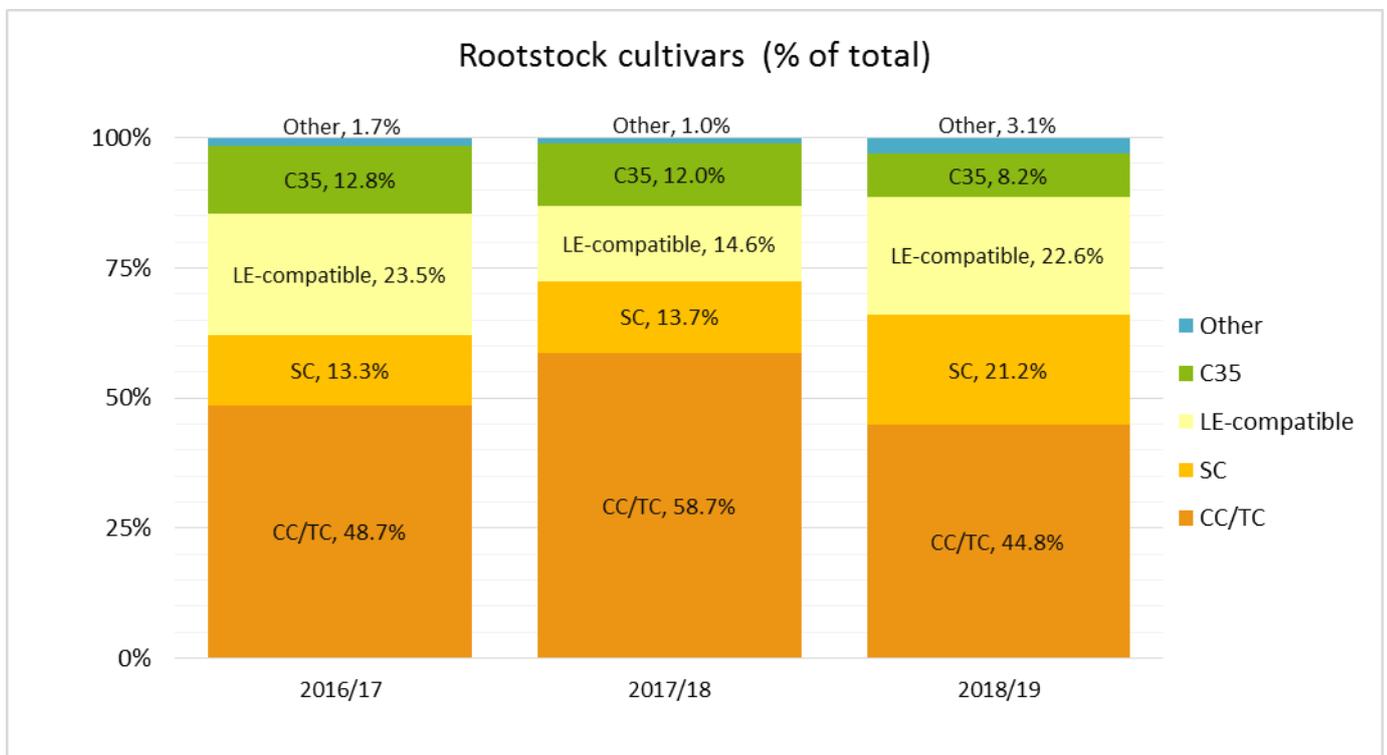


Figure 6.3.1. Rootstock cultivars grouped by type (percentage of total litres of seed per season) supplied by the CFB and seed produced by nurseries (SPIN) during the periods May to April from 2016/17-2018/19.

6.4 Production

Budwood: CFB presently maintains 147 424 multiplication trees of approximately 412 cultivar lines with a potential annual budwood stock of >10.6 million buds. The STG facilities in Nelspruit, CRI has released 7 new cultivars to the CFB. The ARC also introduced 8 new cultivars and re-introduced 6 existing cultivars to the CFB (Table 6.4.1). In order to address budwood demand, multiplication of tree stocks remained a priority for the CFB and 19 115 new multiplication trees, representing 57 cultivar lines, were established in 2018/19. These trees will contribute to an estimated 1.3 million buds in the next 1-2 years. In 2018/19 the top 30 varieties comprised 93.4% of demand. Where BCIN was needed or market demand increase expected, multiplication was planned accordingly. However, due to the continual high demand for specific cultivars, budwood shortages was experienced for 21 cultivars in the top 30. Demand for Royal Honey Mandarin increased by 82% (from 72 thousand in 2017/18 to 414 thousand in this season). Demand for Tango nearly doubled again while demand for ARC Nadorcott LS Mandarin stabilised at 1.19 million compared to 1.08 million. CFB managed to

supply 40% more ARC Nadorcott LS Mandarin budwood than 2017/18. To address space constraints, >10 thousand redundant multiplication trees were removed.

Two trees of 311 cultivars were planted out during October 2016 in newly established evaluation block at the CFB. Another set of trees of 88 cultivars were planted in November 2018 and February 2019. These trees were successfully used for true-to-type evaluation in 2018. As new cultivars are release to the CFB, a set of two trees will be planted out in the evaluation block on an ongoing basis. Two new rows of mother trees were established in Greenhouse 5.

Table 6.4.1. Cultivar introductions from 2014/15 – 2018/19.

Area	2014/15	2015/16	2016/17	2017/18	2018/19
ARC: New introductions	17	15	7	14	8
ARC: Existing lines with new CTV Strain			7	15	6
CRI: New introductions	18		13	7	7
CRI: Reintroductions from the Nucleus Block			8	21	
CFB: Re-multiplication of existing cultivar lines	13	58	35	84	57

Seed: Meeting the continued high demand for seed in 2018/19 remained a challenge. As planned, half of the 1362 high density trees planted in 2014/15 were re-established in adjacent orchards during July/Aug of 2018 and it was estimated that these trees contributed to about 20% of the harvest in 2018. The 318 rootstock trees of high demand and experimental cultivars that were planted in the spring of 2017 is expected to start bearing next season. The planned planting of approximately 729 new trees of the high demand seed cultivars was postponed to Spring 2019. The trees are currently in 10 L bags in the tunnels. Some trees in older orchards will be removed in 2019/20 or 2020/21 to make space for greenhouse expansion. For biosecurity reasons, it would be prudent if no citrus trees at CFB are outside insect-secure structures. To this end, CFB is investigating the acquisition of a farm > 5km away from CFB to establish the seed orchards (10-year plan). The current season's harvest is pointing to an on-year and Gibb sprays will be prioritised during the spring of 2019 as a measure against the possible off-year that may follow. Imidacloprid applications will be applied only after flowering in September 2019 and CFB is investigating the introduction of bees during the flowering season. In 2019, active collection of harvest data from important orchards is underway to assist with future harvest estimates.

6.5 Tree Certification

There were 4 339 244 trees certified during April to March 2018/19 (Table 6.5.1). This is 511 778 more than in 2017/18. Of the applications received, the trees not meeting the certification requirement were 30 898 (0.71% of the applications), 22 798 (0.59%) and 72 753 (1.75%) for the last three consecutive years. This was mostly because of the Phytophthora status or tree age that exceeded 30 months after budding. 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.

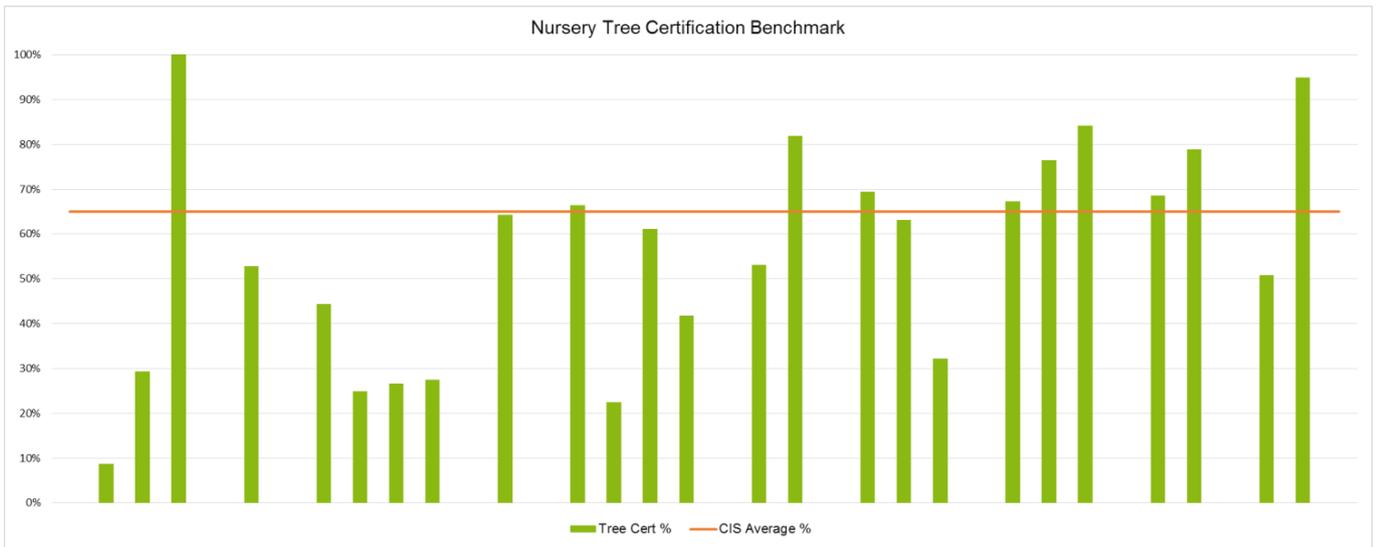


Figure 6.5.1 Nursery trees certified as a percent of the budwood supplied. The data of a specific range of budwood supplied (older than 30) was compared to the trees certified within that range. The overall buds supplied vs trees certified percentage of 65% was used as benchmark.

Table 6.5.1. Trees certified during the period April to March from 2016/17-2018/19.

Variety Type	Year	EC	FS	GP	KZN	LIM	MPU	NC	NW	WC	EXP	Total
Clementine	2016/17	41 071		10 738		9 660	20 970	2 275	1 700	95 639		182 053
	2017/18	68 354				14 900	12 677	1 238		137 085	550	234 804
	2018/19	92 274		5 520		29 470	7 125	5 330		243 465	9 300	392 484
Diverse	2016/17				900	9 420				1 480		11 800
	2017/18									1 700		1 700
	2018/19	575				290	550					1 415
Grapefruit	2016/17			8 830	10 000	18 793	7 800		1 500	150	1 000	48 073
	2017/18			11 948	9 000	50 758	8 690		400	1 600		82 396
	2018/19	6 961		3 970	18 000	102 561	35 499	2 370	20		4 320	173 701
Lemon	2016/17	523 861	1400	4 003	23 470	273 239	321 679	1 700	12 200	311 714	5 800	1 479 066
	2017/18	509 024		3 051	21 250	346 966	138 397		11 181	258 453	17 335	1 305 657
	2018/19	275 083	100	20 044	11 530	98 620	78 612	14 950	24 469	117 881	11 846	653 135
Lime	2016/17	1 000			240	2 100	1 600			150		5 090
	2017/18					500				4 688	200	5 388
	2018/19	155			769	2 814	150			1 612	2 695	8 195
Navel	2016/17	116 221	2230	9 585		90 090	71 431	2 710	8 600	36 816	14 810	352 493
	2017/18	60 945		2 700		20 285	55 312	3 174	6 680	33 538	4 800	187 434
	2018/19	124 265	1660	21 970	6 660	51 098	31 495	3 050	21 585	47 512	19 010	328 305
Satsuma	2016/17	3 173					1 400	10 000		22 071		36 644
	2017/18	1 661				300				12 162	3 650	17 773
	2018/19	12 265				1 813	110			16 394		30 582
Valencia	2016/17	55 603	2581		12 364	271 388	67 629		6 990	32 495	3 420	452 470
	2017/18	40 955		10 620	1 250	168 319	26 000		500	63 264		310 908
	2018/19	106 705		11 754	9 996	236 300	91 443	3 000	4 817	85 768	16 400	566 183
Total		2 040 151	7971	124 733	125 429	1 799 684	978 569	49 797	100 642	1 525 637	115 136	6 867 749

Table 6.5.2. Trees not meeting the certification criteria during the period April to March from 2016/17-2018/19.

Tree Certification	Year	EC	FS	GP	KZN	LIM	MPU	NC	NW	WC	EXP	Total
Not Certified	2016/17	16 232		563		42 310	3 000	499	2	10 097	50	72 753
	2017/18	11 249					177	120		11 252		22 798
	2018/19	15 035			107	204	1 321	3 030		11 196		30 893
Certified	2016/17	1 142 867	8 111	59 786	47 584	839 704	804 095	20 835	97 555	1 030 605	26 814	4 077 956
	2017/18	1 066 009	351	56 524	33 500	986 553	558 165	4 412	51 764	1 042 953	27 235	3 827 466
	2018/19	1 052 232	1 980	74 447	48 255	1 268 734	716 448	42 438	77 885	943 346	113 479	4 339 244

6.6 Nursery Certification

In June 2018, 34 citrus nurseries were visited and all were fully certified. Due to the more frequent complaints received from growers regarding nursery trees with benchroots, additional time was spent at each nursery for a thorough root inspection of the various propagation stages. The undesirable roots were pointed out to the nursery management and an appeal was made to do a stringent screening during each transplanting phase, thereby ensuring that no trees with benchroot are supplied to growers. This will refute grower complaints, when blaming the underperformance of trees on benchroots. Individual nursery audit reports with photos of bare tree roots were sent to each nursery.

In November 2018, 35 citrus nurseries were visited, 34 were fully certified and 1 conditionally. All the nurseries were thoroughly informed about the dreaded Asian greening disease that is heading for South Africa and that they need to plan and budget for insect-secure structures to keep the vector out of the nursery. We implemented a nursery grading system (score out of 5) based on the standard of trees produced in that nursery: the average score was 3.4, with nursery scores ranging from 2.5 to 5. Generally, the standard of the trees is acceptable to good. Due to the high demand for nursery trees, nurseries are under pressure to expedite the supply of trees leading to the supply of very small and/or substandard trees. Growers should be encouraged to visit the nursery in advance, placing orders in writing to specifying the required tree standard and to view the nursery trees before delivery. When the trees are delivered to the farm, poor trees not meeting the standard should be rejected.

Of the 36 nurseries visited in May 2019, 31 were fully certified and 5 were provisionally certified. Conditionally certified nurseries have not fully implemented the quality management systems required for CIS certification. The standard varies from excellent to acceptable and individual reports were sent to each nursery. The average nursery grading score was 3.56 out of 5, with nursery scores ranging from 2 to 5. The tree standards must be reviewed, as some nurseries supply trees topped on triangular wood at 70-80cm and not on round wood. In general, a decline in tree orders are experienced; however, the large nurseries are still fully booked. Most nurseries are still very slow to plan and budget for the required improvements needed for an HLB Safe System. The impression is created that they will react when the vector or disease was incurred in South Africa.

Table 6.6.1. CIS Certified Nurseries in May 2019

Nursery	Town / Province		Contact Person	Tel	Cell	Email
Apapanzi Kwekery	Kirkwood	EC	Nellis Meiring	042 230 1483	082 550 6210	nellis@srvalley.co.za
Attwell 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@augsburnursery.co.za
BF Joubert Kwekery	Kirkwood	EC	Francois Joubert	042 230 0309	084 951 1922	bfjkweek@srvalley.co.za
Cape Grow **	Kraaifontein	WC	Eugene Nepgen		084 416 0184	capegrow@gmail.com
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
Dodhill Nursery **	Chegutu	ZIM	Pete Breitenstein		+263 77 222 1046	dodhill@iwayafrica.co.zw
Du Roi Kwekery	Letsitele	LP	Mariska Benn	015 345 1650	072 475 5568	mariska@duroi.co.za
Du Roi Halls Nursery	Nelspruit	MPU	Scott McKenzie	013 004 0462	083 231 7535	info@duroi-halls.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
Groot Patrysvlei Kwekery	Clanwilliam	WC	Helgard Smit	027 482 2619	084 524 7417	nursery@capspanfarms.co.za
H J Joubert Kwekery	Montagu	WC	Herman Joubert	023 614 2237	082 578 5747	hopewell@breede.co.za
Henley Citrus	Letsitele	LP	André Swanepoel	015 386 0211	084 513 8649	productionmanager@bigday.co.za
Heuers Wholesale Nursery	Brits	NW	David Seewald	012 253 2097	082 887 4269	david@heuers.co.za
Hoedspruit Nursery **	Hoedspruit	LP	Lafras Tremper		083 652 2167	hoedspruitnursery@gmail.com
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
Montana Nursery	Nelspruit	MPU	Dane Ross	079 871 6175	082 808 5661	dane@montananurseries.co.za
Moorland Seedlings	Loerie	EC	Rian Moore	042 286 0605	082 2860 604	info@moorland.co.za
Ngwenya Kwekery	Malelane	MPU	Milanie van der Merwe	013 790 3004	082 418 7693	milanie@riversidefarm.co.za
Nouvelle la Cotte	Letsitele	LP	Riaan Lemmer	015 386 9995	083 253 1586	riaan@nouvellelacotte.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
Paksaam Kwekery	Patensie	EC	Adri Ferreira	042 283 0201	082 923 4412	paksaam@gamtoos.co.za
Parma Kwekery	Hoedspruit	LP	Albert Horn	087 806 5649	072 022 4356	parma@global.co.za
Rietvlei Kwekery	Tzaneen	LP	Lucas McLean	083 630 3236	083 630 3236	rietvlei@global.co.za
R&S Tissue Culture Lab **	Riversdale	WC	Jean Roeleveld	028 713 4113	082 375 2436	jeanroeeveldrs@telkomsa.net
Sondagsrivier Hillside Kwekery	Kirkwood	EC	Willem Truter	042 230 0349	083 227 6655	willem@srvalley.co.za
Stargrow Kwekery	Citrusdal	WC	Andries van der Westhuizen	022 921 2232	082 873 3336	andries@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
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** Provisionally certified – NEW nursery

6.7 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, and the ARC notified NAMC that it support a compulsory statutory CIS.

The new Plant Improvement Act came into force in 2018, which stipulates that public Schemes must be converted to statutory schemes. The Citrus Improvement Scheme schedule has been updated accordingly. A Memorandum of Understanding between the Minister of DAFF and the designated authority, CGA, has been drafted and will be tabled for discussion by stakeholders at CISAC-2019.

6.8 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 has laid a case at the Uitenhage Police station, but the case was deemed a civil matter, not to be prosecuted by the state. DAFF is following up on this case. CRI's Biosecurity Division did a thorough survey of the area and found another 27 properties with citrus trees. These were reported to DAFF.

6.9 PROGRESS REPORT: Citrus Improvement Scheme: Shoot tip grafting and diagnostic services Project 1144 by J.H.J. Breytenbach, C. Steyn, R. Clase and G. Cook (CRI)

Summary

The success of the Citrus Improvement Scheme (CIS) relies on the diagnostic detection of pathogens, their eradication 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 (NB). 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 might 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, relating to graft transmissible diseases, are also conducted. The ongoing activities of these CIS functions are reported. Six new selections were received for STG and seven were released to the CFB and added to the gene source. The gene source maintained at CRI currently comprises 378 accessions. General diagnostic services relating to graft

transmissible diseases were provided and a number of problems addressed, most significantly was the identification of the virus and diagnostic support during the Leprosis-N outbreak in the Eastern Cape.

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 has been used in South Africa since 1977 (de Lange *et al.*, 1981). The STG technique was developed by Murashige *et al.* (1972) and improved by Navarro *et al.* (1975) and de Lange (1978). 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. Some cultivars and selections of the virus-free gene source, maintained at the ARC-ITSC, have been duplicated in part at CRI Nelspruit as back-up sources.

Indexing, or establishing whether GTD disease agents are present, is primarily done by inoculating indicator host plants that are 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 and Costa, 1987). Cross-protection 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. Currently, three CTV sources are used for cross-protection in the CIS depending on the citrus type (von Broembsen and Lee, 1988; van Vuuren *et al.*, 1993a; van Vuuren *et al.*, 1993b; van Vuuren *et al.*, 2000) and pre-immunisation procedures have been adapted to suite South African conditions (Fourie and 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), citrus tatterleaf virus (CTLV) and Citrus Impetratura disease (CID) are done every 10 years.

Objectives

- A. Cultivar introduction (administration, establishment, STG, diagnostics, pre-immunisation, submission to the CFB and NB)
- 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
- G. Facility management

Materials and methods

- A. Cultivar introduction (administration, establishment, STG, diagnostics, pre-immunisation, 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 and Skoog, 1962). Germination takes place in an incubator at a constant temperature of 28°C in continuous darkness. When the seedlings have reached a height of 30 to 40 mm, they are stored at 4°C in darkness.

Scion preparation: Method 1; buds of the source plant are budded on a standard rootstock in the glasshouse. After bud growth and maturation (approximately 3 to 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 32°C and exposed to 16 h light/day. Ten to 14 days later new shoots are harvested and treated as in method 1.

STG: The seedling rootstock is aseptically decapitated about 50 mm above the cotyledons. The cotyledons and their auxiliary buds are removed and an inverted T incision is made, 1 mm vertically and 1 – 2 mm horizontally approximately 10 mm from the top. The cuts are made through the cortex to reach the cambium. A shoot tip consisting of the apical meristem and 2 to 3 leaf primordia is excised from the growth point of the collected shoots under a stereo microscope. The growth tip is placed on the horizontal cut of the incision on the rootstock. The grafted plant is transferred to sterile MS liquid medium and cultured at constant 28°C exposed to 16 h light/day.

STG plant propagation. 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 CTLV. 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 confirm cultivar identifications. The purpose is to ensure that the correct citrus fruit type is produced from each accession and that no mix-ups have occurred.

C. Biological and molecular re-indexing of mother trees and multiplication blocks at the CFB

The population structure of 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 CFB mother 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 of CFB mother trees for CVd 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 of CFB mother trees for CTLV and CPsV is therefore done only every 10 years.

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 tested with Apsca group specific viroid primer sets and viroid-specific primer sets for Citrus bent leaf viroid, Hop stunt viroid, Citrus dwarfing viroid, Citrus bark cracking viroid, Citrus viroid V and Citrus exocortis viroid 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. Screening of CFB multiplication trees are done yearly on a third of the multiplication trees, resulting in all the CFB multiplication trees being screened every third year.

D. Collaboration and duplicate indexing with ARC-TSC laboratory

Shoot tip grafting for the CIS is done at both the CRI and ARC-TSC laboratories. To confirm the pathogen-free status of new accessions prior to release to the CFB, duplicate molecular testing is performed.

Cultivar identification of the NB accessions at the ARC are conducted with the assistance of the CRI cultivar evaluator and CIS-Nelspruit facility manager. The purpose is to ensure that the correct citrus fruit type is produced from each accession and that no mix-ups have occurred.

E. Ad hoc diagnostics for GTDs for 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 outbreaks of graft transmissible pathogens 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.

G. Facility management

Maintenance and improvements at the CIS Nelspruit facilities.

Results and discussion

Objective / Milestone	Achievement
A. Cultivar introduction (administration, establishment, STG, diagnostics, pre-immunisation, submission to CFB and Nucleus Block)	<ul style="list-style-type: none"> • 34 accessions in STG pipeline • 6 new accessions received • 7 accessions released to the CFB
B. Maintenance of the virus-free gene source	<ul style="list-style-type: none"> • 378 cultivars maintained • Citrus types of 171 accessions confirmed by fruit on trees

C. Biological and molecular re-indexing of mother trees and multiplication blocks at the CFB	<ul style="list-style-type: none"> Biological CTV evaluation of rows 10 to 14 of mother trees completed
D. Collaboration and duplicate indexing with ARC-ITSC laboratory	<ul style="list-style-type: none"> All accessions sent to the CFB were duplicate tested prior to release CRI personnel assisted in citrus type verification of fruiting trees in the ARC NB
E. <i>Ad hoc</i> diagnostics for GTDs for growers and external institutions	<ul style="list-style-type: none"> Approx. 160 <i>ad hoc</i> samples analysed
F. <i>Ad hoc</i> investigations as required by CIS	<ul style="list-style-type: none"> Diagnostic support for the Leprosis-N outbreak Possible association of CiVA with Impietratura Seed transmission tests of CTLV in Meyer lemon repeated
G. Facility management	<ul style="list-style-type: none"> Routine maintenance and internal audits done on a weekly basis

A. Cultivar introduction (administration, establishment, STG, diagnostics, pre-immunisation, submission to CFB and Nucleus Block)

Introductions for STG and subsequent releases to the CFB from 2014 to date are summarised in Table 1. Seven new selections of four variety types were submitted for STG in the current year. At the end of this report period, 33 accessions are at various stages in the STG pipeline. A total of 147 STGs were done within this period, including failed grafts. Forty-four STGs were successfully micro-grafted. In the 2017 annual cycle, nine cultivars in the NB tested positive for GTD pathogens which were re-introduced to the STG pipeline for pathogen removal. Two of these cultivars went through STG and the remainder will be processed in 2019.

To facilitate a faster turn-around with the STG process, new introductions are tested directly with RT-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 samples processed are not reported.

Biological indexing of nine successful STGs for CTV and CVd were finalised and confirmed negative by RT-PCR. Biological indexing of fourteen STGs was completed for CPsV and Citrus Impietratura Disease (CID). Of these accessions, a single non-commercial cultivar, re-introduced for STG from the NB, was still positive for CPsV and will again undergo STG. Additionally, biological indexing for CTV, CTLV and CVd of seven successful STGs commenced in this report period. Biological indexing for CPsV and CID is underway for thirty STG's. Twenty cultivars are planned for release to the CFB in 2019 on completion and verification of negative biological indexing.

Table 6.9.1. STG submissions in the pipeline for graft transmissible disease elimination and indexing.

Variety type ²	STG introductions and releases 2014 to 2019 ¹																
	2014			2015			2016			2017/8			2018/9				
	Bf	New	Releases to CFB	Bf	New	Releases to CFB	Bf	New	Releases to CFB	Bf	New	Introductions	Releases to CFB	Bf	New	Releases to CFB	Balance
C	6	0	3	3	0	1	2	0	1	1	1	1	1	1	2	1	2

G	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	1
L	0	1	0	1	0	0	1	0	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	4	0	0	4	1	1	4	2	5	1	3	3	1	1	1	1
N	32	0	13	19	2	2	19	1	4	16	3	1	18	2	3	17
V	4	1	0	5	1	0	6	2	1	7	2	1	8	1	2	7
Or	1	1	0	2	0	0	2	0	0	2	0	0	2	0	0	2
Rs	1	0	1	0	0	0	0	0	0	0	2	0	2	0	0	2
Total	49	3	17	35	4	4	35	6	11	30	11	6	35	6	7	34

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 378 accessions and the number of selections per variety type is presented in Table 2. Two trees of each accession are maintained, each in a separate greenhouse structure. Nine accessions which tested positive for CVd and/or CTV in 2017 are currently in the STG pipeline for pathogen elimination.

A photo record is kept of fruit produced on the NB trees each year and kept in a database. The database is used to confirm the citrus fruit type of each accession to ensure that no potential mix-ups occurred. An additional 23 accessions were verified during the 2018 season. The number of accessions confirmed to be the correct citrus type is presented in Table 6.9.2.

Table 6.9.2. The number of accessions per variety type maintained in the CRI Nucleus Block and the number of accessions confirmed to be the correct citrus type based on fruit produced on the trees.

Variety Type	No. of cultivars at CRI	Citrus type confirmed by fruit
Clementine	32	9
Diverse (Citron, Sour orange, etc.)	2	1
Ellendale	4	
Grapefruit	23	15
Kumquat	1	2
Lemon	23	21
Lime	4	2
Mandarin hybrid	66	32
Midseason	34	17
Navel	88	16
Ornamental	4	4
Pummelo	8	5
Rootstock	23	19
Satsuma	8	6
Valencia	58	22
Total	378	171

C. Biological and molecular re-indexing of mother trees and multiplication blocks at the CFB

The CIS, within a CTV cross-protection programme, introduces different CTV sources to budwood supplied to the CFB for establishment of mother trees. The CTV source applied is determined by the citrus type. The current CIS procedural guide stipulates that the mother trees be assessed for CTV severity by means of biological indexing on 'Mexican' lime every second year. Severity of stem pitting on this indicator host is used as a measure of severity of the CTV in each mother tree. Subsequent to the implementation of this assessment method, more is understood regarding the virus and its influence on the citrus host. Disease expression of CTV is primarily determined by the citrus type, specific cultivar as well as the strains and variants of the virus

present. Symptom expression in a dissimilar host is not a reliable assessment of the effect of the CTV population in the original host. Additionally, shifts in strain populations occur when sources are transmitted to other, dissimilar hosts, invalidating the approach. The assessment on 'Mexican' lime was nonetheless completed for row ten to fourteen of the CFB mother trees. One hundred and sixteen trees were assessed. No severe stem pitting was recorded on any of the 'Mexican' lime hosts.

Budwood of each mother tree used for the inoculations, were also tested by RT-PCR for CTV presence. The presence or absence of CTV symptoms was recorded for the biological indexing and compared to the PCR results for detection sensitivity. Only a 62% correlation was obtained for the 2 methods, but an additional 28% positive CTV plants were identified by PCR. A further 10% were negative by PCR and were inconclusive for the biological assessment. A proposal will be made to CISAC to discontinue the CTV 'severity' assessment on 'Mexican' lime as the effect of the CTV population can best be observed on the mother tree itself. Molecular tests are available for detection and strain determination should that be required.

The screening of the multiplication blocks of the CFB for citrus viroids commenced in this report period, but will be reported on once completed.

D. Collaboration and duplicate indexing with ARC-TSC laboratory

Shoot tip grafting for the CIS is done at both the CRI and ARC-TSC laboratories. To confirm the pathogen-free status of new accessions prior to release to the CFB, duplicate molecular testing is done. The number of accessions tested for specific pathogens are presented in Table 6.9.3.

Table 6.9.3. Sample numbers of duplicate testing for various pathogens prior to final release to CFB.

Pathogen	ARC-ITSC accessions	CRI accessions
CTV ³	12	47
CVd	8	22
CTLV	8	21
CPsV	16	9
'Ca' Liberibacter species	17	18

³ Includes testing to confirm CTV pre-immunization

Cultivar identification of the NB accessions at the ARC was conducted with the assistance of the CRI cultivar evaluator and CIS-Nelspruit facility manager to ensure that the citrus fruit type observed corroborates the database with the aim of detecting potential mix-ups. This evaluation is reported by the ARC.

E. Ad hoc diagnostics for GTDs for growers and external institutions

Approximately 160 samples were received for various *ad hoc* analyses in this report period. Diagnostic samples submitted for citrus greening verification are first tested with a real-time assay for universal detection of all citrus affecting '*Candidatus Liberibacter*' species. If a positive sample is detected, further tests are done to determine the species identity. Seventy-seven suspect samples were received of which 22 tested positive for '*Ca. L. africanus*'. All samples were negative for '*Ca. L. asiaticus*'. Thirty-four samples were submitted for citrus viroid testing. Simple Sequence Repeat (SSR) markers were used to verify rootstocks in 4 instances. In one case the results prevented a grower from planting the wrong rootstock. CTV strain analysis of a grapefruit orchard showing severe stunting and stem pitting was conducted. The investigation indicated that the trees were probably made from field-cut material and not CFB material based on the uniformity of stunting and the CTV strain analysis results.

F. Ad hoc investigations as required by CIS

Symptoms typical for citrus leprosis were observed in Valencia and Navel orange orchards in the Eastern Cape Province. A CL-N associated virus, belonging to the *Dichorhavirus* genus, was detected in samples from 27 affected orchards on five farms. Full genome sequencing of the virus showed it to be a variant of orchid fleck virus (OFV) with closest sequence identity to an isolate described on orchids in the *Cymbidium* genus and not to isolates previously reported on citrus. Orchids were also sampled from nurseries in different

provinces and OFV was identified in samples from three provinces. The full-genome sequence determination of OFV from an infected *Brassia* sp. orchid showed 99% sequence identity to the genome of OFV found on citrus. Phylogenetic analysis of the RNA-dependent RNA polymerase gene showed that OFV found in both citrus and orchids in South Africa cluster separately from OFV isolates from Mexico. CL-N in South Africa is likely to have originated from imported, infected orchids, based on the close sequence identity of OFV found on citrus and orchids.

Grapefruit samples received in 2017 from Nkweleni, showing fruit symptoms typical for citrus Impietratura disease, were inoculated to host plants to preserve the sources. As no disease agent was associated with the disease, RNA was extracted and stored for future reference. Subsequently, citrus virus A (CiVA) was identified in South African material and also detected in these grapefruit samples. Detection of CiVA was recently reported by a number of research groups internationally. Cristacortis, Concave gum and Impietratura are citrus diseases for which aetiology is unknown, but are thought to be associated with viruses of the genus Congovirus, which includes CiVA. Affected trees show characteristic and differential symptoms in field trees, however, sources of these diseases inoculated to sweet orange indicator plants induce similar symptoms, consisting of chlorotic flecking and oak leaf patterns. To identify the causal agent associated to these diseases, a research consortium was established at the 2019 IOCV meeting. Locally, research continues on this virus in Project 1241. This has relevance to the CIS as the identification of the viruses associated with these diseases can eliminate the need for long-term biological screening for these diseases, but only once the aetiologies are confirmed. Molecular diagnostics can in future replace biological indexing and reduce the time to release of commercial varieties. We propose that molecular testing for CiVA be proactively included in the screening of STG accessions and that the gene sources also be tested for the presence of this virus.

SSR markers were used to confirm the identity of a mandarin-hybrid selection at the CFB.

CTV strain analysis was done for a grapefruit selection at the CFB showing severe stem-pitting. Nucleotide sequence data confirmed the presence of components of the GFMS35 cross-protection source only and not of other CTV infections. Results suggest that the selection is a CTV sensitive cultivar.

A single report of citrus tatter leaf virus (CTLV) seed transmission in 'Eureka' lemon was never confirmed. Seed transmission of viruses in citrus is important due to the propagation of rootstocks by seed. Seed was obtained from CTLV positive 'Meyer' lemon trees at the ARC premises in Addo. These trees were visually positive for CTLV and infection was confirmed by biological indexing and RT-PCR. In the 2017/18 report period 893 seedlings were tested for CTLV as pooled samples and the test was repeated on new seedlings of the same seed batch in the current report period. Pooled samples representing 271 seedlings were tested. No seed transmission of CTLV was detected in a total of 1164 seedlings. Although 'Meyer' lemon is not a rootstock, this was as an opportunity to test the seed transmission of CTLV and supports findings that the virus is not seed transmitted in citrus.

G. Facility management

Routine maintenance and internal audits were done on a weekly basis and two external audits were conducted by the CIS manager.

Conclusion

Efficient pathogen detection and elimination enables supply of healthy bud wood to the industry and is the primary objective of this project. Successful elimination of GTDs from new selections were achieved and these were added to the gene source and supplied to the CFB. The CRI gene source is duplicated and maintained in two structures as an ongoing function. Diagnostic services were provided and analysis of industry problems relating to graft transmissible diseases were addressed, most significantly in the diagnostic support during the Leprosis-N outbreak in the Eastern Cape.

Technology transfer

Symposium presentations:

J.H.J. Breytenbach and Z. Theledi. RSA versus USA: comparison of shoot-tip grafting and diagnostics. 2018. 10th Citrus Research Symposium, Champagne Sports Resort, Drakensberg.

C. Steyn, R. Clase, W. Kirkman and G. Cook. Molecular identification of an orchid fleck virus (*Dichorhavirus*) found in citrus in South Africa. 2019. Southern African Society for Plant Pathology (SASPP) Congress, Club Mykonos, Langebaan.

G. Cook, R. Clase, C. Steyn and W. Kirkman. The first case of Citrus Leprosis-N in South Africa. 2019. The Joint Conference of the 21st International Organization of Citrus Virologists and the 6th International Research Conference on Huanglongbing, Riverside, California, USA.

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Publication submitted for review:

G. Cook, W. Kirkman, R. Clase, C. Steyn, E. Basson, P. H. Fourie, S. D. Moore, T. G. Grout E. Carstens and V. Hattingh. Orchid fleck virus associated with the first case of Citrus Leprosis-N in South Africa. European Journal of Plant Pathology.

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6.10 **PROGRESS REPORT: Diagnostic and technical services for the Citrus Improvement Scheme by the ARC-TSC**

Project P030000127 (1 April 2018- 31 March 2019) by E. Jooste, Z. Theledi and N. Hlalele

Quarterly milestones

1. PEQ for citrus propagation material, including pathogen-elimination using shoot-tip-grafting, conventional and molecular diagnostics
2. Maintenance of a virus-free nucleus block of citrus cultivars and germplasm
3. Introduction of cultivars to the CIS Citrus Foundation Block following *Citrus tristeza virus* pre-immunisation
4. CIS diagnostic support for CRI-Nelspruit, including validation of diagnostic tests and improvement of diagnostic protocols
5. Technical CIS support through participation on CISAC and CIS-Pathology committees

Progress on quarterly milestones:

1. PEQ for citrus propagation material, including pathogen-elimination using shoot-tip-grafting, conventional and molecular diagnostics

Progress

- A technical position was filled on 1 July 2018 to assist with PEQ functions at ARC-TSC
- Training of the technical person, Ms. N. Hlalele, was done and is ongoing. She is trained in the molecular detection of pathogens and ongoing training with STG procedures, including establishing of plants, STG and micro grafting.
- 29 new introductions were established, budwood from five introductions were of bad quality and had to be re-imported. Nine seed batches were introduced
- STG are done as shoots are available. A record keeping system of number of STG's per selection was introduced.
- Ten selections are currently in the biological indexing phase, 5 selections are ready for biological indexing and 8 selections had successful STG plants with established nurse plants. Pre-screening of successful STG plants are done before biological indexing commence.
- Reports were submitted to clients on the progress of selections in PEQ.

2. Maintenance of a virus-free nucleus block of citrus cultivars and germplasm

Progress

- Four tunnels at ARC-TSC was upgraded and nucleus block material will be moved to the tunnels, currently underway (we were waiting for tunnel #14 to be functional)
- Ongoing maintenance of trees
- Johan Joubert assisted with variety type evaluations of fruit bearing trees in the ARC Nucleus Block. 57 true-to-type confirmations were done during the reporting time. Ms. Hlalele added photo records of selections to the database.
- DNA fingerprinting was done on suspicious selections identified during the true-to-type identifications. Three selections were identified to be incorrect and further investigations are underway.
- Action plan items are implemented and improvements made after industry audit visits

3. Introduction of cultivars to the CIS Citrus Foundation Block following *Citrus tristeza virus* pre-immunisation

Progress

- 10 selections were released to CFB, five older selections re-released to CFB on demand, 5 seed batch imports released to DAFF

4. CIS diagnostic support for CRI-Nelspruit, including validation of diagnostic tests and improvement of diagnostic protocols

- Ms Hlalele was trained to perform DNA and RNA extractions on citrus and to perform diagnostic tests.
- Twenty-four plants were tested in duplicate tests CRI-Nelspruit that included 9 selections tested for CTV, CTV-pre-immunisation and 6 viroids.
- Thirteen plants were tested for Citrus greening, CPsV and CLBV, respectively.
- A total of 111 duplicate tests were done for validation before release to CFB
- Expansion of PEQ diagnostic tests: 3 additional viruses were identified to be included in screening of imported material; *Citrus mosaic virus*, *Satsuma dwarf virus* and Citrus viroid VII. An outcome from the international travel to UC Riverside was a collaboration on acquiring the expanded protocols used in their high throughput diagnostic laboratory. We are waiting for the signed agreement to establish the protocols in PEQ procedures.

5. Technical CIS support through participation on CISAC and CIS-Pathology committees

- E. Jooste, A. Theledi and N. Hlalele are actively participating in the CIS-pathology meetings
- E. Jooste is representing the ARC in the yearly CISAC meeting.

International travel

CRI partially funded Dr E. Jooste to attend and participate in the 21st Conference of the International Organization of Citrus Virologists (IOCV) and the 6th International Research Conference on Huanglongbing (IRCHLB) set for March 10-15, 2019 at the Riverside Convention Center in Riverside, California, USA. The following oral presentation was presented:

JOOSTE, AEC., THELEDI, Z and HLALELE, N. 2019. Post Entry Quarantine (PEQ) of citrus in South Africa. International organisation of citrus virologists (IOCV), Riverside, California, USA, 10-12 March 2019.

PEQ Executive Report

An executive report of selections in PEQ was compiled. This document contains progress benchmarks that were compiled with data of introductions from 2013 to 2018. The progress benchmarks include information on the average time of introductions in the pipeline, the average time from introduction to interim release, the number of STGs required to establish a nurse plant, the average number of STGs per completed accession and the average time from introduction to first STG attempt.

Acknowledgement

We thank CRI for technical and diagnostic support, during the past year our outputs increased due to the additional technical capacity.

7 INTERNATIONAL VISITS

7.1 Attendance of the Joint Conference of the 21st International organization of Citrus Virologists and the 6th International Research Conference on Huanglongbing. March 10-15, 2019, Riverside, California, USA

Glynnis Cook (CRI, Nelspruit), Hano Maree (CRI & SU, Stellenbosch), Rachele Bester and Dirk Aldrich (SU)

INDEX:

1. Pre-conference visit to the CRB high throughput ACP testing facility, the USDA-ARS National Clonal Germplasm facility and the research laboratory of the Department Microbiology & Plant Pathology, University of California
2. IOCV Conference attendance

3. HLB Conference attendance

4. Post conference visit to Wonderful Citrus Nursery (Glynnis Cook)

5. Post conference laboratory visit to CREC, UF (Prof WO Dawson) (Hano Maree, Rachele Bester & Dirk Aldrich)

1. PRE-CONFERENCE FACILITY VISITS (FRIDAY, 8 MARCH):

Citrus Research Board high throughput laboratory for Asian Citrus Psyllid (ACP) testing in Riverside.

The purpose of this visit was to gauge the requirements for the setting up of a high throughput diagnostic laboratory.

We were received by Dr Qijun Xiang (Laboratory Director) and Geizhar Ramirez (Laboratory Manager). The facility has been operational for one year and four technicians are employed. One thousand samples are processed per week.

ACP from traps are not tested, but are used only to scout for ACP. Traps hang for one month in the field. Scouts monitor the traps for ACP and do follow-up sample collections at positive ACP sites. Samples (either adults or nymphs) are collected in vials in ethanol by field scouts and dispatched to the laboratory for testing.

Samples are received, barcoded and added to a database. Each sample is manually loaded into a single well of a 96 well plate (25 adult ACP can be pooled as a sample or 70 nymphs). The DNA extraction is fully automated using a bead beater, followed by magnetic bead extraction. Liquid handlers are used to load 96 well plates for real-time PCR detection of Las. The primary diagnostic is a probe-based PCR targeting the 16SrDNA (Li, et al, 2009). Positive samples are confirmed with a second probe based diagnostic targeting the gene region for a subunit of ribonucleotide reductase (RNR) (Zheng et al, 2016). The Standard Operating Procedures were obtained from the USDA.

Further details of laboratory equipment and functioning are provided at the end of the report.



f.l.t.r Caleb (CRB general lab manager), Prof HJ Maree, Dr G Cook, Dr E Jooste, Mr D Aldrich, Dr R Bester and Dr Q Xiang (CRB Laboratory director)

USDA-ARS National Clonal Germplasm Repository for Citrus

We were received by the research leader, Dr Marylou Polek, Robert Krueger (horticulturist) and Brittany Moreland, responsible for plant maintenance and biological indexing.

The facility is responsible for the preservation of genetic diversity of citrus and to distribute these sources worldwide on request. Shoot-tip grafting is used to eliminate pathogens from the gene source. Currently 1600 accessions are maintained of which 600 are maintained in two insect-free screen houses.

Various research initiatives include improved pathogen testing and cryopreservation of clonal tissue.

A comparison of Las detection technologies is currently underway which includes standard qPCR, serological detection (ELISA) of a systemic secreted Las protein (Ma), serological detection on tissue imprints of a structural protein of Las and metabolic profiles analysis of trees (H-NMR spectroscopy). The research is ongoing, but results presented thus far show poor correlation between techniques. This was also presented as a poster [IRCHLB-P10-125].

University of California Research Laboratory, Department of Microbiology & Plant Pathology

We were received by associate research scientist, Dr Sorab Bodaghi, who is responsible for the laboratory management.

This laboratory developed high throughput nucleic acid extraction protocols for citrus tissue along with diagnostic assays for the universal detection of citrus viroids and multiplex detection of citrus viruses for the Citrus Clonal Protection Program (CCPP). Dr Bodaghi showed us all the processes followed. The laboratory uses a system whereby students are paid to do the initial sample processing to lessen labour costs. The initial sample preparation is labour intensive and time consuming. Checks and balances are in place to ensure procedures are correctly followed. The high throughput diagnostics, in combination with best management nursery practices, have reduced and maintained citrus viroid finds in citrus nurseries to less than 1% (2015 - 2018: 0.5 - 0.8 %) from relatively high levels (2004 - 2010: 4.3 - 9.5 %) [IOCV-07-01].

Visits to two laboratories conducting high throughput testing was useful to gain insights into requirements for a high throughput laboratory set-up.

2. IOCV CONFERENCE

There were 224 delegates from 23 countries attending the IOCV conference.

IOCV presentations and posters

IOCV sessions were focused on citrus improvement programmes, citrus leprosis, citrus tristeza virus, citrus yellow vein clearing virus, de novo discovery of citrus viruses, citrus viroids and diagnostics.

Diagnostic related presentations of interest:

Citrus psorosis virus (CPsV) sources in China were found to differ in symptom expression and genome sequences. Two clades of this virus were demonstrated. Presently, a single CPsV full-genome is available on Genbank. Molecular diagnostics will need revision once these sequences are published [IOCV-P7-27]

Citrus mosaic virus (CiMV) isolates were fully characterised in China and genome sequences reinforce placement of CiMV in the genus *Sadwavirus* together with satsuma dwarf virus (SDV). Sequence data will enable improved diagnostic development [IOCV-P7-23].

A tissue blot extraction for large scale screening for viroids was developed which shows similar sensitivity to nucleic acid extraction [IOCV-01-04]. This method will be evaluated for an alternate method for the large scale screening of the CFB multiplication blocks. The method excludes the requirement for nucleic acid extraction, decreasing labour and reagent inputs. The methodology was obtained from the researcher.

Next Generation Sequencing (NGS) as routine diagnostic for citrus variety introductions [IOCV-01-07]. The sensitivity of NGS (ribo-depleted RNA Seq) was compared to qPCR for the detection of viruses, viroids and bacteria. NGS was found to be superior but does have the risk of reporting false positives. However, the study lacked several controls and the interpretation of their data is not defensible. In CRI project 1241 we will address

all these issues and hopefully we will be able to define the parameters for routine NGS diagnostics more accurately.

Viruses of quarantine importance

A virus of biosecurity importance is citrus yellow vein clearing virus (CYVCV) which is destructive on Eureka Lemon and reported from Iran, China, Turkey, India and Pakistan. More information regarding this virus was presented, including the lack of seed transmission in Eureka lemon [IOCV-06-02]. The vector was identified as the citrus whitefly, *Dialeurodes citri*, but red spider mite was shown not to be a vector. It was demonstrated that the most severe effects are observed on Eureka Lemon, although other citrus types also display transient vein clearing and can therefore serve as latent infections [IOCV-06-03].

De novo discovery of citrus viruses

Detection of citrus virus A (CiVA) was reported by a number of research groups. Cristacortis, Concave gum and Impietratura are diseases for which aetiology have not been confirmed, but are thought to be associated with viruses of the genus *Conguvirus*. Affected trees show characteristic and differential symptoms in field trees, however, sources of these diseases inoculated to sweet orange indicator plants induce similar symptoms, consisting of chlorotic flecking and oak leaf pattern. To identify the causal agent associated to these diseases a consortium was established named Citrus Coguvirus study group at the IOCV meeting.

Molecular diagnostics for diseases of unknown aetiology, which necessitate long-term biological screening for detection, can in future replace the need for biological screening, greatly increasing the time to release of commercial varieties.

The use of viruses and infectious RNA for therapeutic treatment of field trees

Further CTV-based expression vectors from strains T30 and VT were created which expand the range of tools available for therapeutic use in the USA [IOCV-05-01].

The use of the T36 CTV expression vector for HLB control will be limited by pre-infection with RB strains due to a cross-protection effect [IOCV-05-04].

Dr A. Simon, University of Maryland, reported the identification of stable, infectious RNA (*inRNA*), lacking movement proteins, silencing suppressors or capsid proteins, that traffic in phloem. This *inRNA* is a potential vector for delivery of small RNAs, peptides and proteins apart from CTV. At this point the vector has been shown to replicate in *N. benthamiana* (once). No data was presented on the expression or delivery of any payload by this *inRNA*. In discussions with several prominent researchers on this study they indicated a fair amount of skepticism as to potential applications of this *inRNA*.

Presentations by the CRI and Stellenbosch University Graft Transmissible Disease group:

Detection of a South African variant of a bunya-like virus infecting citrus

by Rachelle Bester, Maryam Karaan, Glynnis Cook and Hans J Maree

Feedback: The Citrus coguvirus study group was founded.



Old tree displaying citrus congave gum disease.

Variants of the CTV T68 strain from the GFMS12 source differ in stem pitting expression in grapefruit
by Glynnis Cook, Beatrix Coetzee, Johan T Burger and Hans J Maree

Feedback: Supportive of our own findings, mention was made of a region in the CTV P33 gene being associated with stem-pitting [IOCV-01-06].

An Australian citrus breeder requested assistance and possible collaboration in a breeding project of Mexican lime hybrids. Tolerance to CTV is one of the traits evaluated and they require assistance with CTV strain identification.

The first case of Citrus Leprosis-N in South Africa

by Glynnis Cook, Rochelle Clase, Chanel Steyn and Wayne Kirkman

Feedback: The talk generated wide interest and requests for collaboration from various Leprosis researchers;

A comment was made regarding the limited host range of OFV reported in South Africa. This finding was contrary to previous findings where OFV was reported to additionally affect mandarins in Mexico.

Dr Avijit Roy (USDA-APHIS) works on orchid fleck virus associated with Leprosis and is currently developing improved diagnostics. Glynnis will be collaborating with him by sending material from the South African Leprosis-N outbreak and by testing the new diagnostics. An offer was made to do mite identifications should we require assistance. Dr Roy would use Dr. Ron Ochoa (USDA-ARS_BARC, Beltsville, MD) for taxonomic identification.

Dr Elliot Watanabe Kitajima is a Brazilian electron-microscopist specialising in Leprosis and tenupalpid mite identification. A post-doctoral student in his group is revising the taxonomy of the tenupalpid mites and has requested mite samples from South Africa for this study. Wayne Kirkman has supplied samples to be sent.

Dr Kitajima also offered to do electron microscopic work on Leprosis samples from RSA. Material will be sent when positive leaf tissue is obtained from the field.

Applying Citrus tristeza virus clones to understand stem pitting development in citrus (POSTER)

by Dirk J. Aldrich, Rachele Bester, Glynnis Cook, Johan T. Burger and Hans J. Maree

Feedback: The poster presentation led to some fruitful discussion following the sessions, with researchers from other continents. The aspects of the poster most noted on were the nano-CT scanned stem pits from a citrus tristeza virus-infected grapefruit plant, and the green-fluorescent protein expression in leaves of *Nicotiana benthamiana* plants infiltrated with CTV infectious clones, which were visually intriguing. The complexity of the citrus-CTV pathosystem was discussed with specific reference to the genetic variability of the virus and how this relates to the prominent biological distinctions we see in citrus-CTV interactions. This is especially true in the case of stem pitting induction, of which there are several distinct disease phenotypes.

3. HLB CONFERENCE

There were 548 delegates at the HLB conference.

Country reports:

Brazil: Juliano Ayres / Silvio Lopes

Since the first detection of Las in 2004 in São Paulo and Minas Gerais, infection rates rose to an average of 18% in 2015. Infection levels plateaued, and are maintained at this point, due to intensive control interventions. HLB Incidence levels are lowest on large farms (> 500 000 trees) at 7% compared to smaller farms (<10 000 trees) at 33 %. Factors attributed to HLB control success in Brazil include nurseries under protected structures, the Fudecitrus leadership and research network, grower experience with CVC and Canker control, laws enforcing infected tree removal and other crop options for small farmers where control is more difficult.

ACP monitoring and alert systems are in place for regional management and awareness campaigns are run in rural communities. The 'Ten Commandment' prescriptions are the cornerstone to control which include planting healthy trees, good nutrition for optimal tree growth, ACP monitoring & orchard inspection to detect HLB infections, removal of symptomatic trees, regional ACP management with specific control applications on orchard borders.

Improvements in vector control:

- Determined lowest efficient spray dose and volume. Spray volumes were reduced by 50-70% and dose/Ha changed to dose/canopy volume.
- Use of processed Kaolin to repel ACP and reduce feeding activity
- Use of *Isaria fumosorosea* as biocontrol agent
- Use of pheromone for ACP monitoring (acetic-acid baited traps)
- Orange jasmine as trap crop on orchard
- Developing models to predict flush growth to optimize timing of sprays

China: Zhou Changyong

There is a northward movement of ACP and HLB from existing areas in Guangxi and Guangdong. An HLB epidemic is predicted for Guangxi as half the nurseries are not virus free. An epidemic in the Guangzhou region of Jiangxi destroyed 50 million trees since 2013. State actions are being introduced including enforcing virus free nurseries, quarantine pest-free zones and other interventions. Drones are being used to spray.

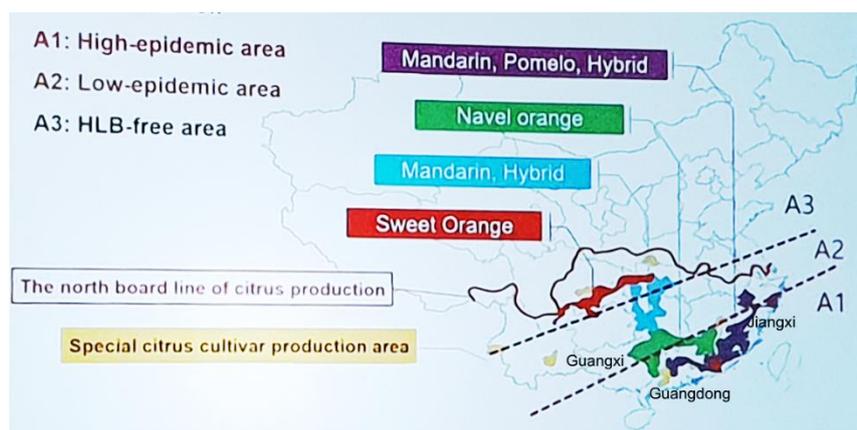


Figure 7.1.1: HLB presence in China since the 1970s to present

Europe: Luis Navarro and Leandro Pena

The Mediterranean Basin produces 23 million tons for the fresh fruit market (20% of total world production) and encompasses 1 million hectares, dispersed over the region. Las has not been reported, but *Trioza erytreae* was reported in Portugal in 2014. Quarantine measures were subsequently reinforced and further prohibitions placed to import HLB, *D. citri* and *T. erytreae* alternate hosts. However, they report a failure to stop the

dissemination of *T. erytrae* in Spain and Portugal due to poor coordination among member countries. There are no common strategies between European Union member countries or non-member countries. Despite import regulations, penalties for illegal introductions are very mild. A Horizon2020 EU project was initiated to prevent HLB epidemics and ensuring citrus survival in Europe.

Australia: Nerida Donovan

No HLB or ACP status, but biosecurity surveillance done on the islands north of Australia in the Torres Straits. Strict quarantine at ports of entry.

USA: Don Seaver (USDA)

ACP in Texas and California. Both states deploy biological control agents in urban and residential landscapes, but also in Mexico across the border from Lower Rio Grande Valley. In Texas, ACP populations were drastically lowered (up to 80%) in area-wide management sites. ACP present, but no HLB detected in Arizona yet. In California ACP is present in all or portions of 27 counties. HLB reported in portions of Los Angeles, Orange, Riverside and San Bernadino Counties.

Actions in California include;

- Detection and delimitation trapping for ACP (traps inspected every 30 days)
- Residential treatment around ACP finds
- Intensive ACP monitoring around find sites and adjacent properties
- Risk based commercial and residential survey for HLB
- HLB positive tree removal followed by mandatory 400m survey and ACP treatment around detection site

Antimicrobials

Various groups report screening antimicrobials against Las as well as methodologies to evaluate their effectiveness. Noteworthy are the following:

A citrus-derived stable anti-microbial peptide, **SAMP**, derived from Australian finger lime, is resistant to plant proteolysis and stable at high temperatures. It shows antimicrobial action against *Liberibacter crescens* and potato zebra chip disease. Trunk injections of SAMP suppressed growth of CLAs in HLB-positive plants. A research consortium has been awarded USDA funding to further research this peptide. <https://news.ucr.edu/articles/2019/04/01/plant-pathologist-leads-research-stop-spread-citrus-destroying-disease>.

Bt toxins with insect-specific, pesticidal proteins, Cry1Ab and Cry1Ba1, were identified for use as ACP control agents. Multiple delivery systems are being investigated for management of ACP including transgenic citrus and transgenic trap plants (*Murraya koenigii*). [IRCHLB-05a-02]

Note: **Cry1Ab** is a small peptide and can be used as a 'payload' in the CTV infectious clone approach.

Zincicide is a Zn-oxide nano-particle formulation that moves systemically in the plant and is effective against citrus canker, with better bacterial inhibition compared to copper. It is not merely a Zinc response. The compound was evaluated in vitro on *Liberibacter crescens* and showed inhibition and cell degradation [IRCHLB-P8-96]. Field evaluation in HLB positive orchards were conducted. Positive effects on yield and fruit size were obtained with treatments in young grapefruit trees, but not in older Valencia trees [IRCHLB-P8-100]. Up to a 3 log reduction in CLAs DNA were found with foliar and soil drench applications. Effects are dependent on dose and repeat applications, but phytotoxicity limits the number of applications [IRCHLB 04b-05].

Mycoshield is one of three antimicrobial products, other than penicillin and oxytetracycline, being evaluated in phase III field trials. Results are inconsistent, but may be due to citrus variety differences and horticultural practices [IRCHLB-P8-95].

CTV vector delivery of defensin gene (field trial at Southern Gardens). Trial is 3 years and differences in tree size, early production and vigor are seen, although details were not divulged. [Tim Eyrich, Southern Gardens Citrus]

Citrus Under Protective Structures (CUPS) & Individual Protective Covers (IPCs)

Grapefruit trials were conducted in screen-houses in-ground and in potted plants. Comparatively good yields reported. Screen-house structures after 5-yrs require significant maintenance. Costs of CUPS is significant factor to consider [IRCHLB-01b-01], [IRCHLB01b-02].

IPCs of polyethylene screen on young Valencia trees reduced HLB infection with added benefits of earlier flushing, higher accumulation of chlorophyll in leaves and faster trunk diameter growth observed. Vapour pressure deficit was also lower in IPC trees. Further research will investigate influence on transition from vegetative growth to reproductive stage [IRCHLB01b-03].

Detection

Las detection in ACP in Texas in 2011 preceded that of plant tissue in 2012 by 10 months. Seasonal variations in Las concentrations in citrus trees as well as in the ACP vector were observed. Higher rainfall was associated with a higher Las detection and lower temperature and humidity with lower Las detection [IRCHLB-03a-01].

Las infected ACP drive HLB spread by infecting flush during feeding and egg-laying resulting in infected progeny. Las was detected on flush 2 weeks after infection [IRCHLB-03a-03].

Note: If trees are sampled with ACP present, collect flush for testing.

Canines are scheduled for deployment to southern California for Las detection validation tests in late 2018-early 2019 [IRCHLB-03a-05].

Pesticide application

Imidacloprid uptake in 4 citrus varieties reached threshold levels in plants within 3-5 days post-application using higher treatment rates and optimal watering (not over watering). This lowers the holding period requirement before shipment of nursery trees and lengthens the field protection period [IRCHLB-04a-03].

Post-harvest treatments for bulk citrus at orchards prior to transport mitigate ACP spread was investigated. Trials with high pressure fogging of aqueous mixtures containing 0.2% Evergreen® (6% pyrethrins & 60% piperonyl butoxide) and 0.5% (v/v) BreakThru® (polysiloxane surfactant) showed good efficacy. This application requires either a tent or a facility, fans, water, but will not be as effective as gas. Fumigation with ethyl formate gas is quicker to apply, requires shorter treatment time and is effective against other citrus pests, but requires air-tight conditions [IRCHLB-04a-04].

Biological control

Tamarixia radiata is mass-reared and released in urban/suburban areas in southern California since 2011, but also along trade routes and the southern border of California covering approximately 8000km². Twelve million *Tamarixia* were released in 2018 at a cost of 25c (US) per insect. A decline in ACP populations over 4 years is reported [IRCHLB-04a-05]. However, low levels of parasitism by *T. radiata* for higher ACP densities on citrus flush shoots were shown in Texas [IRCHLB-04a-06].

Vector management (other)

Orange jasmine (*Murraya paniculata*) was investigated as a trap crop to control *D. citri*. Planting orange jasmine as a border trap crop on newly established commercial citrus orchards reduced the captures of *D. citri* by 40% on yellow stick traps and marker released psyllids by 80% in the citrus orchard. HLB incidence was 43% lower where the trap crop was used. The impact was observed in young plantings, but not in mature orchards [IRCHLB-05a-05].

An **attract-and-kill device for ACP** was developed using a 3-component blend of formic acid, acetic acid and p-cymene, which acts as a phagostimulant. Psyllids that probe and attempt to feed are killed by toxicant deposited in a wax matrix which is attached to the surface in the interior of the device. Psyllids probing the wax matrix are killed and fall off from the trap surface retaining complete trap activity over long periods (months). A colour called bird yellow was found to be an improvement on the yellow colour used for sticky traps. [IRCHLB-05a-06].

<http://citrusindustry.net/2018/10/30/attract-and-kill-device-being-developed-for-psyllids/>

Figure 7.1.2: Attract and Kill Device showing bird yellow colour and reusable plastic frame.



Use of **particle films (kaolin)** to alter incident radiation which repels ACP was found effective, especially for young trees.

Plant growth regulation to manipulate flush timing can form part of ACP management regime to time insecticide applications.

Citrus breeding

The breeding session attracted a lot of attention from the broader audience and resulted in a lively debate. Unfortunately, researchers were divided between the conservative (pragmatic) and the more enthused. The desperation of growers was palpable and seemingly more open to rash decisions. Projects that may deliver solutions in the long run include Identification of tolerant /resistant citrus cultivars or hybrids and conventional breeding using molecular markers. The development of “non-transgenic” HLB resistant varieties using CRISPR-Cas9 is ongoing (DMR6 knock down / knock outs), but it remains to be seen if this technology can be used for European markets (currently not). Use of GMO technologies for the development of breeding lines that contain therapeutic compounds to confer tolerance or resistance are also investigated and include compounds such as an B-caryophyllene (insect repellent), AiiA gene (reduced CLas titre), single chain antibodies to CLas proteins (reduce titre), Thionin (CLas killer? Claims of resistance) and the manipulation of SDE15 expression levels.

It would be in our best interest to follow the progress made in both approaches and critically evaluate any claims made.

Citrus Research Board high throughput laboratory for Asian Citrus Psyllid (ACP) testing in Riverside.

Laboratory flow, equipment and functioning

The building consists of 6 primary areas separated by inter-leading doors;

- 1. Reception**
- 2. Office**
- 3. Boardroom/office**
- 4. Storage and receiving** (photo 9)

Fridges/freezers and racks for consumable stock and sample storage.

- 5. Laboratory room 1** (sample accessioning, psyllid sample loading and mechanical disruption of ACP samples)

Laboratory work benches

Two mechanical bead beaters (photo 4)

Samples are received, barcoded and accessed to a database (photo 1). Each sample is manually loaded into a single well of 96-well extraction plate (preloaded with extraction buffer). This is a laborious and time-consuming process. An absorbent paper is placed on a foil sheet, the psyllid sample contained in ethanol, is poured out on the absorbent paper and the ACP is manually transferred from the paper with a glass pipette to the 96-well extraction plate (photo 2). The paper, glass pipette and foil are used once for a single sample and then discarded. The extraction plates are placed on a rack either for further loading or to be extracted, depending on whether the plates are fully loaded, photo 3). Fully loaded plates are placed in an automated bead beater for sample disruption.

6. Laboratory room 2 (automated DNA extraction and PCR plate loading)

Three automated DNA magnetic bead extraction machines

Two laminar flow cabinets

One PCR-plate centrifuge

One 96-well automatic liquid dispenser

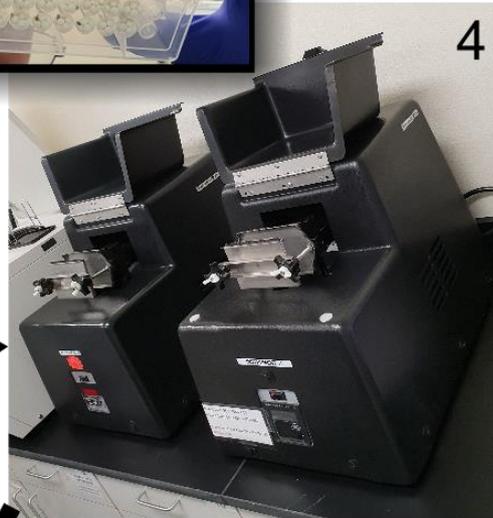
Two ergonomic manual liquid dispensers

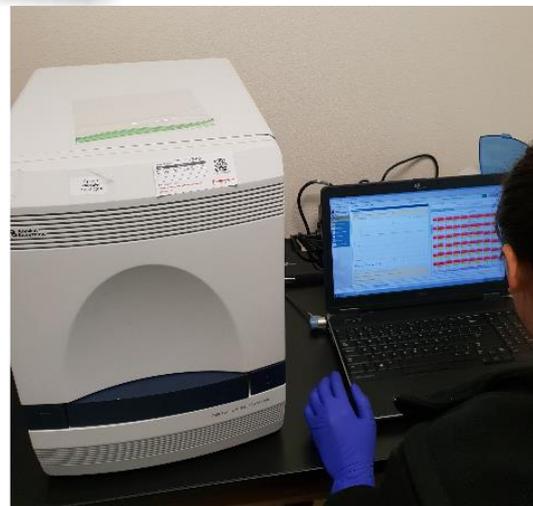
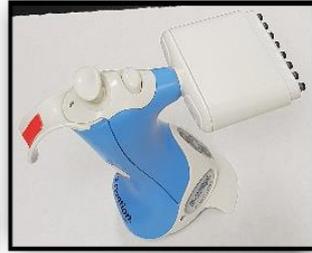
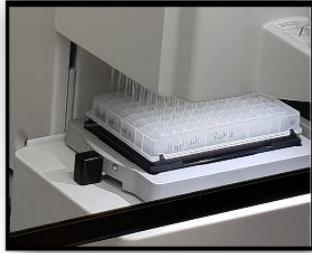
After sample disruption the extraction plates are transferred to the second lab and DNA is extracted by automatic magnetic bead extraction protocols (photo 5). Liquid handlers are used to load 96 well plates for real-time PCR detection of Las (photo series 6-7).

7. Laboratory room 3 (PCR thermal cyclers)

Two 96 well-plate real-time PCR thermal cyclers

Loaded and sealed PCR plates are taken to room 3 for PCR amplification (photo series 8). The primary diagnostic is a probe detection targeting 16SrDNA (Li, et al, 2009). Positive samples are confirmed with a probe detection targeting the gene region for a subunit of ribonucleotide reductase (RNR) (Zheng et al, 2016). An internal PCR is multiplexed with the first PCR to confirm DNA integrity.





4. POST CONFERENCE VISIT TO WONDERFUL CITRUS NURSERY (GLYNNIS COOK)

The nursery is an enclosed, insect-free structure and is situated in close proximity to citrus orchards.

Nursery Enclosed structure



Double door entry to nursery with positive air flow pressure



Seedling tray preparation and sowing are fully automated

Seedling tray preparation



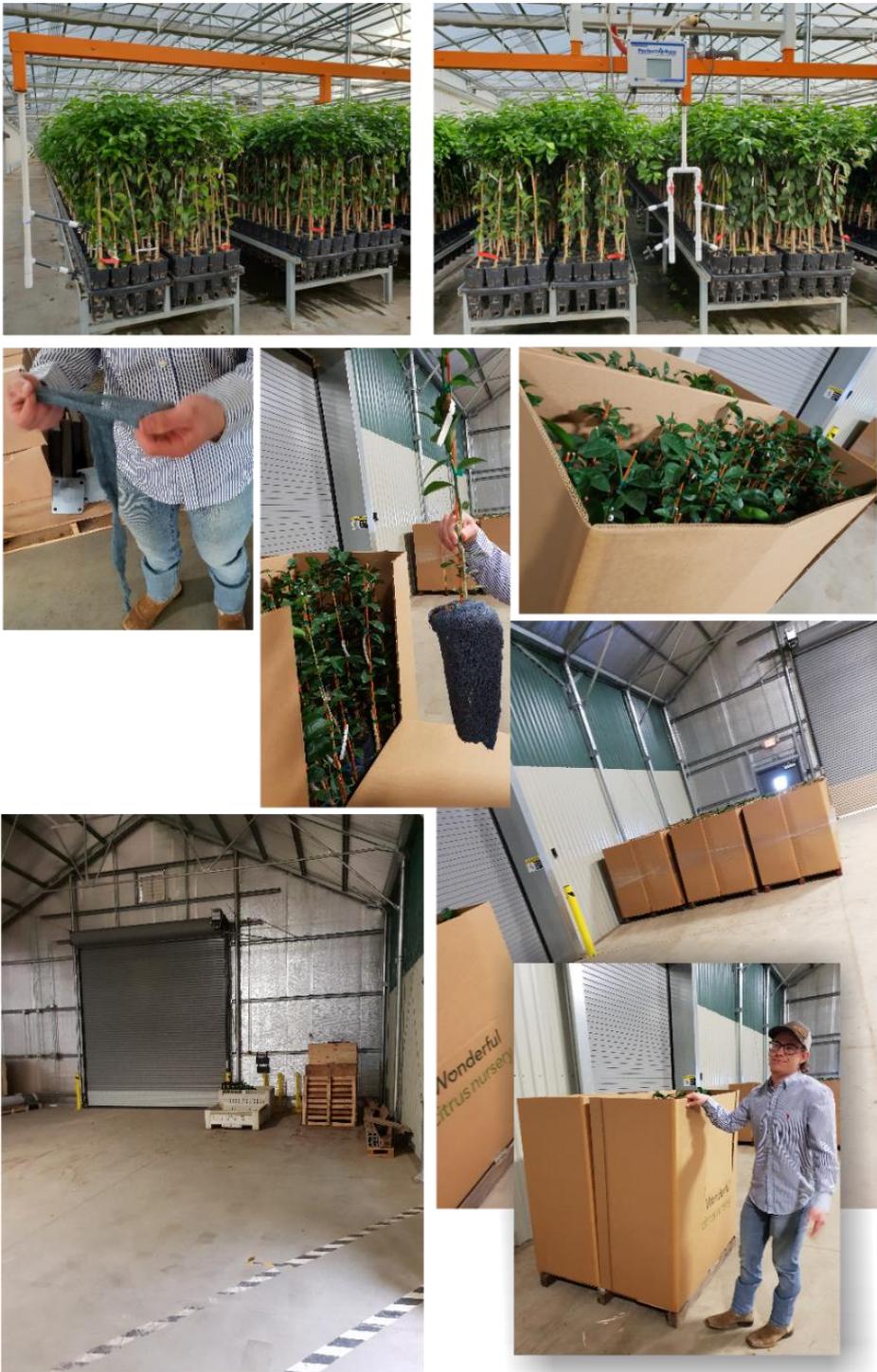
sowing



Tree production is done in one large structure. Budding is done by bending the rootstocks which are cut-back after scion has established.



Trees are moved to an acclimatization room prior to shipment. The plastic pots/cones are removed from the roots and the roots placed in a material socks to maintain root structure. Trees are package in cardboard boxes that are fully closed. Plants are loaded onto trucks at a raised platform. The truck reverses to a garage door of the same dimensions as the truck container for loading to minimize exposure to the outside environment.



5. POST CONFERENCE LABORATORY VISIT TO CREC, UF (PROF WO DAWSON) (HANO MAREE, RACHELLE BESTER & DIRK ALDRICH)

We also had the opportunity to visit our collaborators at the Citrus Research and Education Centre of the University of Florida, Lake Alfred. The main purpose of this visit was to engage meaningfully with Prof Bill Dawson's group and to get hands-on experience on how to successfully infiltrate citrus plants with infectious clones of CTV. This visit also provided opportunities for discussions and explanations on cloning practices that work for CTV, which have been gleaned through years of trial and error by this group.



f.l.t.r. Hano Maree, Rachele Bester, Dirk Aldrich



Dirk Aldrich with Prof Dawson looking at RNAi insect trials



HLB infected orchard with trees of different ages, all infected.

7.2 **10th International Symposium on Fruit Flies of Economic Importance, Tapachula, Chiapas, Mexico, 23 - 27 April 2018**
Aruna Manrakhan (CRI)

Background

The International Symposium on Fruit Flies of Economic Importance (ISFFEI) is a quadrennial event which brings together scientists working on fruit fly pests in different parts of the world. The 10th International Fruit Fly Symposium of Economic Importance took place from the 23 to 27 April 2018 in Tapachula, Chiapas, Mexico.

The objectives of my participation in the 10th ISFFEI were to:

1. Obtain new information on fruit fly biology and management.
2. Meet and connect with other fruit fly workers.
3. Meet with STDF_ PPG_ 567 project partners for discussion on the full project proposal on “Establishment and maintenance of fruit production areas free and under low Prevalence of fruit fly pests in Southern Africa”
4. Present a paper on “Efficacy of existing and new attractants for Afrotropical fruit fly pests” (Session 4 on Tuesday 24 April 2018)
5. Present a poster on “Effects of temperature and intrinsic traits on tethered flight performance of the oriental fruit fly, *Bactrocera dorsalis*” (Session 1 on Monday 23 April 2018)
6. Present a poster on “Assessment of lufenuron-methyl eugenol mixture as a chemosterilant bait for *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae)” (Session 8 on 26 April 2018)

The scientific programme of the symposium covered various aspects of fruit fly biology, ecology and management. The programme consisted of 10 sessions under the following topics (1) Biology, Ecology, Physiology and Behaviour, (2) Morphology and Taxonomy, (3) Genetics and Biotechnology, (4) Chemical Ecology and Attractants, (5) Risk assessment, quarantine and post-harvest treatment, (6) Sterile Insect Technique, (7) Natural enemies and biological control, (8) Other control methods and supporting technology, (9), Area-wide and Action Programs and (10) Social, Economic and policy issues of action programs.

In this report: (1) key points from key oral and poster presentations at the 10th ISFFEI, (2) new ideas for potential research projects on fruit flies based on presentations at 10th ISFFEI, (3) abstracts of oral and poster presentations by Manrakhan et al., (4) outcomes of the STDF_PPG_567 meeting and (5) summary of the field visit to a mango orchard and mango packing house are provided.

1a. Key Points from Key Oral papers

Session 1: Biology, Ecology, Physiology and Behaviour

Slawomir A. Lux: How long can Medfly survive being undetected?

The stochastic model agent 'PESTonFarm' was used to estimate survival and detection of incipient Medfly cohorts under various climates and landscapes. The model uses information on biology and behaviour of the pest and integrates this with climate, landscape, crop phenology and IPM. The results showed that small invading propagules of Medfly would survive and establish under optimal conditions and be detected within a year of establishment. Under suboptimal conditions however, Medfly populations could stay in a cryptic state and there could be no population detection of the pest for a period of up to 3 years.

Christopher W Weldon: Dessication resistance in Tephritid flies

Dessication resistance of *Ceratitis* species was studied in bioassays where flies were deprived of water and food and exposed to dessicants (anhydrous dinitrogen, anhydrous silica gel) for a predetermined time period. Mortality of these flies were assessed. Body water and lipid contents of flies exposed to different treatments were measured. *Ceratitis rosa* was the least dessication resistant compared to *C. capitata* and *C. cosyra*. Wild flies were more dessication resistant than laboratory reared flies. Resistance to dessication might be related to metabolic processes such as lipid catabolism releasing water. A cross tolerance of larval diet and dessication resistance was found for some *Ceratitis* species.

Katherina Merkel: Density dependence on local resource foraging by *Bactrocera tryoni*

In a study on foraging behaviour of *B. tryoni* in a small patch of guava, infestation of guava was found to be negatively dependent on fruit density per tree. The authors found that the lower the fruit density on trees, the higher the fruit fly infestation rate. The authors suggested that when fruit availability was lower, density dependent processes could be higher.

Nikos Kouloussis: Effects of age, sex, adult food and larval food on starvation resistance of Medfly

The authors determined the starvation resistance of Medfly reared on citrus and larval diet. Medfly adults which were reared from citrus endured higher starvation resistance than Medfly adults reared on a protein rich larval diet. The reason why citrus reared flies were more starvation resistant is not known.

Session 2: Morphology and Taxonomy

Allen Norbom: *Anastrepha* diagnostics

Intkey and Lucid keys developed for *Anastrepha* species. These are available online. Information on hosts of *Anastrepha* species are available on the USDA Compendium of fruit fly host information (CoFFHI). <https://coffhi.cphst.org/> [site with restricted access]

Gary Steck: Improving current state of fruit fly larval taxonomy

Currently, descriptions of larvae are available for only 10 out of 95 *Ceratitis* species and only 21 out of over 400 *Bactrocera* species. There is currently a project on description of larvae in the *Anastrepha* genus. The plan is to expand this project to other fruit fly genera.

Scott Geib: Diagnostic tools for Tephritidae

A Tephritid diagnostic tool box is being developed at USDA ARS for species diagnostics and phylogenomics. The objective of the tool would be to identify the species and the source of the flies using population level gene markers.

Session 3: Genetics and Biotechnology

Helene Delatte: Range expansion of *Zeugodacus cucurbitae* in Africa

The population genetic structure of *Z. cucurbitae* in Africa was studied using microsatellite markers. The structure shows Asia as a source population and dispersal from East Africa to West Africa which correlates with first record of the species in 1936 in East Africa and in 1999 in West Africa.

Session 4: Chemical Ecology and Attractants

Nicholas Manoukis: Optimal MAT density for *B. dorsalis* control (less MAT stations may mean more effective control)

The authors tested different densities of methyl eugenol (ME) baited spots (100- 400 spots per km²) for control of *B. dorsalis*. The use of ME baited spots for control of *B. dorsalis* works on the principle of Male Annihilation Technique (MAT) whereby high male mortality is effected by ME baited insecticidal spots which then eventually leads to reduced mating and a reduction in pest population. The authors found that lower MAT densities provided more optimal control. MAT saturation was hypothesized as being the cause for the lower effectiveness of high density MAT. The authors postulated that in an area with high MAT density, males have a high background odour of ME and cannot locate the point source of ME or the ME spot.

William Urrutia: Anamed, new fruit fly bait

Anamed (ISCA Technology) is a new fruit fly bait that is currently being tested on various fruit fly pests: *B. dorsalis*, *B. latifrons*, *C. capitata*, *Z. cucurbitae*. Efficacy of Anamed for control of the fruit fly pests was compared with GF-120. Anamed was found to be more attractive than GF-120 for the target species. Anamed was also found to have a higher rain fastness. A combination of Anamed and MAT is currently being tested on *B. dorsalis* in Burkina Faso.

Session 5: Risk assessment, quarantine and post-harvest treatment

Roger Vargas: Effect of ACP cover sprays on fruit fly

The authors found that cover sprays of Mustang (Zeta-cypermethrin) and Warrior 2 (Lambda cyhalothrin) used for Asian Citrus Psyllid (ACP) were effective in controlling three fruit fly species: *C. capitata*, *B. dorsalis* and *Z. cucurbitae*. However, these cover sprays had a negative impact had on the fruit fly parasitoid: *Fopius arisanus*. The cover sprays have possibly a long residual activity against fruit fly pest species (possibly more than 7 days).

Emilia Bustos Griffin: Commodity tolerance international database to irradiation treatments

Knowledge on commodity tolerance to irradiation is an impediment to the use of irradiation as a post-harvest treatment for fruit fly pests or other cryptic pests. An International Database on Commodity Tolerance (IDCT) was set up: <https://nucleus.iaea.org/sites/naipc/IDCT/Pages/Browse-IDCT.aspx>. Tolerances of various fruit commodities including various citrus types (cultivar specific) to specific irradiation doses are provided in the database. Citrus is radiosensitive (sensitive to specific irradiation doses) except for sweet lime which is radiophilic. Recommendations for pretreatment of the fruit commodity are provided in the database.

Yulu Xia: Fruit bagging & packing house culling on risk mitigation of fruit flies on Citrus

Five fruit fly species are of major concern on citrus in China: *B. dorsalis*, *Bactrocera tau*, *Z. cucurbitae*, *Bactrocera minax* and *Bactrocera tsunenomis*. *Bactrocera tau*, *B. dorsalis* and *Z. cucurbitae* are tropical pests in southern coastal China while *B. minax* and *B. tsunenomis* are found only in temperate regions. Bagging was found to reduce infestation of pummelo by *B. dorsalis*. Packing house culling was found to eliminate infestation of satsumas by *B. minax*. The authors suggested the use of bagging and packing house culling as fruit fly control methods which are part of a systems approach for mitigation of fruit fly risks on citrus produced in China.

Carrol Hicks: Fruit fly data in consulted pest information resources

CABI and EPPO databases are used during Pest Risk Analysis for accessing particular markets. Errors on fruit fly pest's biology and distribution on these databases can impact on trade negotiation. A project was

recently funded by the USDA to identify and address errors on these global databases. An example was given on the export of fruit from USA to South America. The fruit for export was being produced in a pest free area which was 150 km from a detection point of *B. dorsalis* in USA. On the CABI database a wide range of dispersal distances was provided for *B. dorsalis*. More specific information on the dispersal capacity was found in a recent publication by Weldon et al. 2014 and this was used to correct the information on CABI database with regard to *B. dorsalis* dispersal distance.

Session 6: Sterile Insect Technique

Carlos Pascacio- Villafan: Optimization of artificial diet for fruit flies

A Pareto analysis of diet costs was used to determine a more cost effective diet for *Anastrepha* species and *C. capitata*. Various mixtures of diets for these species were evaluated. Effects of diet mixtures on fly performance (pupation and longevity) were determined. An optimization model for diet mixtures (ranges of wheat bran, crushed corn and yeast) was constructed based on various criteria from costs to performance. Such optimization model was used to formulate diet mixtures with the best performance and the least cost.

Session 7: Natural enemies and biological control

Beatriz Paranhos: Field dispersal of parasitoid

In this presentation, the authors presented an interesting technique for studying dispersal of natural enemies of fruit flies. Releases of *Diachasmimorpha longicaudata*, parasitoid of *C. capitata*, were carried out in cherry orchards. The dispersal of the parasitoids was determined by baiting using parasitism units (host fruit fly larvae in artificial diet hung on trees). With this technique, the authors estimated that the parasitoids dispersed over an area of approximately 8000 m² after two weeks.

Session 8: Other control methods and supporting technology

Dong Cha: Non insecticidal property of sugar

The nonnutritive and poorly metabolized sugar: Erythritol (2M) was found to have an insecticidal effect on fruit flies. The effect was confirmed to be not due to starvation. The authors suggested the use of erythritol and sugar in bait spray mixtures for fruit fly control.

Diana Perez Staples: Tephritid bait mixture in a bait station using the Toricelli barometer principle

The authors presented the FC bait station which is a device that uses the Toricelli barometer principle. The bait station consists of a bottle filled with fruit fly bait mixture which is then inverted at the open end onto a Petri dish where the bait mixture flows into. The bottle and Petri dish are contained in an open plastic shell which provides the cover for the bait mixture in the Petri dish. The FC bait station can be used with any fruit fly bait mixture. The station was tested with GF-120 and protein hydrolysate and malathion mixture. The bait station effectively killed flies for 35 days. The bait station also remained attractive for 42 days.

Nikos Papadopoulos: Oviposition deterrent effect of linalool on female Medfly

Linalool is a component of citrus oils. The authors determined the deterrent effects of linalool on Medfly oviposition. In their studies, the authors used pure Linalool and diluted it in ethanol. Concentrations of 15% and 30% linalool were sprayed on tree canopies of bitter orange, apple and nectarines. Sprayed fruit were then picked and offered to Medfly females. Oviposition propensity of Medfly females on sprayed and control fruit (fruit sprayed with ethanol only) was then determined. Oviposition was significantly reduced in linalool treated fruit. A time dependent performance was found for bitter orange and nectarine with further decrease in Medfly oviposition 3 days after linalool application. The authors also found a reduced number of Medfly captures in orchards treated with linalool. However, the authors did not show any data on fruit infestation.

Session 10: Area-wide and Action Programs

Laura Canhanga: IPM optimization for *B. dorsalis* in Mozambique

An optimized IPM strategy consisting of GF-120 sprays and orchard sanitation is being used for *B. dorsalis* suppression in Mozambique. The authors mentioned using an action threshold of 30 *B. dorsalis* males per trap per week.

Muo Kasina: Area-wide management of fruit flies in Kenya

A low prevalence of *B. dorsalis* was maintained in an avocado growing area in Kenya. The population of *B. dorsalis* in the area where a combination of mass trapping, male annihilation technique, orchard sanitation was used was at 0.1 *B. dorsalis* adults per trap per day. Two new control products for *B. dorsalis* were recently registered in Kenya: Bactrolure (ME) and Ceratrap.

Pablo Liedo: Area wide management of fruit flies in Mexico

The authors presented an area wide programme which moved from a research programme to an operational programme in Tapachula, Chiapas state, Mexico. Area wide monitoring programme conducted over years in the Tapachula region demonstrated that the highlands had high fruit fly (*Anastrepha* spp.) populations which moved into the lowland mango growing areas during the mango season. An area wide strategy was adopted with the highland areas of Tapachula being designated as the refugee zone where only fruit fly monitoring was carried out. A buffer zone (5 km wide) was then created between the refugee zone and the lowland mango growing areas. In the buffer zone, augmentative biological control and sterile insect technique (SIT) were used in combination for fruit fly suppression. Fruit fly suppression in the lowland mango growing areas was further achieved by combined use of bait stations or mass trapping, SIT and biological control (releases of fruit fly parasitoids). In the buffer zone and lowland mango areas, the sterile: wild male *Anastrepha ludens* ratio was kept to > 200. This area wide management strategy led to maintenance of wild *A. ludens* population level to well below 0.1 flies per trap per day and reduced fruit infestation.

Session 10: Social, Economic and policy issues of action programs

Rui Pereira: Reorganisation and harmonization of ISPM related to fruit flies

The International standards for Phytosanitary Measures (ISPM) related to fruit flies will be reorganized. ISPM 30 (areas of low fruit fly prevalence) will become part of ISPM 35 (fruit fly systems approach). ISPM No. 26 on fruit fly free areas now has 3 annexes: (1) guidelines on corrective action plans, (2) control measures for an outbreak in a fruit fly free area and (3) phytosanitary procedures used in fruit fly management strategies. There is also a new adopted diagnostic protocol for *Anastrepha* species (<https://www.ippc.int/en/core-activities/standards-setting/ispm/>) and new adopted post-harvest treatment protocols for fruit fly pests on the IPPC portal.

Emilio Hernandez: Irradiation as an alternative post-harvest treatment of mangoes and other fruit commodities

For fruit with no known post-harvest treatments, irradiation can be used as a post-harvest disinfestation treatment for fruit flies given that there are generic doses for Tephritidae and the only information that would be required would be commodity tolerances to irradiation. In mangoes, there were no changes in sensorial parameters due to irradiation (at 150 Gy) and hot water treatment. Irradiated mangoes retained the highest amount of antioxidants. Irradiation, however, delayed ripening of fruit

R. Angulo: Use of drones for sterile releases or releases of biological control agents

In Tapachula, drones are currently used for releases of sterile insects and fruit fly parasitoids. Specialised methods and equipment (Mubarqui system) have been developed for aerial releases of biological control agents. For releases of sterile flies, 380 000 flies can be loaded at once and released at 30 km/h at altitude of up to 100m.

1b. Key Points from selected posters

Valerie Balmes: Import control data for Tephritidae from Africa into Europe

The Plant Health laboratory in France has analysed the species composition of intercepted Tephritidae in mango exported from Africa. Prior to 2003, *C. cosyra* was the most commonly intercepted Tephritidae on

mango imported from Africa. Between 2004 and 2017, *B. dorsalis* mostly dominated among the intercepted Tephritidae. The number of intercepted *C. cosyra* was found to increase in 2009.

Patel et al.: Modified brewery yeast for fruit fly control

Waste brewery yeast slurry from Mauritius breweries was modified into a protein autolysate bait to be used as a fruit fly attractant. Different types of autolysates were prepared with different boiling times and different digesting times with different papain concentrations. Boiling times tested were 24, 48, 72 and 96 hours at 95°C. Digesting times tested were 24, 48, 72 and 96 hours at 65°C. Papain was added as an enzyme for digestion of the yeast waste at different concentrations: 0.1% - 0.4% weight by volume of waste. The most effective protein autolysate bait was prepared by first boiling for 72 hours at 95°C and then digesting by using papain at 0.4% w/v for 72 hours at 65°C. This bait was found to be attractive to *B. zonata* and *Z. cucurbitae*.

Khamis et al.: Comparison of gut transcripts of *Ceratitis quilicii* and *C. rosa*

RNA was extracted from gut tissues of *C. quilicii* and *C. rosa*. Transcript analysis showed that more genes were expressed by *C. quilicii* than by *C. rosa*, in particular for cell growth and immunity. Differentially expressed genes could be used as markers for species diagnosis.

2. Potential fruit fly projects: new ideas

Fruit fly control

- The non-nutritive sugar Erythritol could be explored as a toxicant in protein based baits for fruit fly control
- The use of FC bait station in citrus orchards in South Africa could be tested as an alternative to ground-based bait sprays
- Efficacy of Anamed for fruit fly control in citrus orchards in South Africa could be tested
- Stochastic model 'PESTonFARM' could be used to determine optimal control strategy for fruit fly pests of citrus in Western Cape in particular in areas with mixed crops.

3. Oral and poster presentations by Manrakhan et al. at 10th ISFFEI

3a. Abstract of oral presentation

Efficacy of existing and new attractants for Afrotropical fruit fly pests

Aruna Manrakhan¹, John-Henry Daneel¹, Massimiliano Virgilio^{2,3} & Marc De Meyer

¹Citrus Research International, P.O Box 28, Nelspruit 1200, South Africa. E-mail: aruna@cri.co.za; Royal Museum for Central Africa, Leuvensesteenweg 13, B-3080 Tervuren, Belgium;³ Royal Belgian Institute of Natural Sciences, Vautierstraat 29, B-1000 Brussels, Belgium

Background: Knowledge on responses of many Afrotropical fruit fly species to internationally recommended and new attractants has been scarce. In a 3-year project spanning between 2014 and 2017 under the ERAfrica_NI-027 fruit fly project, a joint Africa and Europe partnership project, studies on the efficacy of existing and new attractants for Afrotropical fruit fly pests were determined in various environments (commercial orchards and natural areas) in two northern provinces in South Africa.

Methods: The efficacy of five food-based attractants and five male lures for monitoring of fruit fly species in commercial orchards and natural areas was determined over a one-year period. The relative responses of fruit fly species to the attractants evaluated were quantified. The taxon coverage of each attractant was also measured in the different environments. The food-based attractants evaluated were three-component Biolure, ammonium acetate+ trimethylamine, ammonium acetate+ putrescine, Torula yeast and Questlure (a protein based attractant). The male lures evaluated were trimedlure, enriched ginger oil (EGO), cue lure, methyl eugenol and zingerone.

The sensitivity of an EGO baited delta trap for detection of *Ceratitis capitata* (Wiedemann), *Ceratitis rosa* Karsch and *Ceratitis cosyra* (Walker) was quantified in mark-release-recapture trials in commercial orchards. Laboratory reared mature males of the three species were released concurrently at different distances (up to 200 m) from a centrally placed EGO baited trap.

Results: The three-component Biolure was the most effective food-based attractant for females of *B. dorsalis* and key *Ceratitis* fruit fly pest species in South Africa. The new male lure zingerone was found to be attractive to two cucurbit infesting *Dacus* species. EGO attracted a wider spectrum of *Ceratitis* species than trimedlure. *Ceratitis capitata*, *C. rosa* and *C. cosyra* responded equally well to EGO in mark-release-recapture trials. Males of the three species released at 200 m from an EGO baited trap were recaptured within one week after release.

Conclusions: Trapping systems with three-component Biolure would be more effective at early detection of females of key Afrotropical *Ceratitis* pests and *B. dorsalis*. Area wide detection programmes targeting *Ceratitis* pests should include EGO baited traps so that a wider spectrum of *Ceratitis* species could be covered.

Key Words: *Ceratitis*, *Bactrocera dorsalis*, *Dacus*, three-component Biolure, EGO, zingerone

Session 4: Chemical Ecology and Attractants

3b. Abstract of poster presentation (Poster 1)

Assessment of lufenuron-methyl eugenol mixture as a chemosterilant bait for *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae)

Aruna Manrakhan¹, John-Henry Daneel¹, Christopher W. Weldon²

¹Citrus Research International, P.O. Box 28, Nelspruit 1200, South Africa. E-mail: aruna@cri.co.za;

²Department of Zoology and Entomology, University of Pretoria, Private Bag X20, Hatfield 0028, Pretoria, South Africa

Background: Mixtures of adult food (protein or sugar) and lufenuron, a chitin synthesis inhibitor, have been shown to confer sterility to females and males of some fruit fly pests following ingestion. The male lure methyl eugenol elicits strong olfactory and feeding responses for males of the Oriental fruit fly, *Bactrocera dorsalis* (Hendel), a pest of economic importance in several parts of the world. The potential of a mixture of methyl eugenol and lufenuron as a chemosterilant bait for *B. dorsalis* was therefore explored.

Methods: Ten-day old virgin sugar and protein fed *B. dorsalis* males were exposed for 24 hours to technical grade lufenuron (99.9%) mixed with liquid methyl eugenol at different concentrations: 5, 10, 30, 60 and 120 mg of lufenuron per g of methyl eugenol. The control group was males fed with methyl eugenol only (no lufenuron). A second control group consisted of males which were not exposed to methyl eugenol (untreated males). Treated and untreated males were paired with virgin untreated 10-11-day old *B. dorsalis* females. Adult mortality, fecundity and fertility were assessed for two weeks following pairing.

Results: None of the lufenuron-methyl eugenol mixtures prevented complete egg hatching. The lowest egg fertility was however recorded from adult pairs with males exposed to lufenuron-methyl eugenol mixture at 120 mg of lufenuron per g of methyl eugenol. Fecundity and adult survival were largely unaffected by treatment.

Conclusions: Findings in this study showed little or no prospect of a male chemosterilisation technique for *B. dorsalis* using the insect growth regulator, lufenuron.

Key Words: Oriental fruit fly, chemosterilisation, insect growth regulator

Session 8: Other control methods

3c. Abstract of poster presentation (Poster 2)

Effects of temperature and intrinsic traits on tethered flight performance of the oriental fruit fly, *Bactrocera dorsalis*

Louisa D. M. Makumbe¹, Aruna Manrakhan², Christopher W. Weldon¹

¹Department of Zoology and Entomology, University of Pretoria, Private Bag X20, Hatfield 0028, South Africa. Email: cwweldon@zoology.up.ac.za. ²Citrus Research International, PO Box 28, Nelspruit 1200, South Africa

Background: Flight plays an important role in the life history of insects by enabling them to search for food, new habitats, mating partners and oviposition sites. It is also important for the surveillance and management of insect pests because it mediates response to traps and colonisation of new areas. Temperature is a fundamental determinant of flight performance due to its effect on metabolic rate and the release of energy for flight muscle function. Morphological traits such as body mass, wing length and width, wing area and wing loading (the ratio of body mass to wing area) also influence flight performance, but these may vary between the sexes and with age. This study sought to determine the effect of temperature on tethered flight performance of virgin female and male oriental fruit flies, *Bactrocera dorsalis*, over a range of ages.

Methods: Measurements of tethered flight by virgin female and male *B. dorsalis* aged 3, 10 and 21 days were recorded for one hour using flight mills. Test temperatures were 12, 16, 20, 24, 28, 32 and 36°C (n=4 for each combination of temperature, sex and age). Body mass of flies was determined before they were attached to the flight mill, and wing length, area and loading were measured after the flight tests. Recordings from each fly were used to calculate the number of discrete flight bouts, total distance flown, mean bout and total flight duration, and mean bout and maximum flight speed.

Results: The mean duration of flight per bout and per hour was 1273.9 ± 93.7 s and 31939.5 ± 64.2 s, respectively. Bout duration peaked at 20°C whereas the total duration of flight increased between 12-16°C but did not change thereafter. Mean bout (0.67 ± 0.05 m/s) and maximum flight speed (3.05 ± 0.23 m/s) was not affected by sex, age or temperature. Body mass and wing loading increased with age whereas wing length and wing area decrease slightly as flies aged. Sex did not influence measured morphological traits. Three day-old flies had more flight bouts than older ones. The furthest total distance flown by *B. dorsalis* was 1559.58 m but the mean was 354.7 ± 28.3 m. Total distance flown increased with fly body mass.

Conclusions: Tethered flight performance traits tended to peak within the range 16-24°C. With the exception of the number of flight bouts, tethered flight traits of females and males across all tested ages were similar.

Keywords: Body mass, Flight mill, Tethered flight performance, Wing loading

Session 1. Biology, Ecology, Physiology and Behavior

4. Meeting on STDF_PPG_567 'Establishment and maintenance of fruit production areas free and under low prevalence of fruit fly pests in southern Africa'

The Standards Trade and Development Facility (STDF) approved a Project Preparation Grant (PPG) to the Department of Agriculture, Forestry and Fisheries (DAFF), Pretoria, South Africa, and associated partners for a project (STDF/PPG/567) entitled "Establishment and maintenance of fruit production areas free and under low prevalence of fruit fly pests in southern Africa". The associated partners for this project were: CRI, Stellenbosch University, Eduardo Mondlane University (Mozambique) and Royal Museum for Central Africa (RMCA), Belgium. The main objectives of the PPG were to undertake in-depth consultations with stakeholders to assess the situation in terms of knowledge and feasibility of the establishment of pest free areas (PFA) and areas of low pest prevalence (ALPP) and formulate a project proposal that would aim to establish PFAs and ALPPs for targeted fruit fly pests in South Africa and Mozambique on the basis of the findings of the PPG. In this PPG, RMCA acted as contractor for this grant and coordinated the different activities. The main activity undertaken by CRI was the write up of the project proposal. A contract agreement between CRI and RMCA was signed for the project proposal write up.

My participation at the 10th ISFFEI was used as an opportunity to meet up with other project partners to discuss progress on the PPG and full project proposal. The only project partner who was also at the 10th ISFFEI was Dr Marc De Meyer, RMCA.

A meeting on the STDF PPG 567 was organized with Dr De Meyer on Thursday 26 April 2018. Points that were discussed were as follows:

1. Project consortium and the involvement of the ARC-TSC as project leader for project management
2. Project objectives and logical framework of the full proposal
3. The second stakeholders' meeting dates, venue and agenda

5. Notes from a field visit to a mango orchard, sterile insect facility and mango packing house in Tapachula, Mexico

Mexico is the main exporter of mango in the world. About 19 000 tons are exported to the USA annually. The production of mangoes per ha is currently at 3-4 tons. Trees in most orchards have high canopies and as such require boom lifts for harvesting.

The Ataulfo variety is the main mango variety produced in Tapachula and is in high demand because of its organoleptic properties and its tolerance to hot water treatment for disinfestation of fruit flies.

The fruit fly control programme in mango orchards includes releases of sterile *Anastrepha ludens* and *A. obliqua* (main mango pests) as well as releases of parasitoids. A male only strain of *A. ludens* is currently being used. For *A. obliqua*, sterile males and females are released. Release rates vary between 1000 – 6000 flies per ha depending on trap catches. The production and releases of sterile flies are under the MOSCAMED programme which receives mainly government funding.

Export of Ataulfo mango to USA requires a hot water treatment. For disinfestation of *Anastrepha* spp. including *A. ludens*, the target temperature of the water in the tank is 46.1°C. The treatment time depends on fruit size. Treatment time can be up to 110 minutes. Temperatures in the tanks are monitored and have to be provided at the end of the treatment.

I visited a mango packing house which had an in house facility for hot water treatment. The facility was certified by USDA. Fruit arriving from the orchard were first sorted into different grades. The fruit were then washed and dipped in a fungicide bath. Export grade fruit were sorted according to weight and packed in baskets. Fruit of particular weight range were dipped in a selected tank with circulating hot water (Fig. 7.2.1 A) at 46.1 °C for a specified time. Soon after the hot water treatment, the fruit were lowered in cool water at 20°C before drying and packing (Fig. 7.2.1 B). The packed fruit were stored in a cool room at 12°C until transportation by road to USA.



Figure 7.2.1: Hot water treatment of mangoes for fruit fly disinfestation in Tapachula, Mexico: (A) Tank for hot water treatment in a mango packing house and (B) Mangoes packed for export to USA after hot water treatment.

Acknowledgments

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8 EXTENSION / VOORLIGTING

By Hannes Bester, M.C. Pretorius, Wayne Mommsen, Keith Lesar, Catherine Savage, David Groenewald, Andrew Mbedzi and Melton Mulaudzi

8.1 VOORLIGTINGOORSIG

8.1.1 Die 2018 Seisoen

Die seisoen het aanvanklik groter uitdagings gehad as wat aanvanklik voorspel was. Daar was nog heelwat vrugte van swak gehalte vanaf Morokko en Egipte teen goedkoop pryse beskikbaar teen die tyd dat die eerste vrugte vanaf Suid-Afrika in die mark geland het. Die Satsumas het baie suurvrot en koueskade gekry en dit was vroeg reeds duidelik daar groter fokus op die bestuur van die koueketting gelê moes word vir die res van die seisoen. Die vruggrootte op nawels was oor die algemeen aan die kleiner kant, wat verder druk op die markte geplaas het. Pomelo's het stadiger verkoop as wat aanvanklik verwag is. Die groot aanbieding van somervrugte in die Noordelike Halfrond het ook bygedra tot die druk wat verkope van sitrus ervaar het. Pryse op suurlemoene was van meet af beduidend laer as die vorige jaar, maar steeds aanvaarbaar. Die swakker wisselkoers het tot 'n mate gekompenseer vir die laer verkoopspryse in die markte.

Die groot toename in uitvoervolumes het SA se probleme t.o.v verkoelingskapasiteit, veral in Durban-hawe, blootgelê. Die situasie gaan in die toekoms beduidend versleg as dit nie so spoedig moontlik na behore aangespreek word nie.

Die Valskodlingmot-bestuurstelsel (FMS) na Europa het uiters suksesvol verloop en daar is slegs nege onderskeppings a.g.v FCM gerapporteer. As in ag geneem word dat die FMS nog nooit voorheen kommersieël getoets is nie, en dat daar in die eerste jaar meer as 750 000 ton sitrus suksesvol onder hierdie stelsel uitgevoer is, kan al die rolspelers gekomplimenteer word vir hul uitstekende bydrae. Die koueskade op die vrugte was nie noemenswaardig hoër as in die verlede nie, en was selfs ietwat laer op pomelo's. Op Midnight Valencias was dit wel hoër waar "step-down temperature" verkoeling nie toegepas is nie en ook omdat die vrugte soms vir lang periodes aan die lae temperature blootgestel was. Die voorkoms van FCM was egter laag gedurende die jaar. Uit 'n fitosanitêre oogpunt het dit dus besonder goed gegaan. Uit die EU, wat baie hoë tegniese eise aan die sitrusbedryf stel, is slegs twee onderskeppings vir swartvlek gerapporteer, wat 'n groot prestasie is as die statistiek van vorige jare in ag geneem word.

Die voorspellingsmodelle vir Suid-Afrikaanse sitrus het 'n paar jaar gelede getoon dat die uitvoervolumes teen ongeveer 2023/24 sou afplat op net meer as 120 miljoen katonne. Die totale verpakking vir uitvoer die afgelope seisoen was reeds 'n rekordvolume van 135.2 mil katonne. Die volumes vir alle sitrustipes was meer as dié van 2017, met verwagte groei van ongeveer 'n verdere 10 mil vir die 2019 seisoen onder gunstige klimaatstoestande, met die grootste toename in sagtesitrus en suurlemoene. Uit verskeie oorde is dit beskryf as die moeilikste bemarkingsjaar sedert die middel negentiger jare. Wat pomelo's betref is die seisoen as 'n ramp beskou. Suurlemoene het beter gevaar as wat verwag is en bemarking van sagtesitrus was bevredigend.

Die vrugkwaliteit was oor die algemeen nie goed nie. Vruggrootte was relatief klein vir meeste sitrustipes. Bederf op veral sagtesitrus en nawels was problematies, en selfs ook op pomelo's en Valencias. Kleurontwikkeling was laat en vrugkleur in die mark was swak, veral in vergelyking met vrugte uit kompeterende lande. Koueskade was wel erger as wat aanvanklik gedink is, wat waarskynlik ten gevolge van swakker vrugkleur is. Daarmee saam was letsels 'n veel groter probleem as vorige jare. Hierdie faktore, in markte wat reeds onder druk was, het bemarking aansienlik bemoeilik.

8.1.2 Die 2019 Seisoen

Die skatting vir sitrus-uitvoervolumes vir 2019 dui daarop dat daar weereens rekordvolumes verwag word, 137.2 miljoen katonne teenoor verlede seisoen se rekordvolume van 135.2 miljoen katonne. Dit is nog onseker hoe die markte hierop gaan reageer. Op die oomblik verkeer verskeie markte nog onder druk, maar

omstandighede kan hopelik positief verander. Nawel-volumes gaan ongeveer dieselfde wees as verlede seisoen, Valencias en pomelo's dui op 'n effense afname en suurlemoene en sagtesitrus dui op 'n betekenisvolle toename in volumes.

As gevolg van droogte in sekere areas en uitermate sterk, warm winde wat net na blom gewaai het, is die geskatte volumes veel laer as die oorspronklike projeksies wat op die boomsensus en oppervlak onder sitrus gebaseer is. Op die oomblik beloop die totale oppervlak onder sitrus in die orde van 84000 ha, met ongeveer 21000 ha wat bome jonger as vyf jaar is. Onder gunstige klimaatstoestande kan 'n geweldige verhoging van uitvoer volumes in die toekoms verwag word.

Daar is verskeie inisiatiewe aan die gang om die druk op die hele logistieke ketting te verlig wat met die toename in uitvoervolumes gepaard gaan. Dit sluit pogings in om meer vrugte per spoor na Durban en Kaapstad te vervoer, meer vrugte deur Maputo uit te voer en om hawe-fasiliteite op te gradeer.

Verder sal daar weer 'n baie sterk fokus op marktoegang wees, veral wat betref die bestuur van die Valskodlingmot-bestuurstelsel (FMS) vir vrugte na Europa. Met 'n paar verbeteringe aan die FMS kan daar met vertroue voortgebou word op die suksesse van 2018. Op hierdie stadium lyk dit of al die fitosanitêre plaë goed onder beheer is in die produksiestreke.

8.1.3 CRI Postharvest Technical Forum

Kartontoetse as deel van die akkreditasie van die kartonvervaardigers het gedurende die eerste kwartaal in alle erns begin. Dit is uiters belangrik dat kartonne wat aan produsente/pakhuis gelewer word, ten alle tye aan die spesifikasies voldoen. Daar word ook voorsiening gemaak vir kartonne wat direk deur produsente en CRI na die laboratorium gestuur kan word. Gedurende die kwartaal was daar vier gevalle waar kartonne deur produsente ingestuur is. In een geval het die kartonne nie aan die spesifikasies voldoen nie en die gevolg daarvan was 'n eis teen die vervaardiger. Die saak is tot tevredenheid van die produsent afgehandel. Die toetsresultate van die kartontoetse vir die res van die seisoen vir akkreditasie was deurgaans goed en die produsente se versoek om kartonvervaardigers te akkrediteer en dat kartonne op 'n gereelde basis deur Sappi se laboratorium getoets moet word, werp beslis vrugte af.

Dit is 'n vereiste dat 'n vervaardigerskode op kartonne gedruk moet word. Klagtes is ontvang van kartonne, sonder enige kodes, wat by die hawens aankom. 'n Ondersoek het getoon dat kartonne sonder vervaardigerskodes deur "kartonvervaardigers", wat nie geakkrediteer is nie, verskaf is. Dit is met hulle en die betrokke produsente/pakhuis opgeneem, en bo en behalwe dit, is al die geakkrediteerde kartonvervaardigers weer daaraan herinner dat hulle vervaardigerskodes op alle kartonne gedruk moet word.

Lewering van kartonne was gedurende die eerste kwartaal in sekere streke 'n probleem. Dit het hoofsaaklik betrekking op pomelo's. A.g.v. kleiner vrugte, kleur en markomstandighede het produsente/pakhuis hulle kartonbestellings gereeld en op baie kort kennisgewing verander. Om van een kartontipe na 'n ander te verander, neem lank en produksietyd gaan verlore. Papierwydtes verander en dit het ook 'n baie nadelige uitwerking op die lewering van papier. Groot lof aan die grootste verskaffer van "liners" en "fluting", wat werklik uit hulle pad gegaan het om hulle produksie-skedules op kort kennisgewing te moes verander, en sodoende het die kartonvervaardigers deurgaans voldoende papier gehad. Kartonvervaardigers het ook deurentyd 'n ernstige beroep op produsente gedoen om verandering van een tipe karton na die ander tot die minimum te beperk.

'n Kleinskaalse proef met plastiese palette is in die Oos-Kaap gedoen. Die palette is gedurende die laaste week van Junie na Kanada verskeep. Daar is ook begin met voorlopige werk op 'n palet wat met 'n kombinasie van hout en plastiek vervaardig word. Vroeë bevindings is dat die palet nie koste-effektief is nie en menings is ook uitgespreek dat 'n kombinasie van hout en plastiek problematies gaan wees wanneer die palette herwin moet word. Daar is heelwat kommer oor die toekomstige beskikbaarheid van hout vir palette. Die toonaangewende paletvervaardigers is van mening dat daar genoeg hout beskikbaar sal wees, maar meld dat produsente 'n hoër prys vir palette sal moet betaal in die toekoms.

CRI-PTF is al vir 'n geruime tyd besig met onderhandelinge met PalletPlast. Voorlopige deurbuiging toetse was suksesvol. Die volgende stap was 'n kleinskaalse uitvoerproef met 20 PalletPlast palette met lemoene vanaf 'n pakhuis in die Oos Kaap. Die 20 palette is by die pakhuis se koelkamers in 'n "Hi-cube container" gelaai, na die Port Elizabeth hawe vervoer en vandaar na Kanada verskeep. Die palette het goed in Kanada aangekom, maar ongelukkig kon die betrokke pakhuis nie enige verdere inligting kry nie. Dis noodsaaklik dat die proef herhaal word met klein suurlemoene in A15C kartonne wat by 'n pakhuis in die Limpopo streek gepak is, wat 'n baie strawwer toets vir 'n palet is. Die pryse van hierdie palette is nog nie beskikbaar nie.

Mpact Plastics het CRI-PTF genader oor die moontlike gebruik van hulle 600x400x170mm plastiese kratte vir uitvoersitrus. Verskeie uitvoer-organisasies is genader om hulle gevoel te toets. Die algemene gevoel was nie baie positief nie en enkele instansies is van mening dat daar dalk 'n baie beperkte mark vir vrugte in plastiese kratte kan wees. Die koste van die kratte is nog nie bekend nie. Daar is tog besluit om 'n kleinskaalse proef by GoGo te doen. Drie palette met industriële graad vrugte is gepak en die palette is gedurende die eerste week in Oktober uitgevoer. Die verpakking en palettisering het heel goed verloop en palette het mooi en stewig vertoon. Tydens die finale evaluering sal daar veral klem gelê word op vrugkwaliteit, moontlike verpoeiering van die waks, koueskade, toestand van die kratte en die stabiliteit van die palette. 'n Volledige verslag sal opgestel word na die oorsese terugvoer.

Ernstige probleme met swamgroeï op palette het weer voorgekom. 'n Video opname wat ontvang is van water wat uit verskepingshouers uitloop, vererger die probleme met swamgroeï wat op palette ondervind word. Dit gaan hoofsaaklik hier oor die verskillende "drain plugs" in die skeepshouers. Daar word tans gebruik gemaak van soliede- sowel as "drain plugs" met gate in die middel wat 'n tipe van 'n sif oor die gate het. "Drain plugs" met die openinge word verkies want die vog/water kan dan heeltyd uitloop. Die hele aangeleentheid is met DAFF, PPECB, sekere uitvoer-organisasies en CGA se logistieke mense opgeneem en geniet tans aandag.

CRI-PTF is genader deur 'n maatskappy, PrimePro Products, met 'n produk wat die raklewe van vrugte verleng. Die vrugte word binne-in die kartonne in plastiese sakke gepak. Dit is binne CRI bespreek en daar is besluit om op 'n baie klein skaal te kyk of die raklewe van sitrus in die sakke wel verleng sal word. Baie vroeë aanduidings is dat vrugte in sakke na twee weke alreeds baie beter lyk as die kontrole.

Tekco Packaging is 'n maatskappy wat in die verlede riffelbord van kartonvervaardigers aangekoop het en dan kartonne vervaardig het. Slegs volwaardige kartonvervaardigers kom in aanmerking vir akkreditasie. Hulle is bewus daarvan en is in die proses om 'n baie moderne nuwe fabriek in Gauteng op te rig. Hulle beplan om in die toekoms hulle eie riffelbord te vervaardig. Sodra hulle nuwe fabriek op en aan die gang is, sal die volledige proses begin om hulle te akkrediteer. Alles dui daarop dat hulle eerste volledig vervaardigde A15C Supervent kartonne in November 2019 gereed sal wees. Die eerste voorlopige laboratorium toetse sal dan gedoen word.

Wonderful Citrus in die VSA ondervind ernstige probleme met hulle kartonne. Hulle het SA besoek om meer uit te vind oor papier, karton-ontwerpe en vervaardiging van SA se volledige reeks uitvoer-kartonne. Tydens hulle besoek is samesprekings gevoer met senior personeel van Sappi en hulle is ook deur die "Sappi Technology Centre" se fasiliteite geneem waar al die papier- en kartontoetse aan hulle gedemonstreer is. Hulle is ook deur Houers Koöperatief se fabriek in Letsitele geneem. Hulle het tydens hulle besoek ook 'n paar sitruspakhuse in Limpopo, Mpumalanga en die Wes-Kaap besoek.

Mpact Paper het in die verlede meer gefokus op die vervaardiging van "Fluting" en wit papier. Die produkte staan bekend as "Bayplex Fluting" en "Baywhite Liners". Hulle het besluit om in die toekoms ook meer te fokus op die vervaardiging van 'n hoë gehalte bruin "Linerboard" vir bruin (Kraft) uitvoerkartonne. Die produkte staan bekend as "Baykraft" (BK). In samewerking met Houers is daar eksperimentele A15C kartonne met 170g/m² BK vervaardig. Let daarop dat die ander vervaardigers se "Liners" se basiese massa, 175g/m² is. Wat die tipiese waardes betref vergelyk die 170g/m² BK baie goed met 175g/m². Die produksie personeel by Houers was heel tevrede met die vervaardiging van die riffelbord en die kartonne. Die kartonne is na Mpact se laboratorium in Springs geneem waar stapelsterkte toetse gedoen gaan word. Die ligter 170g/m² BK behoort die kartonne ook effens meer koste-effektief te maak.

"Tree Wrap" (TW) wat met 300g/m² Sappi Kraftpride (KP) vervaardig was, is nie meer beskikbaar nie. Die KP TW was regtig suksesvol en daar is heelwat versoeke ontvang om die vervaardiging van TW wat met die swaar papier vervaardig word, weer te ondersoek. In samewerking met Houers is 'n kleinskaalse proef by

Laeveld Sitrus gedoen met 170- sowel as 300g/m² KP TW wat met die hand gesny is. Die doel op hierdie stadium is om te bepaal hoe lank die 170g/m² gaan hou. Intussen word verskeie metodes om die papier in groottes van 340x210mm te sny en te verpak, ondersoek.

Op versoek van een van die toonaangewende produsente is 'n "mulching" proef met 300g/m² "Kraftpride Linerboard" in een van hulle boorde met nuutaangeplante Bennie-Valencias gedoen. Dit gaan hoofsaaklik oor waterbesparing (voginhoud in die grond) en onkruidbeheer. Die proef beslaan 2,2 hektaar. Voginhoud van die grond en onkruid sal op 'n gereelde basis gemonitor word. Die verwagting is dat die papier vir minstens twee jaar sal hou.

Die jaarlikse Verpakkingswerkgroep vergadering het gedurende November plaasgevind. 'n Volledige voorlegging oor alle verpakkingsaangeleenthede gedurende die 2018 seisoen is gedoen en tydens die vergadering het Sappi aangekondig dat hulle gedurende 2019 sal voortgaan met die akkreditasie toetse van die kartonne. Dit sal, net soos in die verlede, weer op Sappi se koste gedoen word. 'n Skrywe waarin die bedryf se dank en waardering uitgespreek is, is aan Sappi gestuur. In samewerking met Sappi is die skedule vir 2019 opgestel en na die geakkrediteerde kartonvervaardigers gestuur. Net soos verlede jaar sal daar gedurende 2019 ook weer meer kartonne by pakhuis getrek word.

Met die doel om kostes te besny het twee produsente versoek dat tuimelverpakking (jumble packing) van tellings 105 en 125 lermoene ondersoek word. Tesame hiermee is die vraag ook gevra wat die effek van die gebruik van toedraaipapier op verkoeling is en of daar nie daarmee weggedoen kan word nie. Wat die effek van die gebruik van toedraaipapier op verkoeling betref, is dit 'n feit dat enige vorm van toedraai van vrugte 'n nadelige invloed/effek op verkoeling het. Sekere kopers voel egter baie sterk oor toedraai van vrugte vir hulle markte. Dit is iets wat tussen die produsente, hulle uitvoermaatskappye en die oorsese kopers hanteer moet word en CRI-PTF kan ongelukkig nie hierby betrokke raak nie. Tuimelverpakking is op A15C, D15C, E15C en D15D ondersoek met die doel om die "payload" te verbeter. Met alles inaggenome (massa per karton, massa per palet, bruto massa per skepshouer, ens.) was die D15C karton die mees geskikste karton wat tuimelverpakking betref. Die nadeel was dat die koste per palet ±R70 hoër is as verpakking per telling (plekverpakking) in die A15C karton. Dit is a.g.v, die hoër prys van die D15C karton. Wat die "payload per pallet" betref bly plekverpakking in die A15C karton die beste opsie. 'n Volledige verslag is op aanvraag beskikbaar.

Verskeie samesprekings is vroeg in 2019 met 'n nuwe kartonvervaardiger gevoer wat graag geakkrediteer wil word as 'n verskaffer van sitruskartonne. Die voorlopige proses is begin, die laboratorium toetse is gedoen en fase twee (die pak en uitvoer van kartonne) is beplan en sal gedurende die tweede kwartaal 'n aanvang neem.

Gedurende 2018 is daar op versoek van OptiFlo Freezer Spacers 'n voorlopige ondersoek gedoen met Freezer Spacers (FS). Hulle het die stelling gemaak dat die gebruik van FS die verkoelingstyd van vrugte met tot soveel as 50% sal verkort. Bogenoemde ondersoek het ongelukkig die teendeel bewys. Afgesien van die negatiewe effek van die gebruik van FS op verkoeling sal die gebruik daarvan die sitrusbedryf miljoene kos. Hulle is egter versoek om die FS se ontwerp aan te pas sodat daar slegs een direk bo-op die paletbasis, in plaas van een op elke laag kartonne, geplaas kan word. Verskeie gesprekke is met hulle gevoer en hulle het 'n aangepaste prototipe beskikbaar gestel. Toetse met die gewysigde FS sal gedurende April gedoen word.

CRI-PTF is deur 'n maatskappy, "NanoProtect", genader om uitvoer-sitruskartonne met "Nano Coating" te behandel om die kartonne vogbestand te maak. 'n Voorlopige ondersoek het getoon dat die behandeling tydens die vervaardigingsproses geheel en al onprakties is en dat die koste verbonde hieraan ook baie hoog is.

8.1.4 **Produksiestreke**

Uit 'n produksie-, oes- en verpakkings-oogpunt was dit 'n jaar met vele uitdagings; laat kleur-ontwikkeling, bleek vrugkleur, baie letsels, bestuur van die nuut geïmplimenteerde FMS, arbeidsprobleme en vele meer. Heelwat navrae om vrugkleur te verbeter het laat in die seisoen deurgekom. Aangesien dit meesal aan klimaat

toegeskryf kan word, is daar weinig wat aan vrugkleur gedoen kan word, behalwe miskien vir kleiner aanpassings t.o.v bemestings- en besproeiingsbestuur.

Arbeids-onrus in dele van die Oos-Kaap, veral die Sondagsriviervallei, het veroorsaak dat die seisoen op 'n moeilike noot afskop, maar na samesprekinge tussen die onderskeie partye is die ongelukkigheid en gepaardgaande onsekerheid betyds uit die weg geruim.

Welkome reën gedurende die middel van 2018 in die opvanggebiede in die Oos-Kaap het groot verligting van die erge droogte van die afgelope paar jaar gebring. Die Kouga dam was op slegs 5% en alle watervoorsiening aan produsente was gestaak, maar binne 'n paar weke na die goeie reën het die watervlak na 50% gestyg. Goeie reënval het meer dikwels in die Wes-Kaap voorgekom en die stand van damme aansienlik verbeter.

Die meer gereelde reënval en koue in die Wes-Kaap het hoë voorkoms van Botrytis veroorsaak, veral op sekere sagtesitrus-tipes. In sekere manderyn-boorde naby Porterville het feitlik al die blomme reeds afgespeen a.g.v Botrytis. Daar is egter geen chemiese produkte vir beheer van Botrytis op sitrus geregistreer nie en registrasieproewe is dringend aan die gang gesit. Hierdie situasie dwing produsente egter om van ongeregistreerde produkte gebruik te maak.

Hoewel pryse nie so hoog soos die 2017 seisoen was nie, was produsente in die suide tevrede dat die seisoen relatief goed afgegaan het. Die groot aanplantings wat steeds plaasvind is 'n aanduiding dat produsente vertroue in die toekoms het en ook nie in vrees leef vir politieke kwessies wat grondbesit raak nie.

Die watersituasie vir 2019 lyk belowend vir die suidelike produksiegebiede en geen krisis word voorsien nie. Hoewel damvlakke steeds relatief laag is, is daar genoeg water beskikbaar om die oes deur te dra. In die suidelike produksiestreke, veral die Oos-Kaap, het stormsterk winde gedurende November nie net heelwat vrugval veroorsaak nie, maar ook baie windskade op die vrugte tot gevolg gehad. Dit sal beslis 'n negatiewe impak op die uitvoerpersentasie van die vrugte inhou. Die volumes van nawels en Valencias sal laer wees, maar die verhoogde volumes suurlemoene en sagtesitrus sal daarvoor opmaak.

Valskodlingmot-druk deur die afgelope jaar was besonder laag, maar het teen die einde in feitlik alle produksiegebiede begin toeneem, wat beteken dat FCM-beheer en die totale bestuur van die FMS meer nougeset hanteer sal moet word. In sommige areas is bladspringer vanjaar skielik 'n probleem, maar andersins bleik meeste plaë onder beheer te wees.

Die produksie in die sentrale produksie streek was 'n gemiddelde oes met uitsonderings van hoër opbrengste in sekere boorde in van die areas. Die meeste areas het weer 'n uitgerekte blom vir die 2018 seisoen ervaar, wat die bestuur daarvan 'n uitdaging gemaak het. Produsente in van die areas het in Februarie die vruggies wat bly hang het na die November vrugval fase met die hand uitgedun.

Oor die algemeen was die oesladings in die sentrale produksie gebied op al die kultivars heelwat meer as gedurende 2017 seisoen. Die interne gehalte van die vroeër kultivars was uitstekend. Die pomelo's was weens swak kleur bietjie later gepluk en daar was ook heelwat meer kleiner vrugte as wat verwag is weens die hoër oeslading. Heelwat skilprobleme, veral kraakskil, is veral op die nawel kultivars waargeneem en vrugval het ook in meeste van die produksiestreke voorgekom. Swaar drag op meeste van die kultivars het die plukseisoen heelwat onder druk geplaas en heelwat produsente het gesukkel om oeste in die normale pluktydperk af te kry.

Daar was 'n hoër insidensie van windskade en blaaspootjie merke asook rooidopluis wat op vrugte waargeneem is. Die FCM en vrugtevlug druk was aansienlik laer as wat verwag is, maar produsente moet ook krediet kry vir hul hoë vlak van bestuur en insette om hierdie uitdaging die hoof te kon bied. Meeste produsente was oor die algemeen tevrede met die seisoen, ten spyte van skilprobleme in sekere areas op nawels en sekere Valencias voorgekom het. Daar was minder fito-skade oor die algemeen, maar na-oes koueskade weens die nuwe EU regulasies is wel aangemeld.

Die Sentrale produksie gebied het 'n goeie blomperiode vir 2019 gehad, alhoewel uiterste temperatuurverskille voorgekom het. Temperature sou vir 'n paar dae tot middel dertigs gestyg het, om net die volgende dag of twee onder twintig grade Celsius te beleef. Goeie vroeë reënval het voorgekom, maar die buie is nie opgevolg met goeie opvolg reën nie. Damvlakke was teen die einde van die jaar baie laag, maar die verwagting was dat goeie somerreëns wel sou volg.

Hael het vroeg in 2019 wydverspreid voorgekom in Groblersdal/Marble Hall asook Burgersfort, Ohrigstad en Nelspruit areas. Burgersfort het vyf haelbuie met skade vroeg in die nuwe seisoen beleef en van die ander areas tot soveel as drie keer. Die reënval was maar ondergemiddeld gedurende die eerste helfte van die seisoen maar het wel 'n piek bereik gedurende die tweede helfte van die seisoen. Die meeste opgaar damme het voldoende water vir die volgende seisoen. Die Nkweleni area het vir heelwat weke uiters hoë dagtemperature beleef.

Vrugtevlug-druk was in meeste van die areas hoër as gewoonlik. Lugbespuitings is ingespan om met area-wye bespuitings die getalle onder beheer te bring. Laat blaaspoottjie druk was hoog in meeste van hierdie areas.

In die Noorde het Pomelo oeste eers later afgeskop in die Letsitele gebied as gevolg van 'n vertraging in opkleur van die vrugte op die bome. Tesame met 'n massiewe oeslading het 'n bottelnek ontstaan, met die groot aantal kartonne wat alles gelyk uit die Noorde na die hawe toe gestuur is. Hierdie bottelnek asook baie klein vrugte het 'n negatiewe impak op die Europese mark m.b.t pryse van kartonne in die mark gehad en daar was kommer dat dit moontlik 'n president sou skep vir die Valencias wat moes volg.

Zimbabwe produsente het geen probleme gehad met die verpakking van die Pomelo's nie. Die druk in die hawe het wel 'n groot impak gehad op die vervoer van die vroeë Valencias. Daar was op 'n stadium geen trokke beskikbaar nie en dit het die oes van vrugte vertraag. Vruggehalte en uitpakte was goed in hierdie streek.

Die Letsitele gebied was bekommerd oor die watersituasie. Die gebied het baie lae reënval ontvang en opgaardamme is alreeds leeg. Die ander Noordelike streke was gelukkig in 'n baie beter situasie wat water betref.

Die Noordelike gebiede het laathangende vrugte hergroen. Dit het in sommige gevalle die uitpakte dramaties verlaag. Met laathangende vrugte kon die nuwe groeistuwing en vroeë blom nie ordentlik beskerm word teen insekte en siektes nie, wat 'n groot risiko geplaas het op 2019 seisoen se uitvoervrugte. Daar was baie vrugval op Midnights as gevolg van dun skille en op Turkeys was kraakskil 'n probleem gedurende 2018.

Dit is duidelik dat moeilike klimaatsomstandighede, gepaardgaande met die massiewe oeslading, 'n effek gehad het op skilrypheid en gehalte oor die algemeen. Heelwat terugvoer was voor oes ontvang van vrugte met verskillende vorms van brandskade en skilafbraak. Oesmanipulasie word meer belangrik vir die toekoms om die vrugset en lading van vrugte te kan bestuur en dit sal natuurlik die vruggroote, skilkondisie en vruggehalte positief beïnvloed.

In die Noordelike produksiegebied word gemiddeld tot ondergemiddelde oeste vir 2019 verwag. Water was 'n beperking veral in Letsitele. Daar is gebiede waar soute in die ondergrondse water opgebou het. Dit sal oeste in sommige gebiede vir 2019 beïnvloed. Verder is daar ook laat Valencia boorde met baie min vrugset as gevolg van die laat hang van vrugte. Produsente sal hierdie boorde reg moet bestuur, veral in situasies waar die CBS bespuitings buite die voorgeskrewe periode gespuit is.

Suurlemoen volumes uit die Noorde het 'n bietjie afgeneem en aanvraag in die mark op die oomblik is hoër as die aanbod. Die vroeë pomelos uit Tshipise en Hoedspruit is vanjaar van goeie gehalte en uitpakte bo die 90% word behaal. Dit gee 'n aanduiding van die verwagtinge wat kwaliteit hierdie seisoen betref, wat hopelik sal opmaak vir die afname in volumes.

Vrugtevlug druk is hoog in die Letsitele en Hoedspruit gebiede. Dit is nodig om meer gereeld te spuit, saam met lugbespuitings, om die getalle af te bring. FCM tellings was laag in die eerste kwartaal. Die Tzaneen damvlak staan tans op 18% en daar is regtig groot kommer oor volgende seisoen se produksie.

Zimbabwe het 'n nuwe kwekery op die been gebring. Die kwekery is nuut gebou onder insekvrue net en is in Chegutu geleë. Skoon sitrus-enthout sal van Suid-Afrika ontvang word en die kwekery neem deel aan die SVS. Thys Du Toit het in middel Maart die kwekery besoek om die oudit te doen. Dodhill kwekery is in finale stappe om akrediasie te kry. Die produsente in die Noorde werk ook nou saam met die regering op Biosekuriteit, veral op die invoer van enige plantmateriaal wat risiko inhou op sitrus. Sitrus-aanplantings in Zimbabwe gaan nie drasties in die kort tot medium termyn groei nie.

Daar is heelwat aanvraag van produsente vir opleiding van sitrus scouts. CRI het in die Brits/Rustenburg area asook in Patensie opleiding aangebied. Die terugvoer was positief, alhoewel daar nogsteeds 'n leemte is vir meer opleiding in verband met die interpretasie van scouting data.

8.1.5 Na-oes Voorligting

Pakhuis is gedurende 2018 op 'n een-tot-een basis besoek en die houding en terugvoering was weereens baie positief, met goeie interaksie en samewerking met die pakhuis. Pakhuisbestuurders is meer tegemoetkomend en gewillig om hulle idees en vertroulike informasie i.v.m. terugvoering oor bederf, residu-resultate ens. te bespreek, en die pakhuis is bereid om die nodige aanbevole veranderinge aan te bring. Dit was baie positief om te sien dat waar daar aanbevelings tydens die 2016/7 pakhuisbesoeke aanbeveel is, die veranderinge aangebring is, met 'n positiewe uitwerking op die pakhuisstelsels.

Die 2018 seisoen het oor die algemeen goed begin. Dit het met die interne gehalte van die Satsumas oor die algemeen goed gegaan, behalwe vir 'n geweldige toename in suurvrot infeksies. Bederf was meer van 'n probleem in sekere van die Noordelike streke, veral op die vroeë kultivars (Satsumas en Clementines) en op die nawels, weereens as gevolg van swaar reënval voor die plukseisoen, en wisselvallige reënval tydens pluk. Haelskade in een of twee van die streke het ook hoë bederf op die vroeë nawels veroorsaak en baie bederf is in die uitskot-vrugte waargeneem. Die meerderheid bederf was as gevolg van die wondpatogene, groen- en blouskimmel en suurvrot. Die latentepatogene het ook voorgekom, veral op die suurlemoene. Diplodia stingelent verrotting was die probleem in hierdie geval.

Kraaskil op nawels, en ander kultivars was ook 'n geweldige probleem wat waargeneem is. Ander skilprobleme was ook op sekere kultivars waargeneem. Oleo, endokserose en blomment skilafbraak is op suurlemoene waargeneem, en dan ook baie koue- en vriesskade, en ook "pitting" op Bennies en ander Valensia-tipes.

Swak paletisering was ook nog 'n probleem in enkele van die pakhuis. Party palette se hoekstukke loop van bo af tot heel onder die palet tot op die vloer. Ander palette se hoekstukke loop tot in die middel van die palet se voetstuk, en ander hoekstukke eindig by die onderste karton en ander raak aan die begin van die voetstuk van die palet. Te veel palette met gebuigde hoekstukke is ook waargeneem. Daar is geen eenvormigheid nie en ook geen konsekwentheid nie. Paletisering lyk in te veel pakhuis nog slordig. Sekere pakhuis kla ook oor die vertraagde aflewering van kartonne.

Baie produsente het oor die algemeen met hoë volumes klein vrugte gesukkel. Die vrugte oor die algeheel was klein en dit het die volumes, verpakking (gemiddeld piek-groottes van 88 tot 105 op lemoene), hantering en uitvoer moeilik gemaak. Die eksterne gehalte van baie vrugte was wisselvallig a.g.v laat opkleur van die vrugte op die bome, die hoë temperature, laathangende vrugte, en dan die pluk en ontgroening van oorryp vrugte. Dit het dan die aankoms van baie oorryp en sagte, pofferige vrugte in markte veroorsaak.

Nogtans is die 2018 seisoen met 'n suksesvolle rondte van besoeke/konsultasies aan 54 pakhuis afgesluit. Die mikpunt vir die seisoen was om by plus-minus 100 pakhuis uit te kom, maar weens die tyd wat sekere

pakhuis vereis het, en die lang afstande se ry op slegte paaie, was dit nie maklik om by al hierdie pakhuis uit te kom nie.

Dit is bevredigend om waar te neem hoeveel van die pakhuis nou poog om hulle kritiesebeheerstelsels reg te bestuur, veral die sanitasie komponent wat noemenswaardig verbeter het, en dit ook hand in hand met die bestuur van pakhuisbehandelings en die toediening van die regte konsentrasies en residuladings. Die gevaar van bestandheid teen die swamdoders, wat die afgelope paar seisoene beklemtoon is, het beslis 'n verskil in bestuurspraktyke veroorsaak.

Tydens die pakhuisbesoeke is 'n punt daarvan gemaak om swamspoor-pluise van besmette uitskotvrugte en retensie monsters van besmette vrugte in die vrugwasstelsels te neem en die monsters by die Diagnostiese laboratorium te ontleed vir moontlike verlies van sensitiwiteit (bestandheid). Die eerste tekens van verlies van sensitiwiteit het al voorgekom. Hierdie praktyk sal meer gereeld by die pakhuis toegepas word en die pakhuis sal aanbeveel word om hulle eie monsters in te stuur vir ontleding vir moontlike verlies van sensitiwiteit. 'n Groot verbetering nou is dat baie pakhuis nou hulle eie spoormonsters instuur vir verlies van sensitiwiteit ontleding.

Waksaanwending is ook 'n kritiesebeheerpunt wat baie wisselvalig in pakhuis 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 oor terugvoer van die markte. Die vogverlies simptome lyk soos skilafbraak in en om die blomkelk weefsel waar daar heelwat minder waks aangewend word. Heelwat koueskade simptome op vrugte in foto's uit die markte is ook waargeneem.

Sporadiese bederf, veral suurvrot, was meer van 'n probleem in sekere gebiede, veral op die vroeë sagtesitrus (satsumas en clementines) en op die nawels, weereens as gevolg van swaar reënval voor die plukseisoen, en wisselvallige reënval tydens pluk. Haelskade in een of twee van die streke het ook hoë bederf op die vroeë kultivars veroorsaak, en baie van die wondpatogeen-infeksies, veral suurvrot, is in die uitskotvrugte waargeneem. Min latentepatogeen infeksies het voorgekom. Hoë dag- en nagtemperatuur in sekere produksiegebiede tydens die 2018 pakseisoen het die vrugte op die bome verhoed om ordentlik op te kleur. Dié toestande was die oorsaak van laat-hangende vrugte van oorryp en swak gehalte.

Residulading van chemikalieë was wel 'n bekommernis in die bedryf hierdie jaar, veral in sekere markte wat minder chemie en residu op uitvoer vrugte vereis. In baie gevalle was daar 'n verlies van chemiese beheer ontdek. Dit word nou 'n ernstige probleem omdat die chemie onder druk geplaas word, en weerstand begin intree. Daar is 'n ringtoets gedoen om die verskillende laboratoriums te evalueer en nie een van die laboratoriums het voldoen aan 'n bevredigende resultaat nie. Daar is 'n toename in die getalle residu-monsters wat vir toetse na die EU gestuur word.

Packers are naturally nervous about the potential loss of imazalil as a postharvest fungicide but overall the feeling is positive and there is trust in CRI and CGA that new developments and alternatives will be communicated to them. In the interim, there is much emphasis being placed on sanitation with packhouses improving their processes and including steps such as bin washers and regular cleaning schedules. A concern is the number of sanitation products on the market. Packhouses are not always sure which product is the best to use in order to avoid residues, blockages, or corrosion. There is no one product that is suitable to use over the whole operation. Some packhouses sacrifice using a superior product to avoid a potential accidental residue carryover by labourers or equipment.

Packhouses across the country have spent the offseason upgrading their lines with many packhouses completely overhauling the line and increasing the size of the packshed. This 2019 season will see at least one brand new packhouse in operation. In all packhouses there is a drive for more automation with many installing automated dosing systems for sanitation as well as electronic graders, not only for the final packing but also for the pre-sorting of fruit. Automatic place packers are still rare with the majority of packhouses using people to pack the cartons.

Sour rot on the early satsumas is a concern but the early navels, lemon and grapefruit crop that has been packed looks good with very little disease. The majority of the packhouses will only be fully operational in the next quarter.

8.1.6 10de CRI Sitrusnavorsingsposium

Die 10de CRI Sitrusnavorsingsposium wat van 19-22 Augustus by Champagne Sports Resort naby Winterton aangebied is, het navorsers van CRI en ander navorsingsvennote die geleentheid gebied om hul terugvoer oor hul navorsing van die afgelope twee jaar aan die sitrusbedryf te gee. Altesaam 72 praatjies van hoogstaande gehalte, waarvan tien deur buitelandse sprekers, en 22 plakkaat, is aangebied. Bywoning het die rekordgetal van 704 bereik, waarvan sitrusprodusente 26% en borge 32% uitmaak. Dit moet egter in ag geneem word dat die doel van die simposium nie voorligting is nie, maar navorsingsterugvoer, en gevolglik is die teikengroep hoofsaaklik konsultante en tegniese raadgevers wat die inligting kan verwerk in 'n formaat om uit te dra na die produsente. Die tegniese personeel van die borge, chemiese maatskappye, DAFF, uitvoermaatskappye, ander landboukundige maatskappye en kwekerye maak saam met die konsultante deel uit van die teikengroep wat primêr baat vind by die simposium.

Die simposium het uitstekend afgeloop as die komplimente, van die persone wat bygewoon het, ernstig opgeneem word. Daar is egter paar aspekte wat aandag moet kry voor die volgende simposium. Waar daar in die verlede heelwat praatjies weggevoer moes word a.g.v beperkte tyd, moes daar hierdie keer geraap en skraap word om sekere sessies te vul, ten spyte van die feit dat daar 'n sessie minder was en sommige sessies korter was as met die vorige simposium.

Die groeiende aantal borge en bywoning is ook 'n aanduiding dat die sitrusbedryf groot baat vind by die simposium. Sommige borge het terugvoer gegee dat hulle uitstekende blootstelling gekry het. Een borg, Farmtrace, het gerapporteer dat hy nou met 'n personeeltekort sit, direk a.g.v die uitstekende blootstelling wat hy by die simposium gekry het. Die uitdaging wat nou voorlê, is hoe daar op die volgende simposium verbeter kan word.

'n Totaal van 92 gholfspelers het deelgeneem aan die gholfdag wat op Sondag 19 Augustus 2018 gehou is en die terugvoering hier was ook baie positief. Plusnet was die hoofborg en hulle het hulle tevredenheid uitgespreek en aangedui dat hulle in 2020 graag weer die gholfdag sal wil borg.

8.1.7 CRI Geïntegreerde Plaagbeheer (IPM) en Siektebestuur (DM) werksinkels

Die CRI IPM & DM werksinkels is gedurende September in Limpopo, Mpumalanga, Oos- en Wes-Kaap aangebied. Die fokus was op marktoegang, biosekuriteit, die FMS, en siekte-en plaagbestuur. Die portfoliobestuurders het ook oor hul onderskeie vakgebiede terugvoer oor die aanbiedinge by die afgelope CRI Sitrusnavorsingsposium gegee. Die bywoning by al vyf werksinkels was goed en die terugvoer was werklik baie positief.

8.1.8 Sitrusverbeteringskema

Die doel waarvoor die Sitrusverbeteringskema destyds begin is, was om boomkwaliteit in die Suid-Afrikaanse Sitrusbedryf te verbeter en produsente teen minderwaardige plantmateriaal te beskerm. Gedurende die laat tagtiger en vroeë negentiger jare is die kwekerye voortdurend op 'n gereelde basis deur kwekery-adviseurs besoek om kwekerye te adviseer en boomkwaliteit te monitor ten einde die verskaffing van gesertifiseerde bome aan produsente te verseker. As gevolg van verskeie faktore het die stelsel verander tot die punt waar kwekerye toegelaat is om self-oudits van hul bome te doen, en daarvolgens word dan besluit of bome sertifiseerbaar is, al dan nie.

Daar is die afgelope paar jaar verskeie kere melding gemaak van die agteruitgang van kwekery-bome se kwaliteit. Heelwat klagtes van produsente oor swak boompies is die afgelope jaar ontvang, en in baie gevalle kan die swak kwaliteit van boompies in die areas, wat kort tevore uitgeplant is, waargeneem word. Die groot

oorsaak hiervan is dat die vraag na nuwe boompies so hoog is dat kwekerie nie betyds kan voorsien nie en gevolglik heeltemal te swak en klein boompies aan produsente gelewer word. Die grootste klagte van produsente in hierdie verband is dat indien hulle nie tevrede is met die kwaliteit nie, hulle nêrens anders beskikbare bome kan kry om in die reeds voorbereide grond te plant nie, en kwekerie dan bloot hul bome aan die volgende produsent verkoop.

Die feit dat baie bome op die oomblik in die sitrusbedryf gesertifiseer word wat glad nie aan die minimum vereistes vir sertifisering voldoen nie, is regtig 'n brom tot kommer. Dit is duidelik dat die self-oudit stelsel van kwekerie nie aan die verwagtinge voldoen nie en die SVS gevolglik nie in sy doel slaag om bome van hoogstaande gehalte aan die bedryf te verseker nie. Hierdie saak behoort dringend aandag te geniet.

8.1.9 CRI Na-oes werksinkels

Die jaarlikse CRI Na-oes werksinkels vir 2019 is gedurende Februarie afgehandel en daar was weer rekord bywonings-getalle in totaal. 'n Baie wye reeks onderwerpe is gedek om die pakhuisse en ander na-oes rolspelers van die nuutste inligting te voorsien voor die aanvang van die pakseisoen. Die feit dat verteenwoordigers uit die bedryf betrokke is by die beplanning van die werksinkels werp beslis vrugte af, aangesien onderwerpe aangespreek word wat die beste in die behoeftes van veral die pakhuisse en produsente kan voldoen.

8.1.10 Biosekuriteit

Na vergaderings in Namibië met AMTA in 2017 en MAWF in 2018 is 'n opvolgvergadering weer met MAWF gehou om samewerking met die Suid-Afrikaanse Sitrusbedryf te bewerkstellig. Die uitsluitlike doel is om suidelike Afrika teen eksotiese sitrussiektes te beskerm tot voordeel van alle lande. Aangesien Namibië nie 'n plantkwarantynstelsel in plek het nie, is hulle bereid om plantmateriaal van Suid-Afrika in te bring vir toekomstige aanplantings.

Die feit dat hulle geen kwarantynstelsel in plek het om te verseker dat siektevrye plante die land ingebring word nie, is werklik 'n bron tot kommer, veral in die lig gesien dat daar sterk gerugte is dat China 8000 ha sitrus in Namibië wil vestig. Daarom is dit van kardinale belang dat uitstekende samewerking tussen CRI Biosekuriteit en MAWF bewerkstellig word. Die voorstel is op die tafel gelê dat verskeie van hul sleutelpersoneel uitgenooi word om die CRI IPM & DM werksinkels by te woon, asook 'n besoek aan die CFB te bring, om op hoogte te kom met die werksaamhede en suksesse van die CIS.

Verskeie vergaderings met bestaande en voornemende sitrusprodusente is die afgelope drie jaar gehou, waartydens hulle van die gevare van eksotiese siektes bewus gemaak is. Produsente is aangemoedig om alle plantmateriaal vanaf gesertifiseerde kwekerie in Suid-Afrika te bestel, en ook slegs vanaf kwekerie in swartvlekvrye en vergroeningsvrye gebiede.

8.1.11 Opsomming van aktiwiteite vir die periode April – Junie 2018

Datum	Studiegroep/Aktiwiteit	Onderwerpe/Aksies	Betrokkes/Sprekers
3 Apr 18	Sappi Technology Centre	BCT toetse op kartonne	Dawid Groenewald
3 Apr 18	Mafroda Bestuurder Noord	Borge reël vir TTG's	Wayne Mommsen Johan Goosen
4 Apr 18	Innovation Hub	Paletvergadering: Peter Brouns	Dawid Groenewald
4 Apr 18	Onderberg produsente	Produsent besoeke in Onderberg area –Chris Kellerman	MC Pretorius Chris Kellerman Johan Joubert
7 Apr 18	Sappi Technology Centre	BCT toetse op kartonne	Dawid Groenewald
9-10 Apr 18	Letsitele Pakhuise:	CRI Na-oes besoeke	Wayne Mommsen

	Christie Landman De Nysschen Letaba		Keith Lesar Catherine Savage
11–13 Apr 18	Letsitele & Hoedspruit pakhuis: Laeveld Sitrus Nouvelle La Cotte Rooister Merite CPJ Group 91 Moletele	Pakhuis besoeke	Keith Lesar Catherine Savage
12 Apr 18	TTG Noord-Wes	Studiegroep vergadering in Brits	Wayne Mommsen Mariana Le Roux Willem Van Schalkwyk Clint Lawson Sean Thackeray
9-13 Apr 18	Laeveld Agrochem Seilsafari en Landbouskou	Verskeie onderwerpe en aktiwiteite	Hannes Bester MC Pretorius Vaughan Hattingh
13 Apr 18	Sappi Technology Centre	BCT toetse en Sappi tegniese vergadering	Dawid Groenewald
16 Apr 18	Nelspruit area	Probleem boorde besoek – Flip Walters	MC Pretorius James Warrington
17 Apr 18	Jacques Coetzee	Pallet netting	Hannes Bester
17 Apr 18	Nelspruit	Pakhuis besoek beplanning	MC Pretorius Keith Lesar Catherine Savage
	Innovation Hub	Karton-ontwikkeling vergadering	Dawid Groenewald Wimpie Mostert
	Seminaar	Nvirotek opening van Sitrus MRL laboratorium	Wayne Mommsen
17-18 Apr 18	Fort Beaufort pakhuis: Kat River Citrus Eden Riverside	Pakhuis besoeke	Keith Lesar Catherine Savage
19 Apr 18	Groblersdal: Schoonbee Landgoed Moosrivier Nyawa Boerdery	Produsente-besoeke	Hannes Bester MC Pretorius
	Sappi Proewe	Lignosulphonate proewe by Ambrosia Sitrus	Wayne Mommsen Coenie Scheepers Barend Olivier George Breytenbach
20 Apr 18	Groblersdal: Roslé Boerdery De Wagendrift	Produsente-besoeke	Hannes Bester MC Pretorius
23 Apr 18	Nelspruit	Simposium beplanning	Hannes Bester MC Pretorius Liezl vd Linde
	Karino Koöp	Navorsingsbehoefte t.o.v bemesting	Hannes Bester MC Pretorius Pieter Raath

	Groep91 en Denyschen Broers	IPM opleiding oor VKM en VV	Wayne Mommsen Andries Joubert
24 Apr 18	Hoedspruit: Ambrosia Unifrutti Letaba	Produsente-besoeke t.o.v Navorsingsbehoefes	Hannes Bester Wayne Mommsen Pieter Raath
24 Apr 18	Karino	Larten probleemboorde	MC Pretorius
25 Apr 18	Karino	Thermal imaging drone fotografie van ekspiment 1092 boorde	MC Pretorius
25 Apr 18	Letsitele: Laeveld Sitrus Bosveld Sitrus Mahela Boerdery Groep 91	Produsent-besoeke t.o.v Navorsingsbehoefes	Hannes Bester Wayne Mommsen Pieter Raath
26 Apr 18	Mesina/Tshipise: Noordgrens Alicedale	Produsent-besoeke t.o.v Navorsingsbehoefes	Hannes Bester Wayne Mommsen Pieter Raath
2 Mei 18	Nelspruit	Dr Mieke Daneel vergadering – aalwurmtellings en data	MC Pretorius
3-4 Mei 18	ADAMA Werkswinkel	Werkswinkel om ADAMA registrasies vir Sitrus	Wayne Mommsen Tim Grout Aruna Manrakhan MC Pretorius Sean Moore Hardus Hern Roelf Swart Lourens Oellerman
4 Mei 18	Sappi Technology Centre	Oesskattings en papier volumes vergadering	Dawid Groenewald
7-9 Mei 18	Swaziland and Pangola Ngonini Estate Tambuti – Swaziland Mvutshini Mhlati Farm	Produsent besoeke: boorde, kultivars en pakhuisbesoeke saam met Chris Kellerman	MC Pretorius Chris Kellerman Johan Joubert Keith Lesar
10 Mei 18	Sondagsrivier: SRV Pakhuisforum-vergadering	Agenda	Hannes Bester
16 Mei 18	CFB	CFB Evaluasies CIS-CC vergadering	Hannes Bester Paul Fourie Johan Joubert Werner Swiegers Thys Du Toit
	Innovation Hub	PrimePro produkvergadering	Dawid Groenewald
	Moletete Pakhuis besoek	Na-oondersoek vrugte bederf op limonera suurlemoene	Wayne Mommsen Martin Mentis Pierre Fourie Chris Botes Keith Lesar
	Bothaville	Nampo – borge en uitstallers	MC Pretorius
17 Mei 18	Pretoria	Mike Holtzhouzen besoek	MC Pretorius

17 Mei 18	At van Schalkwyk	Nuwe bemestingsprodukte	Hannes Bester
21 Mei 18	Tom Burke	Kwekery probleem bome op suurlemoen bome besoek	MC Pretorius
22-24 Mei 18	Northern Cape Pakhuise: Mosplaas (Karstens Bdry) Groenheuvel Renosterkop Augpad Lemoenkop Sitrus Saamfarm	Pakhuis besoeke	Keith Lesar Catherine Savage
22 Mei 18	Nelspruit	Pieter Raath – grondontledings laboratorium besoek asook vergroenings boorde op Croc Valley	MC Pretorius Pieter Raath
23-24 Mei 18	CSR	Simposium- beplanningsvergadering	Hannes Bester Dawid Groenewald Liezl vd Linde MC Pretorius Wayne Mommsen
28 Mei 18	Nelspruit	Borge vergaderings: Werner Felco en Jan Landman Citroshine	MC Pretorius
29 Mei 18	Ryton, Twycross, Karino, Croc Valley	Pakhuis besoeke – focus op waks saam met Jan Landman (Citrashine)	Keith Lesar Catherine Savage
30-31 Mei 18	Nelspruit/Machadodorp	Villa/CRI werkswinkel	Hannes Bester MC Pretorius Wayne Mommsen Tim Grout Sean Moore Aruna Manrakhan Charl Kotze Jan van Niekerk
1 Jun 18	Witrivier	Timac Agro – bemestings werkswinkel	MC Pretorius
4-8 Jun 18	FMC Werkswinkel Livingstone	FMC (Du Pont Aquisition) Registrasies en Posisioneering van sitrus produkte	Wayne Mommsen Tim Grout Sean Moore MC Pretorius
12-14 Jun 18	KZN Pakhuise: Bolton Citrus Estates FruitStar Carissbrooke Sun Valley Estates	Pakhuis besoek	Keith Lesar Catherine Savage
12 Jun 18	Stellenbosch: Cooling Working Group vergadering	Agenda	Hannes Bester Dawid Groenewald
	Pieter Raath	Bemestingsnavorsing	Hannes Bester Pieter Raath
13 Jun 18	Stellenbosch: JBT – Jaco Theron	Borgskap	Hannes Bester
	Burgersfort	Probleem boorde besoek – Ruan Potgieter	MC Pretorius

		Studiegroep voorsitter – Albert Winterbach	
14 Jun 18	Nelspruit Onderberg	Flip Walters probleem boord besoek Hael boorde besoek: Karino en GFC	MC Pretorius
18 Jun 18	Nelspruit	Timbale – ontwikkelings Konsultant vir klein boere vergadering	MC Pretorius Andrew Mbezi Johan Joubert
19 Jun 18	Nelspruit	IPM Research werkswinkel DM Research workshop	Hannes Bester MC Pretorius Wayne Mommsen
	Sappi Technology Centre	Midseisoen sitrus- vergadering: Craig Zorab	Dawid Groenewald
20 Jun 18	Nelspruit	IPM & DM Res workshop Wetenskaplike komitee paScientific Committee vergadering Biosekuriteits vergadering	Hannes Bester Wayne Mommsen
20& 21 Jun 18	Pretoria	Thilivali – registrateur besoek en NSSA vergadering	MC Pretorius
25 Jun 18	Vaalharts: Danie Mathewson Retha Greyling Michael van Niekerk	Produsentebesoeke	Hannes Bester
25-26 Jun 18	Zim Suid Pakhuise	Na oes besoeke by BK Cawood en Knottingham Estates	Wayne Mommsen Keith Lesar Catherine Savage
26 Jun 18	Britz	Rustenburg studiegroep snoeidemonstrasie	Hannes Bester
	Innovation Hub	Corruseal Karton kwaliteit vergadering: Rajiv Mehta	Dawid Groenewald
27-28 Jun 18	Tshipise and Weipe Packhouses: Alicedale Maswiri (Bonaire) Christo Vorster (Nel Packhouse) Depo Weipe Noordgrens Overvlakte	Pakhuis besoeke	Keith Lesar Catherine Savage
28 Jun 18	Groblersdal	Henry Pieterse	Hannes Bester

Opsomming van aktiwiteite vir die periode Julie – September 2018

Datum	Studiegroep/Aktiwiteit	Onderwerpe/Aksies	Betrokkenes/ Sprekers
5 Jul 18	Lignen Proewe	Ambrosia Sitrus Lignen Proewe van SAPPI	Wayne Mommsen Coenie Scheepers Barend Olivier George Rautenbach
5 Jul 18	Pakhuis besoek	Ngonini – eSwatini	Keith Lesar

		Follow up training on chlorine management	Catherine Savage
6 Jul 18	Plaasbesoek	CC Coetzee Boerdery Blaarsimptome ondersoek	Theuns Coetzee Wayne Mommsen
16-19 Jul 18	Pakhuis besoeke	West Limpopo (Bufland; Floris van Wyk; Limpopo citrus; Noordgrens; Sirkel N; Soutpansberg; Waterberg; Zebediela)	Keith Lesar Catherine Savage
17 – 19 Jul 18	Oos Kaap – CFB evaluasies	Jaarlikse GVB evaluasies	MC Pretorius
18 Jul 18	Sappi Technology Centre (STC)	Neem Arno Erasmus en kollegas van Wonderful Citrus deur die STC fasiliteite. Groepsbespreking oor die vervaardiging- en fisiese eienskappe van “Linerboard” en “Fluting”	Dawid Groenewald
18 Jul 18	Windhoek: MAWF	Vergadering met MAWF oor biosekuriteit	Hannes Bester
19 Jul 18	Houers, Letsitele	Neem Arno Erasmus en kollegas deur Houers se fabriek. Groepsbespreking oor die vervaardiging van kartonne	Dawid Groenewald
23 Jul 18	Kakamas: Oseiland Bdy	Boordbesoeke	Hannes Bester
24 Jul 18	Kakamas: Groenheuvel Bdy Karsten Bdy	Boordbesoeke	Hannes Bester
24 Jul 18	Nelspruit	James Warrington – vergadering siesoen bespreking	MC Pretorius
25 Jul 18	Grobbersdal/Marble Hall	Piet Engelbrecht – vergadering	MC Pretorius
25 Jul 18	Kakamas: Krisma Bdy Augrabies Zwartbooisberg	Boordbesoeke	Hannes Bester
25-26 Jul 18	Pakhuis besoeke	Zimbabwe Noord	Wayne Mommsen Catherine Savage
26 Jul 18	Hectorspruit	GFC besoek	MC Pretorius
26 Jul 18	Innovation Hub	Samesprekings met Tekco Packaging. Moontlike toelating as geakkrediteerde kartonverskaffer	Dawid Groenewald
27 Jul 18	Nelspruit	Seisoen bespreking: Chris Kellerman, James Warrington, Faan van Vuuren	MC Pretorius
27 Jul 18	Pakhuis besoek	Doreen Pakhuis Tshipise	Wayne Mommsen Catherine Savage

1 Aug 18	Pretoria	UP Prof Nico Labuschagne	MC Pretorius
2 Aug 18	Ohrigstad	Mahela – Seisoen beplanning en nabetrugting: Eddie Vorster/ Sean Colyn Kobus Beetge	MC Pretorius
2 Aug 18	Pakhuis en Boordbesoeke	Noordgrens Landgoed, Hope Farm Tshipise, Robbertse Lemmietjies Waterpoort	Wayne Mommsen
3 Aug 18	Pakhuis besoek	Joubert & Sons	Keith Lesar Catherine Savage
7 & 8 Aug 18	TTG, Boordbesoeke en Kwekery besoek	Constantia Studiegroep, Letaba Estates en Mahela Sitrus Kwekery	Wayne Mommsen MC Pretorius
17-22 Aug 18	Champagne Sports Resort	CRI Simposium	Dawid Groenewald Hannes Bester Liezl vd Linde MC Pretorius Wayne Mommsen Catherine Savage
25 Aug 18	Kakamas	Begrafnis: Dawid Spangenberg	Hannes Bester
29 Aug 18	Houers, Letsitele	Beplanning van proef met Mpact-/Mondi Paper se 170g Baykraft. Francois van Rooyen se roudiens	Dawid Groenewald
29 Aug 18	Depot Weipe Sitrus	Jong Boom Phytophthora ondersoek	Wayne Mommsen Thys Du Toit
30 Aug 18	Subtropico, Pretoria Mark	Verpakking van Lemoene en Pomelo's in eksperimentele Mpact plastiese kratte.	Dawid Groenewald
4-5 Sept 18	Eiland: Limpopo 1 CRI IPM & DM werkswinkel	Agenda	Hannes Bester Wayne Mommsen MC Pretorius Catherine Savage Elma Carsten Sean Moore Wayne Kirkman Aruna Manrakhan Tim Grout Charl Kotze Providence Moyo Jakkie Stander
6-7 Sept 18	Loskopdam: Limpopo 2 CRI IPM & DM werkswinkel	Agenda	Hannes Bester Wayne Mommsen MC Pretorius Catherine Savage Elma Carsten Sean Moore Wayne Kirkman Aruna Manrakhan Tim Grout

			Charl Kotze Providence Moyo Jakkie Stander
10 Sept18	Innovation Hub	Samesprekings oor voorlopige proewe met Mpact plastiese kratte	Dawid Groenewald
10-11 Sept 18	Nelspruit: Mpumalanga CRI IPM & DM werkswinkel	Agenda	Hannes Bester Wayne Mommsen MC Pretorius Catherine Savage Liezl vd Linde Sean Moore Wayne Kirkman Aruna Manrakhan Tim Grout Charl Kotze Providence Moyo Jakkie Stander Jan van Niekerk
13-14 Sept 18	Addo: Oos-Kaap CRI IPM & DM werkswinkel	Agenda	Hannes Bester Wayne Mommsen MC Pretorius Karin Nel Liezl vd Linde Elma Carsten Sean Moore Wayne Kirkman Aruna Manrakhan Tim Grout Charl Kotze Providence Moyo Jakkie Stander Jan van Niekerk
17 Sept 18	Mpact Paper Mill, Springs	Stapelsterkte toetse van eksperimentele 170g Baykraft kartonne	Dawid Groenewald
18-19 Sept 18	Simondium: Wes-Kaap CRI IPM & DM werkswinkel	Agenda	Hannes Bester Wayne Mommsen MC Pretorius Karin Nel Liezl vd Linde Sean Moore Wayne Kirkman Aruna Manrakhan Tim Grout Charl Kotze Providence Moyo Jakkie Stander Jan van Niekerk
19 Sept 18	Vaughan Hattingh	Een-op-een bespreking	Vaughan Hattingh Hannes Bester
19 & 20 Sept 18	Stellenbosch	Soilborne disease interest group meeting	MC Pretorius

19 Sept 18	Houers en Laeveld Sitrus	Vervaardiging van eksperimentele A15C 170g Baykraft kartonne en "Tree Wrap" proef by Laeveld Sitrus	Dawid Groenewald
20 Sept 18	TTG	Noord Wes Studiegroep Brits. Kultivar en onderstam seleksies	Wayne Mommsen Johan Joubert
20 Sept 18	Pieter Raath	Bemesting navorsing en voorligting	Hannes Bester Pieter Raath
21 Sept 18	Boordbesoeke	Brits Noord Wes	Wayne Mommsen Johan Joubert
25 Sept 18	Letsitele Tegniese komitee vergadering	Mahela Raadsaal	Eddie Vorster Henk Van Rooyen Pierre Smit Johan Gubbitz Gerhard Vorster Desiree Fourie EC Landman Wayne Mommsen
25-26 Sept 18	CRI Nelspruit	CRI Facility Takeover Event	Dawid Groenewald Hannes Bester MC Pretorius Liezl vd Linde
26 Sept 18	Magoebaskloof Orchids	Monsterneming vir CLN navorsing.	Wayne Mommsen Claudia Smith

Opsomming van aktiwiteite vir die periode Oktober – Desember 2018

Datum	Studiegroep/Aktiwiteit	Onderwerpe/Aksies	Betrokkeses/ Sprekers
2 en 3 Okt 18	Houers en Bosveld Sitrus, Letsitele	Tuimelverpakking proef, toedraai van vrugte en vergadering met Houers.	Dawid Groenewald
5 Okt 18	Boordbesoek Letaba Estates	Laat valentias erg chemise brand	Wayne Mommsen Harry Grove
9 Okt 18	Letsitele Studiegroep	FCM beplanning	Eddie Vorster Wayne Mommsen Johan Joubert
9 Okt 18	Vergadering – Marblehall citrus	Voorstelle vir 2019 seisoen	Catherine Savage Keith Lesar
	Nelspruit	Corteva besoekers – boordbesoeke	MC Pretorius
10 Okt 18	Paarl: CMF vergadering	Agenda	Hannes Bester Vaughan Hattingh
	Nelspruit	CAD Mapping – boordopnames met Drone tegnologie	MC Pretorius
10 Okt 18	Waterberge Studiegroep	Snoei aanbieding	Wayne Mommsen Joa Hoogenboezm Danie Janse Van Vuuren
11 Oct 18	Nelspruit	Mieke Daneel - LNR	MC Pretorius
12 Okt 18	Ohrigstad	Mahela – produksie besoek	MC Pretorius

16 Okt 18	Stellenbosch: CFQM Navorsing Komitee Vergadering	Agenda	Hannes Bester Tim Grout Paul Cronje Pieter Raath
	Karino	Produsent besoeke + Croc Valley	MC Pretorius
17 Okt 18	Stellenbosch: Kultivar Evaluasie Komitee vergadering	Agenda	Hannes Bester Tim Grout Paul Cronje Johan Joubert Werner Swiegers
17 Okt 18	Nelspruit	Beplanning vir Pongola besoek	MC Pretorius Chris Kellerman
18 Okt 18	Paarl: ETP vergadering	Agenda	Hannes Bester Liezl vd Linde Catherine Savage Wilma Du Plooy Keith Lesar Dawid Groenewald
	Paarl: Cooling Working Group vergadering	Agenda	Hannes Bester Catherine Savage Wilma du Plooy Keith Lesar Dawid Groenewald
22 Okt 18	Johannesburg: IPM Navorsings Komitee vergadering	Agenda	Hannes Bester Tim Grout Sean Moore Wayne Kirkman Solomon Gebeyehu
23 Okt 18	Johannesburg: DM Navorsings Komitee vergadering	Agenda	Hannes Bester Tim Grout Paul Fourie Jan van Niekerk Wayne Kirkman Solomon Gebeyehu
	Nelspruit	Gasspreker – Bayer - vrystelling van nuwe aalwurmdoder	MC Pretorius
24 Okt 18	Letsitele	Gasspreker – Bayer – vrystelling van nuwe aalwurmdoder	MC Pretorius
	Loskopdam	Sitrus Koördineering vergadering	Wayne Mommsen Elma Carstens Wayne Kirkman Vaughn Hatting Solomon Gebeyehu
25 Okt 18	Groblersdal/Marble Hall	Gasspreker – Bayer - vrystelling van nuwe aalwurmdoder	MC Pretorius
26 Okt 18	Letsitele Pakhuisforum vergadering	2018 nabetragting	Wayne Mommsen
29 – 31 Okt 18	KZN – Pongola	Pongola produsent/ boord en nuwe boord besoeke	MC Pretorius Chris Kellerman Johan Joubert

30 Okt 18	Addo/Kirkwood: Deon Joubert André Combrinck Etienne van Greunen	Reël CGA Pre-congress tour	Hannes Bester
30 Okt 18	Patensie: Phillip Dempsey	Reël CGA Pre-congress tour	Hannes Bester
	Letsitele vergadering	Daff Roadshow	Wayne Mommsen
31 Okt 18	CGA Marketing labelling requirement workshop	Update information on carton labelling requirements	Catherine Savage Wilma du Plooy
	Nelspruit	CIS Soilborne vergadering	MC Pretorius Paul Fourie Jan v Niekerk Elaine Basson
	Hoedspruit	Daff Roadshow	Wayne Mommsen
1 Nov 18	Nelspruit	Biosekuriteits vergadering	Biosekuriteits komitee
5 Nov 18	Karino	Studiegroep beplanning	MC Pretorius Hannes Breedt Johan Joubert
6 Nov 18	Sappi Technology Centre	2018 Akkreditasie toetse en beplanning vir 2019.	Dawid Groenewald
	Brits	Sitrus Scout Opleiding	Wayne Mommsen
7 – 8 Nov 18	LNR	Mieke Daneel – Data verwerking	MC Pretorius
8 Nov 18	Stellenbosch: FMS Working Group meeting	Agenda	Hannes Bester Vaughan Hattingh Paul Cronje Elma Carstens
8 Nov 18	Hoedspruit Pakhuis Forum vergadering	Na-seisoen Forum vergadering	Wayne Mommsen Catherine Savage Keith Lesar
9 Nov 18	Nelspruit	Besoek moontlike perseel vir CRI bestuursvergadering	MC Pretorius Jon Pinker
12 Nov 18	Nelspruit: Voorligting- beplanningsvergadering	Agenda	Hannes Bester Dawid Groenewald MC Pretorius Wayne Mommsen Catherine Savage Liezl vd Linde
13 Nov 18	Nelspruit: CRI Na-oes werkswinkels beplanningsvergadering	Agenda	Hannes Bester Dawid Groenewald MC Pretorius Wayne Mommsen Catherine Savage Liezl vd Linde Wilma du Plooy
14 Nov 18	Polokwane: Elé Boeredag	Agenda	Hannes Bester Dawid Groenewald Wayne Mommsen Johan Joubert Werner Swiegers

15 Nov 18	Pretoria: Packaging Working Group meeting	Agenda	Dawid Groenewald Hannes Bester Catherine Savage Tarl Berry
19 Nov 18	Hylgard Lourens	Pakhuis ontwerp vergadering	Catherine Savage Keith Lesar
	Depot Weipe	Boordbesoek: Water Kwaliteit	Wayne Mommsen Danie Erasmus
	Pretoria	UP Prof Nico Labuschagne + Wilma Augustyn – Data bespreking	MC Pretorius
20 en 21 Nov 18	Houers en Bosveld Sitrus, Letsitele.	Vorbereiding vir “Mulching” proef by Houers en proef self by Bosveld Sitrus	Dawid Groenewald Wayne Mommsen
21 Nov 18	Onderberg studie groep	Voor seisoen en Pakhuis vergadering	MC Pretorius Catherine Savage Keith Lesar Johan Joubert
22 Nov 18	Nelspruit studie groep	Voor Seisoen en Pakhuis vergadering	MC Pretorius Catherine Savage Wilma du Plooy Johan Joubert
26-29 Nov 18	KZN	Stugiegroepe en produsent besoeke – Nkwaleni en Ixopo	MC Pretorius Johan Joubert
4 Des 18	Burgersfort – Morone	Pakhuis besoek	Wilma du Plooy Catherine Savage Keith Lesar
4 & 5 Des 18	Patensie	Sitrus Scout Opleiding	Wayne Mommsen
5 Des 18	Johannesburg: CRI BOD meeting	Agenda	Hannes Bester Vaughan Hattingh Jon Pinker
5 Des 18	Pretoria Mark	Swamgroeie op palette ondersoek.	Dawid Groenewald.
11 Des 18	Swellendam: Sarel Neethling	Volg probleme met Oase Kwekery se swak bome op.	Hannes Bester

Opsomming van aktiwiteite vir die periode Januarie – Maart 2019

Datum	Studiegroep/Aktiwiteit	Onderwerpe/Aksies	Betrokkeses/Sprekers
10 Jan	Innovation Hub, Pta	Behandeling van kartonne met “Nano Coating”	Lindi Strydom (NanoProtect) Dawid Groenewald
11 Jan	Overvlakte Landgoed, Noordgrens, Weipe	Boordbesoeke	Wayne Mommsen Arthur Lilford Francois Dillman
16 Jan	Karino	Na-oes werkswinkel bespreking met Andrew Muller, Pakhuis besoek asook + Boordbesoeke	MC Pretorius
29-30 Jan	Limpopo 1 CRI Na-oes werksinkels	Agenda	Hannes Bester Dawid Groenewald

			MC Pretorius Wayne Mommsen Liezl vd Linde Catherine Savage Sean Moore Wilma du Plooy Lindo Mamba Jade North Tarl Berry
31 Jan – 1 Feb	Limpopo 2 CRI Na-oes werkswinkels	Agenda	Hannes Bester Dawid Groenewald MC Pretorius Liezl vd Linde Catherine Savage Sean Moore Wilma du Plooy Lindo Mamba Jade North Tarl Berry
4 Feb	Voorligting Beplanning	Agenda	Hannes Bester MC Pretorius Wayne Mommsen Liezl vd Linde Catherine Savage Keith Lesar
5-6 Feb	CRI Bestuurs Komitee vergadering	Agenda	Hannes Bester Wayne Mommsen MC Pretorius
11-12 Feb	KZN & Swaziland CRI Na-oes werkswinkels	Agenda	Hannes Bester Dawid Groenewald MC Pretorius Wayne Mommsen Catherine Savage Sean Moore Wilma du Plooy Lindo Mamba Paul Cronje Tarl Berry
13 Feb	Sappi Technology Centre	Beplanning van die 2019 akkreditasie toetse en opstel van die skedule	Sappi senior personeel Dawid Groenewald
14-15 Feb	Mpumalanga CRI Na-oes werkswinkels	Agenda	Hannes Bester Dawid Groenewald MC Pretorius Liezl vd Linde Catherine Savage Sean Moore Wilma du Plooy Lindo Mamba Paul Cronje Tarl Berry
19-20 Feb	Oos-Kaap CRI Na-oes werkswinkels	Agenda	Hannes Bester Dawid Groenewald Wayne Mommsen

			Liezl vd Linde Catherine Savage Sean Moore Wilma du Plooy Lindo Mamba Paul Cronje Tarl Berry
21 Feb	Noordwes Brits	Studiegroep vergadering Spuut bedekking	Wayne Mommsen Mariana Le Roux Charl Kotze van Hygrotech
21-22 Feb	Wes-Kaap CRI Na-oes werkswinkels	Agenda	Hannes Bester Dawid Groenewald MC Pretorius Liezl vd Linde Catherine Savage Sean Moore Wilma du Plooy Lindo Mamba Paul Cronje Tarl Berry
26 Feb	Innovation Hub, Pta	OptiFlo Freezer Spacer beplannings vergadering	Mike Cohen Dawid Groenewald
	Nelspruit en Croc Valley	Floppy sprinkler + Drone application of fruit fly bait demonstration	MC Pretorius Aruna Manrakhan Peter Stevens
27 Feb	Letsitele DNB Pakkers	Pakhuisforum: Seisoen opening funksie	Wimpie Mostert Frikkie Van Wyk Novon: Koos van Wyk Mareli Mouton Jacques Nel Andrew Jackson Nicholaas Gubbitz Evert Louw
28 Feb	Onderberg	GFC – Ngwenja kwekery	MC Pretorius
5 Mrt	Patensie: Phillip Dempsey & Stephen Meeding	CGA Pre-summit tour	Hannes Bester
5 Mrt	Letsitele	Studiegroep vergadering Voor-oes plaagbeheer	Wayne Mommsen Johan Visagie LAC De Wet van QMS
	Burgersfort	Produsent besoeke – haelskade en boordbesoeke	MC Pretorius
7 March	Lourenza	Pakhuis besoek	Catherine Savage
8 March	Morone	Pakhuis besoek	Catherine Savage Keith Lesar
8 Mrt	Letsitele CPJ Erasmus	Voorligting: Kultivars en onderstamme	Wayne Mommsen Johan Joubert
10 -12 Mrt	Patensie: CGA Pre-summit tour	Toerprogram	Hannes Bester MC Pretorius Wayne Kirkman Johan Joubert

13 Mrt	Innovation Hub	Verpakkingsnavorsing - 2019	Rajiv Metha, Corruseal Dawid Groenewald
13-14 Mrt	CGA Citrus Summit	Agenda	Hannes Bester MC Pretorius
18 – 20 Mrt	Zimbabwe Harare	Kwekery Oudit, Scout opleiding, ACP moniteering	Wayne Mommsen Thys du Toit
19 Mrt	Karino	Vrugtevlieg proewe – beplanning	MC Pretorius Aruna Manrakhan Hannes Breedt LA Visagie James Warrington
20 Mrt	Bambi	Pieter Ahlers -boordbesoek	MC Pretorius
19-22 Mrt	<u>Namibië:</u> Windhoek: MAWF Biosekuriteits vergadering. Outjo: Produsente vergadering	Agenda Agenda & boordbesoeke	Hannes Bester Wayne Kirkman
21 – 25 Mrt	<u>Groblersdal</u>	Produsent besoeke	MC Pretorius
26 Mar	Bishopstone	Pakhuis besoek	Catherine Savage
27-29 Mar	Moletete, Ambrosia, Rederberg, Moriah, Alicedale, Nel Pakkers, Doreen, Rooister, Group 91	Pakhuis besoek	Catherine Savage Wayne Mommsen
27 Mrt	Hoedspruit	Studiegroep en DAFF & PPECB roadshow	Wayne Mommsen Hannes Meintjies DAFF & PPECB
28 Mrt	Innovation Hub, Pta	Akkreditasie van nuwe kartonvervaardiger	Raymond Lund Dawid Groenewald.
28 Mrt	Knysna: John Stanwix	Boordbesoeke: IPM, DM en algemene praktyke	Hannes Bester Sean Moore
	Onderberg	GFC Boordbesoeke – jongboom aanplantings probleme	MC Pretorius
29 Mrt	Patensie	Boordbesoeke met Gerhards v Vuuren (Die Koöp)	Hannes Bester
	Nelspruit	Tegniese algemene gesprek	MC Pretorius Chris Kellerman James Warrington Fanie van Vuuren

8.2 OTHER MEANS OF TECHNOLOGY TRANSFER

8.2.1 SA Fruit Journal by Tim G Grout (CRI)

Table 8.2.1.1. S A Fruit Journal articles in 2018-19 besides Extension Briefs.

Issue	Pages	Title	Author/s	
April/May	17(2)	79--81	Entomopathogenic fungi to control false codling moth in citrus orchards	Candice Coombes, Martin Hill and Sean Moore
		82-84	Metamitron: Novel Mandarin thinning agent	Jakkie Stander

Jun/Jul	17(3)	68-70	Excellent results in the control of false codling moth in field trials using entomopathogenic nematodes	AP Malan and S.D. Moore
		72-73	Produksie van Midnight Valencia in die Nelspruit / Karino area.	James Warrington
		74--75	Create Soil pH that Ensures Long-term Citrus Orchard Performance	Pieter Raath, Aisla Hardy & Vincent van der Berg
		76-77	Excellent Participation at 2018 CRI Postharvest Workshops by All Postharvest Role Players	Catherine Savage; MC Pretorius, Liezl van der Linde, Wayne Mommsen and Keith Lesar
Aug/Sep	17(4)	66-67	Postgraduate qualifications in citrus entomology and microbiology	Anonymous
Oct/Nov	17(5)	69	Hannes de Lange – Why he received a CRI Technical Merit Award	Anonymous
		70-73	Effect of Phosphonate Applications, for Phytophthora Brown Rot Control, on 'Nadorcott' Mandarin External Fruit Quality	Jan van Niekerk, Charl Kotze, Jade North and Paul Cronje
		74-77	Production of summer lemons in Sicily, Italy	Jakkie Stander
Dec/Jan	17(6)	78-79	Report back on a meeting of fruit fly scientists in Mexico in April 2018- Notes on the 10th International fruit fly Symposium	Aruna Manrakhan
		76-77	Postharvest treatments aid control of Citrus Black Spot (CBS)	Wouter Schreuder, Wilma du Plooy, Arno Erasmus, Catherine Savage, Elaine Basson, Cheryl Lennox and Paul H. Fourie,
Feb/Mar	18(1)	50-52	Monitoring of fruit fly pests in commercial citrus orchards: temporal patterns of male and female catches	A. Manrakhan, J-H Daneel, R. Beck, M. Virgilio, K. Meganck and M. de Meyer
Apr/May	18(2)		'Nadorcott' mandarin: keeping growth in check and maintaining productivity through pruning	Regina Cronje, Christo Human and Innocent Ratlapane

8.2.2 CRI website by Tim G Grout

Table 8.2.2.1. Visits and page requests on www.cri.co.za since April 2018

Month	Unique visitors	Number of visits	Pages	Hits	Bandwidth
Total 2017/8	46 890	85 648	263 056	996 075	22.48 GB
Apr 2018	4 279	6 680	16 890	82 727	2.39 GB
May 2018	4 379	7 588	22 860	95 968	2.72 GB
Jun 2018	3 728	5 957	21 491	86 657	2.08 GB
Jul 2018	4 410	7 275	20 514	91 761	2.27 GB
Aug 2018	4 408	7 504	22 940	103 534	2.43 GB
Sep 2018	3 798	6 586	21 078	90 522	2.33 GB
Oct 2018	4 064	7 927	22 780	91 644	2.59 GB
Nov 2018	4 239	8 808	21 135	85 330	2.44 GB
Dec 2018	3 910	7 795	22 578	75 039	1.69 GB

Jan 2019	4 591	11 038	35 954	137 490	3.23 GB
Feb 2019	3 954	8 500	25 994	114 109	3.39 GB
Mar 2019	3 404	5 881	20 152	100 358	2.70 GB
Total 2018/9	49 164	91 539	274 366	1 155 139	30.26 GB

8.2.3 CRInet by Tim G Grout

Table 8.2.3.1. Numbers of messages circulated per month on CRInet.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
2019	1	2	1										3
2018	18	3	2	1	27	11	22	1	1	7	2	2	97
2017	0	0	4	7	0	4	2	14	3	5	3	1	43
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

8.2.4. Cutting Edge by Tim G Grout (CRI)

Table 8.2.4.1. Cutting Edge issues during 2018-19.

No.	Title	Month	Author/s
247	Consumer Assurance Update	May	Paul Hardman (CGA)
248	CL-N	Jul	Wayne Kirkman, Vaughan Hattingh, Elma Carstens, Paul Fourie, Sean Moore, Glynnis Cook and Tim Grout
249	Consumer Assurance Update	Jul	Paul Hardman (CGA)
250	Buprofezin Precautionary note	Aug	Paul Hardman (CGA)
251	Reduction in size of navel-ends	Sep	Jakkie Stander
252	Crop Manipulation	Oct	Jakkie Stander
253	FCM control guidelines	Oct	Sean Moore and Vaughan Hattingh
254	Updated CBS Spray Programmes	Oct	Providence Moyo, Jan van Niekerk and Paul Fourie
255	Customer Assurance Update	Oct	Paul Hardman (CGA)
256	Dichlorprop Notice	Oct	Paul Hardman (CGA)
257	Bactrocera dorsalis surveillance monitoring data	Oct	Aruna Manrakhan, Elma Carstens and Vaughan Hattingh
258	Update CBS spray programme	Nov	Jan van Niekerk, Elma Carstens, Providence Moyo, Paul Fourie and Paul Hardman
259	Customer Assurance Update	Nov	Paul Hardman (CGA)
260	Consumer Assurance Update	Dec	Paul Hardman (CGA)
261	FMS revised	Dec	Sean Moore, Vaughan Hattingh, Elma Carstens, Paul Hardman and Paul Cronje
262	CRI-PhytRisk developments	Jan	Providence Moyo and Paul Fourie
263	FCM colour plates	Feb	Sean Moore and Peter Stephen

264	Managing fruit fly pests in the northern citrus production areas of South Africa	Feb	Aruna Manrakhon, Wayne Mommsen and MC Pretorius
265	Leafhopper alert	Mar	Sean Moore, Wayne Kirkman and Tim Grout