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1 MARKET ACCESS TECHNICAL COORDINATION

By Vaughan Hattingh and Elma Carstens (CRI)

1.1 SUMMARY

The Citrus Fruit Fly Management System (FFMS) that CRI has developed as an alternative to postharvest disinfestation (cold treatment) was implemented for citrus exports to EU for the first time in 2019. In response to the 19 EU FCM interceptions on citrus from South Africa, several improvements were made to the FMS in December 2019. Five CBS interceptions on citrus from South Africa were notified by the EU in 2019. Eleven years of exchange of scientific information led to the finalisation of a workplan for export of fresh citrus fruit to the Philippines. Research on the host status of lemons and fruit flies led to an amended protocol for citrus exports to China. The protocol included improved fruit fly mitigation measures for lemons. However, due to the COVID-19 pandemic, the amended protocol could not be signed. There was no progress on the long outstanding market access issues with Japan and the USA. CRI supported the eSwatini National Plant Protection Organisation with technical inputs on the Pest Risk Analysis (PRA) conducted for access to the USA market and on compliance measures for the new EU regulations pertaining to FCM and fruit flies. CRI provided Zimbabwean citrus growers with inputs on a draft protocol to export fresh citrus fruit to China and supported Zimbabwean National Plant Protection Organisation to comply with the amended EU regulations pertaining to Fruit Flies and FCM.

OPSOMMING

Die Sitrus Vrugtevlieg Bestuurstelsel (FFMS) wat CRI ontwikkel het as 'n alternatief vir na-oes disinfestasie (koue-behandeling), is vir die eerste keer in 2019 vir sitrusuitvoere na die EU geïmplementeer. In reaksie op die 19 EU VKM onderskepings op sitrus vanaf Suid-Afrika, is daar verskeie veranderinge in Desember 2019 aan die FMS aangebring. Vyf SSV-onderskepings op sitrus vanaf Suid-Afrika is in 2019 deur die EU gerapporteer. Elf jaar van uitruiling van wetenskaplike inligting het tot die afhandeling van 'n werksplan vir die uitvoer van vars sitrusvrugte na die Filippyne gelei. Navorsing oor die gasheerstatus van suurlemoene en vrugtevlieë het tot 'n gewysigde protokol vir die uitvoer van sitrus na China gelei. Die protokol sluit verbeterde bestuursmaatreëls vir vrugtevlieë op suurlemoene in. Weens die COVID 19-pandemie kon die gewysigde protokol egter nie onderteken word nie. Daar was geen vordering met die lang uitstaande marktoegangskwessies met Japan en die VSA nie. CRI het eSwatini se Nasionale Plantbeskermingsorganisasie met tegniese insette oor die Pes Risiko Analises (PRA) wat uitgevoer is vir toegang tot die VSA-mark, ondersteun, asook met die nakoming van maatreëls vir die nuwe EU-regulasies rakende VKM en vrugtevlieë. CRI het tegniese insette aan die Zimbabwiese sitrusprodusente op 'n konsepprotokol om vars sitrusvrugte na China uit te voer, gelewer, en het die Zimbabwiese Nasionale Plantbeskermingsorganisasie ondersteun om aan die gewysigde EU-regulasies rakende vrugtevlieë en VKM te voldoen.

1.2 EUROPE (EU)

FCM

The new EU Commission Implementing Directive (EU) 2019/523 of 21 March 2019 that amends Annexes I to V to Council Directive 2000/29/EC required non EU trading partners to comply with the regulations as of 1 September 2019. The Commission required non EU trading partners to inform the Commission of the measures that will be implemented to comply with the new set of rules. For FCM, 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”. CRI provided DALRRD with the relevant scientific publications demonstrating the effectiveness of the FCM Systems Approach for communication with the EU. DALRRD submitted the scientific information on 13 August 2019. The EU acknowledged receipt on their website.

On 16 August 2019, South Africa received a letter from the European Commission indicating concern about the FCM interceptions. The commission requested a report on investigations conducted and corrective actions planned and/or implemented. CRI provided inputs and DALRRD submitted a response.

CRI convened meetings of the FMS Core Team and the FMS Working Group in October 2019 and FMS amendments were agreed upon. CRI presented the FMS changes to DALRRD on 29 October 2019. This led to some further amendments and the FMS was thereafter approved by DALRRD for implementation. The FMS changes were communicated at the Annual Citrus Coordinating meeting in November and on 02 December DALRRD provided CRI with official confirmation of the approved FMS to be implemented for the 2020 export citrus season. A Cutting Edge was sent out in December highlighting all the changes to the FMS.

CRI and DALRRD shared information on the investigations conducted in response to 2019 EU FCM interceptions. DALRRD submitted a letter and the investigation reports to the Commission on 14 November 2019. On 02 December 2019, CRI received a request from DALRRD to provide inputs on further questions posed by the Commission pertaining to the FCM Systems Approach. CRI submitted feedback on 04 December 2019. CRI provided further inputs on the last nine FCM interceptions reports. DALRRD submitted the reports with a cover letter to the EU on 23 December 2019. In this letter DALRRD indicated that a response to the EU concerns pertaining to the FCM Systems Approach (received on 26 November 2019) will be forthcoming.

DALRRD submitted all the investigation reports with a cover letter to the EU on 23 December 2019. On 3 February 2020 DALRRD sent a copy of the amended FMS for the 2020 citrus export season and a detailed response on the EU questions.

CBS

Nine notifications of CBS interceptions were received by the end of the 2019 citrus export season. DALRRD submitted the investigation reports with CRI inputs to the EU (five on 14 November 2019 and four on 23 December 2019).

Changes to the CBS RMS were discussed at the industry FMS Working Group meeting in October 2019 and at the Annual Citrus Coordinating meeting amendments to the CBS RMS were announced. The changes were to align the CBS RMS with the FMS for the 2020 export season.

A draft CBS RMS document was submitted to DALRRD on 27 January 2020 and the final version of the CBS RMS was circulated on 20 March 2020. CRI also provided inputs on additional phytosanitary declarations for fruit destined for the EU, as required by the new EU Plant Health regulatory legislation.

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 included a new set of rules 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. DALRRD and the implicated industries, PPECB, FPEF and SA-DAFF met and 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 DALRRD on 20 May 2019.

DALRRD approved the Citrus FFMS on 08 August 2019. Cutting Edges to inform the industry about the detail and the implementation of the Citrus FFMS were circulated. DALRRD submitted the Citrus Fruit Fly Management System Approach (FFMS) to the Commission on 13 August 2019 and indicated that South Africa will comply with Implementing Directive (EU) 2019/523 of 21 March 2019, 16.5 (d). Compliance will be achieved through the implementation of the Citrus FFMS. The EU acknowledged receipt by updating their website indicating how non EU trading partners will comply with the amended regulations. DALRRD also published new declarations for the phytosanitary certificates for Citrus consignments that were shipped to the EU as of 1 September 2019.

By the end of the 2019 citrus export season, 3 notifications of Fruit fly interceptions in the EU had been received. At the Annual Coordinating Meeting discussions took place and it was agreed that no changes are needed to the FFMS for the 2020 citrus export season.

1.3 **JAPAN**

In 2019 there was no response from Japan-MAFF on the three long outstanding market access requests: access for all mandarins (except Satsumas), under the current protocol for Clementines (pending from November 2009); revision of 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 inclusion of all navel orange cultivars in the current protocol (pending from September 2016). Their explanation for the lack of response remains the same – Japan-MAFF only work on one market access request from a country at a time and in 2019 Japan-MAFF worked on a protocol for South African Avocado exports to Japan.

On 19 December 2019, DALRRD sent a letter to Japan-MAFF indicating that the next market access priority for SA, is to conclude the three long outstanding citrus issues. In the letter they indicated that SA's request to include all mandarins (excluding satsumas) and navels are technically justified. The justification is based on the agreement reached by the Technical Panel on Phytosanitary Treatments (TPPT) that is responsible for recommending phytosanitary treatments to the Standards Committee of the IPPC. The TPPT agreed "that there are no fruit fly population differences in relation to cold treatment and no varietal or cultivar effects". In the same letter DALRRD also pointed out that SA's request to adopt a revised cold treatment of 1.4°C or lower for 16 days for all citrus types is within Phytosanitary Treatments (PT) standards for Mediterranean Fruit Fly (Medfly) in citrus fruits, as adopted by the International Plant Protection Convention (IPPC). They also pointed out that the time temperature conditions covered by these PTs, the cold treatments accepted by Japan for import of citrus from other countries, and the Medfly cold treatment protocols accepted by other countries for import of citrus fruit, include time temperature options similar or equivalent to the 1.4°C or lower for 16 days as validated in the South African trial results. Feedback from Japan was pending by the end of this reporting period.

1.4 **USA**

None of the long outstanding matters – the equivalence between USA domestic CBS regulations and USA import regulations (access for fruit from CBS areas in South Africa); recognition and access for CBS pest-free places of production in an area of low pest prevalence; and inclusion of other Western Cape magisterial districts in the export programme; the updated pest list and an updated work plan, had been concluded despite follow ups by DALRRD and other options pursued by Industry.

A new point for discussion with USDA-APHIS was the request from SA to include additional ports of entry for export of citrus fruit from SA. Industry forwarded a request to DALRRD on 12 December 2019 to include two additional ports for entry of SA citrus in the current workplan. The Port of Savannah, Georgia and Port of Charleston, South Carolina were identified. The inclusion of the ports will enable the SA Citrus industry to better meet the needs of the retail customers in the USA and to serve the needs of the markets. DALRRD submitted the request to USDA-APHIS on 20 December 2019. DALRRD requested further inputs and after discussions with the Industry on 10 February 2020, a response was submitted to DALRRD on 13 February 2020. DALRRD submitted feedback to USDA-APHIS in March 2020 and on 1 April 2020, USDA-APHIS published a notice in the Federal Register. The Notice included a proposed revision to Import Requirements for the Importation of Fresh Citrus from South Africa into the United States. The revision proposed that the restrictions on ports of entry for SA citrus fruit be removed. All comments on or before 1 June 2020 will be considered before a final decision will be made.

1.5 **CHINA**

The request submitted to GACC to exempt lemons from the current cold treatment requirement (24-days) by recognising the non-host status of lemons for fruit flies remains pending. On 26 July 2019 DALRRD received feedback from GACC on the information submitted by DALRRD on 9 November 2018. Despite the information provided, they still declined to accept that commercial export grade lemons produced in South Africa are not a host for fruit flies. They proposed using ISPM28-PT26: *Cold treatment for Ceratitis capitata on Citrus limon* 3°C or below for 18 consecutive days for lemons from South Africa to China. They again indicated that for South Africa to continue pursuing recognition of non-host status for lemons, trial results in accordance with ISPM37: *Determination of host status of fruit to fruit flies (Tephritidae)* should be submitted to the IPPC to be included into an international standard.

In order to resolve this issue, representatives from CRI and Fruit SA visited China (the CIQA) in August 2019 to discuss a possible trial to affirm the non-host status of commercial export grade lemons for fruit flies and to evaluate the impact of the prescribed cold treatment (3°C or below for 18 continuous days) on the quality of the fruit. The CIQA however indicated that they cannot commit to a trial procedure without the GACC being involved.

Further consultation between DALRRD and CRI resulted in DALRRD submitting a response to GACC on 18 October 2019. DALRRD accepted inclusion of the ISPM 28-PT26 cold treatment of *Citrus limon* for *Ceratitis capitata* (3°C or below for 18 continuous days), in the current protocol and requested GACC to agree to continue with ongoing bilateral discussions to exempt South African export grade lemons from a cold treatment based on non-host status.

On 6 November 2019, DALRRD received feedback from GACC. They provided an amended protocol for fresh citrus fruit to China which included the ISPM 28-PT26 cold treatment of *Citrus limon* for *Ceratitis capitata* (3 °C or below for 18 continuous days). They however included other changes to the current protocol as well – Citrus exported must come from pest free production sites (orchards) for *Ectomyelois ceratoniae* (Carob moth) following the guidelines of ISPM 10, new wording for the sticker that must be affixed to each carton and the region and province in which the PUC is located must be included on the carton label. A meeting took place between DALRRD, CRI, PPECB and FPEF and DALRRD provided feedback to GACC on 22 November 2019 which included a request to keep the labelling of the cartons as in the current protocol as well as maintaining the *E. ceratoniae* requirements as in the current protocol.

On 14 January 2020, CRI received a request from DALRRD to provide inputs on the response received from GACC regarding proposed revisions to the protocol with inclusion of a provision for improved conditions for lemons. GACC agreed to remove the requirement for information about region and province on the carton labels. Although GACC agreed with DALRRD on the quarantine pest control measures for *E. ceratoniae,* it was not reflected in the draft protocol received. DALRRD responded to GACC on 17 January 2020 with changes to the protocol reflecting what was previously agreed to regarding *E. ceratoniae*. DALRRD's attempts to finalise the protocol despite Covid-19 travel bans before the start of the 2020 export season have not as yet been successful, but continue with high level political engagements.

1.6 **SOUTH KOREA**

In August 2019, CRI received a request from the Western Cape citrus producers for inspection, pre-cooling and loading of citrus fruit destined for South Korea from Cape Town. The industry submitted a proposal to DALRRD on the 14 August 2019. DALRRD indicated that the detail of the request (how many PUCs and inspection points involved) should be discussed at the Annual Citrus Coordinating Meeting held in November 2019. DALRRD also agreed to include the request in the invitation to the Korean inspector for the 2020 citrus export season. At the Annual Citrus Coordinating Meeting and the Market Access meeting held in November 2019 it was agreed that the request needed further discussion between FPEF, CRI and DALRRD in January 2020. A telecom took place on 5 February 2020 and DALRRD confirmed that there is a facility in the Western Cape that complies with the requirements. At that time DALRRD was awaiting the letter from South Korea to confirm the arrival dates of the inspectors and the feedback on the past season and it was agreed that a follow up telecom will take place to discuss the way forward. Unfortunately, due to the COVID-19 situation the approval for inspection, pre-cooling and loading of citrus from Cape Town could not be discussed further.

The COVID-19 pandemic also affected global travel and South Africa's decision to suspend travel from high risk countries, DALRRD sent a letter to South Korea to propose alternatives for conducting phytosanitary preclearance inspections and calibration of equipment for in transit cold treatments. For the South Korean export programme the phytosanitary inspections are jointly conducted by inspectors from South Korea and SA. CRI provided inputs into the letter. By the end of April the South Korean Authorities informed DALRRD that exports can commence without the South Korean inspectors present but that this is only an interim measure that will be evaluated monthly. They also indicated that on arrival inspections will be intensified.

1.7 **INDIA**

During this reporting period there was no response from the Indian Authorities on the trial consignments sent in 2017 and 2018 for India to accept in-transit cold treatment for fruit flies in all crops exported from SA to India. Despite several follow ups by DALRRD, no feedback on the erroneous report of *Elsinoë australis* interception on citrus fruit from South Africa and the reported presence of *Phyllosticta citricarpa* in India was received.

1.8 **VIETNAM**

The Pest Risk Analysis for fresh citrus fruit exports to Vietnam remained incomplete. The outstanding issues to agree upon are the finalizing of the list of quarantine pests and packhouse procedures. Despite several follow ups by DALRRD and Industry, no communication was received during this reporting period from the Plant Protection Department of the Ministry of Agriculture and Rural Development of Vietnam (PPD) on a response sent by DALRRD in March 2018. The first response received from the PPD since January 2018, was on 14 January 2020. They sent a request to DALRRD for a site visit to the South African citrus industry in June 2020. CRI was consulted and on 17 January 2020 DALRRD confirmed the proposed date (June 2020) with the PPD. Since then there was no response from the PPD. Due to the COVID-19 pandemic, the South African Borders for international visits were closed on 27 March 2020.

1.9 **THE PHILIPPINES**

In April 2019 DALRRD received the draft phytosanitary requirements from the BPI. On 16 May 2019 a meeting took place between CRI and DALRRD to discuss the draft and many problems were identified. According to the draft phytosanitary requirements, citrus from South Africa should be sourced from FCM and fruit fly pest-free-places of production and be subjected to an in transit 24 day cold treatment. DALRRD provided CRI with the first draft of the response on 10 June 2019 and CRI provided feedback to DALRRD on 16 June 2019. The response was submitted to the BPI on 10 July 2019 which acknowledged receipt of the information.

DALRRD received a response from the BPI on 30 August 2019. Although the BPI accepted the quarantine pest list and most of the corrections, they still requested that fruit should be supplied from FCM and Fruit fly free orchards, that consignments must be subjected to a 22 day cold treatment and that a 5-10% fruit sample will be inspected on arrival. CRI provided feedback on 27 September 2019 and DALRRD submitted the response to the BPI on 1 October 2019. In the feedback, DALRRD indicated that mitigation measures and fruit sampling procedures must be according to International Standards for Phytosanitary Measures.

On 8 November 2019 DALRRD provided CRI with the feedback received from the BPI. They no longer requested that fruit should be from FCM and fruit fly free orchards. They also accepted DALRRD's proposal that pre-export quarantine inspection shall be conducted by DALRRD to ensure freedom from the all the Philippines' quarantine pests of concern, particularly, *Ceratitis capitata, C. rosa* and *Thaumatotibia leucotreta* and in case of interception of these species during the export inspection, DALRDD will reject the consignment (whereas BPI had previously proposed banning of the orchard) and shall ensure that the fruit will not be allowed to be exported to the Philippines. The BPI also accepted DALRRD's proposal for the monitoring of FCM and fruit flies in orchards but called for clarification of the verification process to be followed by DALRRD. They however did not agree with DALRRD's proposal for a 2% sample size for inspection on arrival in the Philippines and proposed a 5% sample size. CRI provided feedback to DALRRD after a meeting on 18 November 2019 with the Directorate Inspection Services. DALRRD submitted a response to the BPI on 26 November 2019, providing detail of the verification process and indicating that they accepted the 5% sample size.

In November 2019, a Fruit South Africa delegation (including CGA and CRI) accompanied by DALRRD, along with the Charge Affairs of the South African Embassy in Manila and the South African Agricultural Counsellor in the region, met with the BPI to engage with a view to accelerate market access for SA produce. At this meeting the draft phytosanitary requirements for citrus from SA were discussed.

On 25 February 2020, DALRRD sent CRI feedback received from the BPI. In the letter an indication was given that BPI is in the process of drafting the workplan and that the work plan will be sent to DALRRD for comments and review. Attached to the letter was the final draft PRA and the phytosanitary import conditions. The phytosanitary import conditions however did not reflect all the agreements reached. DALRRD sent a letter to the BPI on 6 March 2020 to confirm that the BPI did accept the following condition – “Pre-export quarantine inspection shall be conducted by the DALRRD to ensure freedom from the all the Philippines’ quarantine pests of concern, particularly, *Ceratitis capitata, C. rosa* and *Thaumatotibia leucotreta* and in case of interception of these species during the export inspection, DALRDD will reject the consignment and shall ensure that the fruit will not be allowed to be exported to the Philippines”. On 6 April 2020, the BPI confirmed in a letter that they did agree to the procedure for the pre-export quarantine inspection but no mention was made of when DALRRD can expect to receive the final work plan. On 8 April 2020 DALRRD sent a letter to the BPI to enquire about the status of the workplan.

Although the BPI and DALRRD agreed on all the outstanding issues pertaining to the phytosanitary requirements, the final work plan was not received by the end of this reporting period.

1.10 **eSWATINI**

In 2019 CRI supported the eSwatini National Plant Protection Organisation with technical inputs on the Pest Risk Analysis (PRA) conducted for access to the USA market. Concerns were raised about the inclusion of the fruit fly *Ceratitis cosyra*. Further technical inputs were provided in March 2020 to support the exclusion of *C. cosyra*. Reliable reports were provided to support the claim that export grade lemons and limes are not hosts. Furthermore, no reliable reports are available that commercial export grade grapefruit, oranges and mandarins are suitable hosts. Evidence was also provided that the proposed cold treatment schedule T107-e would ensure risk mitigation for all the listed pests of quarantine concern namely *T. leucotreta*, *B. dorsalis*, *C. capitata*, *C. rosa* and *C. cosyra*.

CRI also supported the eSwatini National Plant Protection Organisation to comply with EU Commission Implementing Directive (EU) 2019/523 of 21 March 2019 that amends Annexes I to V to Council Directive 2000/29/EC which required non EU trading partners to comply with the regulations as from 1 September 2019 pertaining to FCM and fruit flies. The regulations included new rules for fruit flies.

Concerns were raised by the NPPOZA about the high number of *Bactrocera dorsalis* catches next to the eSwatini borders. Issuance of import permits for citrus fruit were put on hold until new import conditions were released. As eSwatini has no pest free areas for *B. dorsalis*, inputs were provided to DALRRD to align the Citrus imports conditions with SA conditions in place to move citrus fruit from *B. dorsalis* infested areas to free areas. The inputs were accepted and new import conditions were released in March 2020. Permits were issued based on the new import conditions.

1.11 **ZIMBABWE**

In January 2020 CRI provided inputs to the Zimbabwean producers on the draft **PROTOCOL OF PHYTOSANITARY REQUIREMENTS FOR THE EXPORT OF ZIMBABWE FRESH CITRUS TO CHINA BETWEEN GENERAL ADMINISTRATION OF CUSTOMS OF THE PEOPLE'S REPUBLIC OF CHINA AND THE MINISTRY OF LANDS AGRICULTURE WATER AND RURAL RESETTLEMENT OF THE REPUBLIC OF ZIMBABWE.** CRI also supported the Zimbabwean National Plant Protection Organisation to comply with

EU Commission Implementing Directive (EU) 2019/523 of 21 March 2019 that amends Annexes I to V to Council Directive 2000/29/EC which required non EU trading partners to comply with the regulations as from 1 September 2019 pertaining to FCM and fruit flies. The regulations included new rules for fruit flies.

2 BIOSECURITY

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

2.1 SUMMARY

A draft Biosecurity Master Plan was prepared, currently undergoing further revision, with a final working version to be completed in 2020. With funding from APHIS a regional training on citrus greening and vectors is planned at ICIPE, Kenya for 10-12 individuals from selected countries in eastern and southern Africa in 2020. The list of the Top 10 Biosecurity Threats had been discussed at the BAC meeting and approved, import conditions for Citrus spp. vegetative propagation material had been updated accordingly.

The HLB and ACP Action Plan and HLB Safe System for Nurseries has been developed to a final draft after consultations with key stakeholders, and was adopted as the official working version in May 2019, the HLB SC was also constituted from key stakeholders.

During the reporting period, two surveys for ACP (*Diaphorina citri*) and HLB were done in Mozambique, as well as one in Angola. No HLB (Las) and ACP were detected. Similarly a survey for the detection of HLB and ACP was conducted in Ethiopia in December 2019. The results showed that 11 of 29 citrus leaf samples analysed tested positive for HLB. No ACP or ACT (*Trioza erytreae*) were detected.

In this reporting period eight pest reports were sent to the IPPC. Four of the reports were on detections of *B. dorsalis* (previously *B. invadens*) in pest free areas in South Africa. Three of the reports were to notify that *B. dorsalis* was eradicated from some of the pest free areas. The other report was on the first-time detection of Citrus Leprosis-N in South Africa.

The discovery of Citrus Leprosis (CL), 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. Random orchards on the affected farms were surveyed in 2019 for the presence of symptoms and mites, but none were found. All affected farms will be officially audited for compliance to the CLRP in July 2020, and the outcome will determine whether the status of all orchards on the farms will change.

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. The meeting scheduled for March 2020 had been postponed due to the COVID-19 pandemic. Concerns were raised by the NPPOZA about the high number of *B. dorsalis* catches next to the eSwatini borders. Issuance of import permits for citrus fruit were put on hold until new import conditions were released.

A delimiting and a detection survey was conducted in the Knysna magisterial district in the Greening free buffer zone in the Western Cape Province in June 2019. DALRRD issued orders to remove the trees at the end of October 2019. DALRRD visited the owners in February 2020 and all the trees had been removed.

Monitoring surveys to cover the 5 km radius around the CFB were conducted in April and May 2019, and further farms and home gardens with citrus trees were found. Information was provided to DALRRD and in June, DALRRD issued orders to all the implicated owners to remove the trees. DALRRD did follow up visits to all the owners to whom orders were issued. By the end of the reporting period 16 of the implicated owners removed the trees.

OPSOMMING

'n Konsep meesterplan vir Biosekuriteit is opgestel, wat tans verder hersien word, met 'n finale werksweergawe wat in 2020 voltooi moet word. Met befondsing vanaf APHIS word 'n streeksopleiding oor sitrusvergroening en vektore by ICIPE, Kenia, vir 10-12 individue uit geselekteerde lande in Oos- en Suider-Afrika in 2020 beplan. Die lys van die Top 10 Biosekuriteitsbedreigings is tydens die BAC-vergadering bespreek en goedgekeur. Invoervereistes vir *Citrus* spp. vegetatiewe voortplantingsmateriaal is dienooreenkomstig opgedateer.

Die HLB- en ACP-aksieplan en die HLB-veilige stelsel vir kwekerye is in 'n finale konsep ontwikkel, ná konsultasies met verskeie belangrike rolspelers, en is in Mei 2019 as die amptelike werksweergawe aanvaar. Die HLB Bestuurskomitee is ook uit belangrike rolspelers saamgestel.

Gedurende die verslagtydperk is twee opnames vir ACP en HLB in Mosambiek, asook een in Angola, uitgevoer. Geen HLB (Las) en ACP is opgespoor nie. ʼn Soortgelyke opname vir die opsporing van HLB en ACP is in Ethiopië in Desember 2019 uitgevoer. Die resultate het getoon dat 11 van die 29 sitrusblaarmonsters wat ontleed is, positief vir HLB (*Liberibacter asiaticus*) getoets het. Geen ACP of ACT is opgespoor nie.

In hierdie verslagtydperk is agt verslae na die IPBK gestuur. Vier van die verslae was oor die opsporing van *B. dorsalis* (voorheen *B. invadens*) in pesvrye gebiede in Suid-Afrika. Drie van die verslae was om aan te dui dat *B. dorsalis* in sommige van die pesvrye gebiede uitgewis is. Die ander verslag handel oor die eerste opsporing van Sitrus Leprosis-N in Suid-Afrika.

Die ontdekking van Sitrus Leprosis (CL), wat daarvoor bekend is om in Suid- en Sentraal-Amerikaanse lande voor te kom, is op drie plase in die Addo-omgewing in die Oos-Kaap, in Mei 2018, soos vereis, by die Departement van Landbou, Bosbou en Visserye (DAFF) aangemeld. Opnames is in verskeie boorde op die betrokke plase in 2019 vir die teenwoordigheid van simptome en myte uitgevoer, maar niks is gevind nie. Alle geaffekteerde plase sal in Julie 2020 amptelik geoudit word om aan die CLAP te voldoen, en die uitslag sal bepaal of die status van alle boorde op die plase sal verander.

CRI het insette by die Phyto Risk Forum gelewer en in hierdie verslagtydperk het twee vergaderings plaasgevind. Tydens beide vergaderings is terugvoering oor sitrus-opnames gegee. Die vergadering wat vir Maart 2020 beplan is, is weens die COVID-19-pandemie uitgestel. Die NPPOZA het kommer uitgespreek oor die groot aantal vangste van *B. dorsalis* langs die eSwatini-grense. Die uitreiking van invoerpermitte vir sitrusvrugte is tydelik gestaak totdat nuwe invoervoorwaardes opgestel is.

'n Afbakenings- en 'n opsporingsopname is in Junie 2019 in die landdrosgebied van Knysna, die vergroeningsvrye buffersone in die Wes-Kaap provinsie, uitgevoer. DALRRD het teen einde Oktober 2019 lasgewings uitgereik om die bome te verwyder. DALRRD het die eienaars in Februarie 2020 besoek en al die bome is verwyder.

Moniteringsopnames om die 5km radius rondom die SGB te dek, is in April en Mei 2019 uitgevoer en verdere plase en tuine met sitrusbome is gevind. Inligting is aan DALRRD verskaf, en in Junie het DALRRD lasgewings aan al die betrokke eienaars gegee om die bome te verwyder. DALRRD het opvolgbesoeke aan al die eienaars aan wie lasgewings uitgereik is, gebring. Aan die einde van die verslagtydperk het 16 van die betrokke eienaars die bome verwyder.

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 African 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 were reviewed. A draft Master Plan appropriate for the context of the southern African citrus industry was then written and submitted to VH for comment. Comments and inputs that suggested review of the Master Plan were received in November 2019, to produce a second and improved draft. A second draft resulting from substantial review of the first draft has since been sent to VH in March 2020 for comment. The next step will be to incorporate comments from VH, and send a revised and improved draft to the Biosecurity Advisory Committee for inputs, with a final working version of the Master Plan to be produced by June 2020.

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 short regional training workshop on citrus greening diseases and associated vectors at ICIPE/Kenya, and subsequently secured support for the proposal. APHIS agreed in principle, requested preparation of a concept note and budget required to implement the training. SG prepared the concept note and budget in consultation with ICIPE, and submitted these to Mr McKim. A positive feedback was received in September 2019 that the proposal was accepted for funding amounting to $40,000. The plan is to train 10-12 individuals from selected countries in eastern and southern Africa, and the training is tentatively scheduled to take place in July 2020, provided that current travel restrictions due to COVID-19 are lifted in the coming few months allowing travel to Kenya.

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

Following extensive communication with contacts at University of Eduardo Mondlane and the NPPO of Mozambique, WK visited Mozambique in May 2019 to initiate detection surveys of the Asian citrus psyllid (ACP) and HLB in southern Mozambique. The trip was successful and from the spot surveys it was clear that ACP was not yet present in Maputo Province. ACP traps were placed and retrieved after some weeks in the field, confirming results from physical scouting that ACP was not present in Maputo Province. A follow up trip was coordinated with the same contacts in Mozambique to extend detection surveys for ACP and HLB to the Inhambane province, leading to a second successful trip in March 2020 by EC and WK. Yellow sticky traps were deployed in the field and collected after a few weeks, and the results from those traps were pending at the time of this reporting.

Extensive email communication with collaborators in Angola resulted in a trip to survey for ACP and HLB in Angola in late July/early August 2019 (detail under project 5 below). Extensive email and telephonic communication was made with two academics/researchers from Wolyata Sodo University in Ethiopia to arrange a field trip to a commercial citrus growing region in the Rift Valley to conduct HLB and ACP surveys that resulted in a successful field survey in December 2019 (detail under project 6 below).

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

The list of the Top 10 Biosecurity Threats had been discussed at the BAC meeting of 3 September 2019 and was approved. The import conditions for Citrus spp. vegetative propagation material had been updated to include the Top 10 pests. At the meeting it was recommended that mini PRAs should be conducted on the Top 10 Biosecurity Risks. By the end of this reporting period a draft PRA framework was developed to compile relevant scientific information on each pest and disease from available literature including the likelihood of entry into South Africa, likelihood of establishment and spread, management options, as well as other relevant data that would assist in risk assessment. Accordingly, the PRA framework was used to compile scientific information starting with Citrus yellow vein clearing virus and *Xylella fastidiosa* which was then sent to the BAC for comment and input. If the BAC supports the mini PRA framework, the plan is to follow the process and conduct PRAs on all of the Top 10 priority pests in the course of the year.

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

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

The HLB and ACP Action Plan and HLB Safe System for Nurseries has been developed to a final draft after consultations with key stakeholders, and was adopted as the official working version (subject to periodic amendments as required) on the occasion of the foundational meeting of HLB Steering Committee on May 30, 2019 in Pretoria in the presence of key stakeholders-CGA, SACNA, SANA, ARC and relevant directorates of the Department of Agriculture, Land Reform and Rural Development (DALRRD). Terms of Reference for the HLB SC were subsequently drafted, tabled for review and finalized. In the course of the reporting period, the HLB SC met four times. At these meetings SG served as the Secretary responsible for minutes which were drafted and circulated within a week of the date of the meeting. The 4th meeting convened in Pretoria on 19 February, 2020.

As part of the preparatory steps to implement the Action Plan, CRI trained 12 DALRRD officials in November 2019 on basic survey and sampling methods for the detection of HLB and ACP in non-commercial settings, border posts and residential areas. CRI has also increased trap reading capacity by hiring a dedicated trap reader in Nelspruit.

A follow up engagement was made with Jan-Hendrik Venter of DALRRD to progress the emergency registration of insecticides to control Asian citrus psyllid, ACP in the event of early detection in South Africa. A list of suggested chemicals for emergency registration for use against ACP had been forwarded to DALRRD in December 2019. Positive response was received and communication copied to the chemical registrar for initiating the process of registration.

2.6.2 Project 5. Ensure that HLB and ACP surveillance is undertaken in Eastern and Southern Africa

During the reporting period, two surveys for ACP and HLB were done in Mozambique, as well as one in Angola.

<u>Angola</u>
CRI became aware of the locations of large orchards planted with trees imported from Brazil, particularly Minas Gerais, where the nurseries are not housed in insect-secure structures. A survey visit to Angola was planned by Paul Fourie and Wayne Kirkman to assess the status of the trees and the HLB risk that they pose to southern African citrus production. Angola Alliance and Edifox were again extremely helpful in the logistical planning of the surveys, and also in providing locations and contact details of other farms with imported trees. Meetings with the Angolan Ministry of Agriculture were arranged to follow up on previous engagements and present the survey schedule, and also to debrief the Ministry of the findings.

Angola Alliance also arranged for Drs Daniel Bassimba (Instituto de Investigación Agronómica, Chianga, Huambo) and Camillo Jose (PlantCare, Luanda), both trained plant pathologists, to participate in the surveys and meetings.

Given the time constraints, all citrus trees/orchards could not be inspected. Owners/managers of farms were requested to take the survey team to diseased or problem areas on the farms. On smaller farms, orchards were surveyed in a transect pattern, while on larger farms, orchards were inspected from the back of a truck to identify possible survey points. Trees were visually inspected for signs and symptoms of citrus pests and diseases, particularly those that resemble HLB and its vectors, and other exotic diseases, such as Citrus Variegated Chlorosis, Citrus canker, Sudden Death, Leprosis Virus, and post-bloom fruit drop.

Leaf and twig samples with suspect symptoms were taken for laboratory analysis at CRI in South Africa. Yellow sticky traps for insects were also placed at the various localities. These were collected approximately 3 weeks later by Nelus de Waal and sent to CRI for inspection. All plant and insect samples were transported to South Africa with the relevant permits from both countries.

Various tests were conducted on the leaf and twig samples and three laboratories participated in different aspects to confirm results. DNA/RNA was extracted from petiole and midrib tissue of leaves and various PCR and sequencing analyses conducted using approved methods (see attached reports).

Following the surveys, but prior to the sample analyses, a debriefing meeting was held with the Secretary of State for Agriculture, Jose Carlos Lopes da Silva Bettencourt, and National Director of Agriculture, Antonio Sozinho. This meeting was attended by the survey team, as well as Rui Lopes of Edifox.

Dr Bassimba explained the preliminary findings of the survey, highlighting the concern that we found typical HLB / Greening symptoms in some of the orchards planted with trees from Minas Gerais. To our knowledge and that of Drs Bassimba and Jose, African Greening was not yet described in Angola, and given the remote locations of some of these farms, we dreaded that finds were HLB.

The following recommendations were made to the Ministry by CRI:

1. Refrain from granting import permits for citrus trees from countries with exotic diseases.
2. Urgently compile a list of all such previous imports and conduct thorough surveys to ascertain whether any exotic pests or diseases might have entered with the trees (these surveys should be repeated annually for at least 3 years, since the symptoms might be latent).
3. Complete analyses on the 2019 survey samples, and implement control measures accordingly. It was noted that we could not be certain whether the typical symptoms were caused by HLB or African Greening.
4. Nurseries in Angola should participate in the SA-CIS to ensure that certified and disease-free citrus propagation material is used. It was suggested that a delegation of key stakeholders should visit South Africa and the SA-CIS to promote this initiative.

Mr Bettencourt agreed to write an order immediately to ban imports of citrus material from Brazil. He also requested that a project proposal be compiled for surveys of all suspect orchards in the North and South of Angola. A workshop should also be held with all relevant role players. Dr Fourie recommended that the Ministry invite the relevant experts from Fundecitrus (Sao Paulo, Brazil) to assist them with this workshop; Fundecitrus is world-renowned in their HLB management systems and importantly also speaks Portuguese. Mr Bettencourt also agreed that Citrus should be added onto the list of crops in a development programme with special government funding. This should assist in supporting biosecurity initiatives, particularly control measures where needed.

Dr Fourie pledged CRI's ongoing support for citrus biosecurity initiatives in Angola, but stressed that CRI does not have the capacity to conduct the required surveillance throughout Angola. The Ministry should invest in building the required capacity for surveillance and diagnostics. CRI could support these initiatives through technical support and training in South Africa or Angola. Dr Fourie noted that Drs Bassimba and Jose's participation in the surveys was a highlight and that they are ideally equipped to lead the survey project.

The meeting also discussed problems regarding the availability of agrochemicals. It was proposed that Angola recognises South African registrations, to which Mr Sozhino agreed. A preliminary laboratory report was provided to the survey team on 12 August 2019, which indicated that HLB was not detected, but that African Greening was detected in some samples. It was noted that the laboratory was doing some more confirmatory tests as well as tests for other pathogens. Comprehensive analyses of the samples were concluded (see attached reports), which indicated that African Greening was detected, but no HLB or CVC was detected. All ACP traps were collected and evaluated. No *D.citri* nor *T. erytreae* were detected.

Mozambique

The presence of *D. citri* (Asian Citrus Psyllid/ACP) near the Naliendele Research Institute (10˚ 21' 14.33" S, 40˚ 10'05.62" E) in the Mtwara district of Tanzania, approximately 30 km north of the Mozambique border was unofficially communicated to CRI/Tim Grout in February 2019 by Dr Chris Materu at Naliendele, due to the close proximity, it is possible *D. citri* may be present in northern Mozambique. Surveys in this region were not possible due to security concerns arising from political unrest, and it was decided to first survey the Maputo region of southern Mozambique. If no *D. citri* are found in this region, further surveys will be conducted further north, towards the Beira – Chimoio – Manica transect to determine the current southernmost frontier of ACP in Africa.

Sampling was carried out in 10 regions of the Maputo districts in Mozambique from 21 to 23 May 2019. Citrus trees were targeted, as well as *Murraya* sp*.* plants. At each location between 2 and 14 double sided sticky lime-green card (14.0 x 18.4 cm) ACP traps (Alpha-Scents, USA) were placed. In total 44 traps were placed at various sites. GPS coordinates and date of placement were recorded. All or at least 10 trees in the vicinity of the traps which contained green soft flush were visually examined and tap sampled for the presence of *D. citri* and *T. erytreae.* Leaves which showed possible symptoms of Las and Laf were collected for analysis. No *D. citri* or *T*. *erytreae* were found at any of the sites by visual inspection or tap sampling. Traps were collected one month after hanging, and were sent to CRI for evaluation. No *D. citri* and *T. erytreae* were detected on any of the traps.

A second survey was conducted in Mozambique in 2020. Sampling was carried out in three transects around Inhambane in Mozambique from 04 to 06 March 2020. Citrus trees were targeted. At each location, one double sided sticky lime-green trap was placed. GPS coordinates and date of placement were recorded and plotted on Google Earth. All or at least 4 trees in the vicinity of the trap which contained green soft flush were visually examined and tap sampled for the presence of *D. citri* and *T. erytreae.* Leaves which showed possible symptoms of Las or Laf were collected for analysis.

Only one psyllid species was found during tap sampling, and was identified as *Pseudophacopteron* sp. In total 80 traps were hung out. Traps were collected after four weeks by Elias Cambula and couriered to CRI, Nelspruit. Leaf samples were collected at nine sites with possible greening symptoms. Samples were tested at CRI and none of the nine samples tested positive for Las or Laf. Only 51 out of the 80 traps placed were recovered. No *D. citri* or *T. erytreae* were found on the traps. Other *Diaphorina* species like *D. punctulata* and *D. zebrana* were identified, as well as a few other *Psylloidea* specimens. The *Diaphorina* species recorded were kept in alcohol for confirmation if required.

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

SG conducted a field trip to survey for the detection of HLB and ACP in a commercial citrus production valley in Ethiopia in December 2019. Useful contact was established with a plant pathologist and an entomologist from Wolayta Sodo University who accompanied SG to the field. A valuable contact was also established with Mr Barry Smales, a South African who is the operations manager of Africa Juice, the farm where samples were collected. Citrus leaf samples were brought back to SA and submitted to CRI Diagnostics Centre for analysis. The results showed that 11 of 29 citrus leaf samples analysed tested positive for HLB. No ACP or ACT were detected during field scouting, but sticky yellow traps were left with the operations manager for deployment in the field. The plan was for Mr Smales to bring and deliver the traps to CRI in May 2020. The plan has been disrupted by travel restriction due to COVID-19 as of the writing of this report, and discussion is underway regarding an alternative plan.

2.6.4 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 eight pest reports were sent to the IPPC. Four of the reports were on detections of *B. dorsalis* (previously *B. invadens*) in pest free areas in South Africa. Three of the reports were to notify that *B.*

dorsalis was eradicated from some pest free areas. 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 other report was on the first-time detection of Citrus Leprosis-N in South Africa.

This project will ensure that the relevant Acts and Regulations are aligned with the relevant action plans. In this reporting period Amendments to Regulation R.110 had been communicated to DALRDD. The amendments included deletion of certain host plants of HLB from Tables 3, 6 and 12. Inputs were also made to a new Regulation pertaining to Control Measures relating to Asian citrus psyllid (*D. citri*) and Citrus greening: Asian and American strains (*Candidatus* Liberibacter asiaticus and *Candidatus* Liberibacter americanus*).* This new regulation had been approved by the Minister in March 2020 to solicit public comments. A delayed response is expected due to the COVID-19 pandemic and the lockdown.

2.6.5 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.

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 communicated 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. No flat mites were found in any of the affected orchards during the reporting period.

Subsequently the disease was identified on a fifth farm in the Addo area, namely Mooidam, were nine orchards tested positive. These, along with adjacent orchards, were treated according to the CLRP. Several other suspicious sites were inspected but samples tested negative for leprosis. The other affected farms in the Sundays River Valley, Kleinplaas, Halaron, Bellevue, and Elim East were audited for compliance to the CLRP in July 2019, with the assistance of Luke Cousins, who was employed by CRI for one month, after being intensively trained by WK. The audits focussed on pruning, spray programmes, weed control, mite presence, controlled movement of people and record keeping.

Halaron complied fully with all requirements of the CLRP, and it has been recommended that the Red1 status be downgraded. There were no major non-compliances on Bellevue and Elim East. Bellevue was subsequently sold to SRCC, and the Delta and Midknight Valencia orchards where leprosis was first discovered were removed and destroyed. The remaining affected orchards, Cara Cara navels, which were not as severely infected, complied with the requirements of the CLRP. Once again the major finding at Kleinplaas was that the weeds and ground cover (Wandering Jew) had not been removed. The grower was again given three months to rectify this by CRI and SRCC, but the deadline was not met, and so the status of the entire farm remains Red 1. The farms will be audited again in July 2020.

Random orchards on the affected farms were surveyed in 2019 for the presence of symptoms and mites, but none were found. All affected farms will be officially audited for compliance to the CLRP in July 2020, 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 growers and distributors. WK convened a meeting of the Eastern Cape Technical Association (ECTA) on 23 May 2019, where a presentation was made on CL. 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”.

2.6.6 Project 9: Phytosanitary Risk Forum

In this reporting period two meetings took place. The meeting scheduled for March 2020 had been postponed due to the COVID-19 pandemic. Concerns were raised by the NPPOZA about the high number of *B. dorsalis* catches next to the eSwatini borders. Issuance of import permits for citrus fruit were put on hold until new import conditions were released. As eSwatini has no pest free areas for *B. dorsalis*, inputs were provided to DALRRD to align the citrus import conditions with SA conditions in place to move citrus fruit from *B. dorsalis* infested areas to free areas. The inputs were accepted and new import conditions were released in March 2020. Permits were issued based on the new import conditions.

2.6.7 Project 10: Greening surveys (African greening - *Candidatus* Liberibacter africanus & Asiatic greening - *Candidatus* Liberibacter asiaticus)

A delimiting and a detection survey was conducted in the Knysna magisterial district in the Greening free buffer zone in the Western Cape Province in June 2019. The delimiting survey focussed on the area where positive trees were found in October 2018. DALRRD issued orders to the implicated owners and the trees were removed. The detection survey was conducted in other neighbourhoods in Knysna and in Karatara. Samples were submitted to the DALRRD’s laboratory in Stellenbosch and five (5) samples tested positive for Laf. The Department issued orders to all the implicated owners in September 2019 and with CRI conducted a follow up delimiting and detection survey. Trees from two other neighbourhoods in Knysna were sampled. Thirty-three (33) samples were submitted to the Department laboratory in Stellenbosch and seven (7) of the 33 samples tested positive for Laf. DALRRD issued orders to remove the trees at the end of October 2019. DALRRD visited the owners in February 2020 and all the trees had been removed. DALRRD also put traps out for *D. citri* on their way to Knysna and in specific sites in Knysna. Fifty-nine (59) traps were collected on their way back. Thirty-five (35) traps were analysed at DALRRD’s laboratory in Stellenbosch and no *Diaphorina* spp were identified. Twenty-four (24) traps were sent to CRI. By the end of this reporting period results were pending from CRI.

2.6.8 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. Surveys were conducted in 2011 to identify farms 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. In November 2019 further information was provided to the Senior Prosecutor on request. By the end of the reporting period no outcome on the reported case was available.

Monitoring surveys were conducted in late 2018 and early 2019 under the auspice of DALRRD in the transect of the 5 km citrus free zone outside the CFB. Several places with citrus trees were found and the decision was taken that the monitoring survey should be repeated to cover the 5 km radius. Orders were issued to all the implicated owners. Monitoring surveys to cover the 5 km radius were conducted in April and May and further farms and home gardens with citrus trees were found. Information was provided to DALRRD and in June, DALRRD issued orders to all the implicated owners to remove the trees. DALRRD did follow up visits to all the owners to whom orders were issued. By the end of the reporting period 16 of the implicated owners removed the trees.

3 PORTFOLIO: INTEGRATED PEST MANAGEMENT

3.1 PORTFOLIO SUMMARY

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

The Integrated Pest Management (IPM) Portfolio consists of three programmes: False Codling Moth (FCM), Fruit Flies and Other Pests. These are coordinated respectively by Sean Moore, Aruna Manrakhan and Tim Grout. At least every second year, CRI's extension team solicit's the citrus growers' opinions on their research needs and priorities, a process that is conducted region by region. This is combined with CRI's knowledge of market access forces and impending biosecurity threats. Research proposals are finally vetted by specialist research committees with strong CGA and citrus technical representation. Through this process, the main research priorities within the IPM Portfolio have remained FCM and fruit flies. However, the importance of mealybug research has increased over the last couple of seasons, with notably elevated levels in the field and increased interceptions of infested fruit destined for the South Korean market. Additionally, focus on the imminent arrival of the Asian Citrus Psyllid, and its ability to vector the devastating Huanglongbing greening disease, has shifted attention to this pest over the last few years. Overlaid onto these very pressing research priorities, is the increasing demand, particularly by European markets and retailers, for reduced levels and numbers of residues. Understanding the potential conflict between increasing market intolerance for phytosanitary pests and diseases and blemished fruit on the one hand, and increasing market intolerance for chemical residues on the other hand, the demand by the citrus industry for a strong focus on research for biological alternatives, was greater in 2019 than ever before. CRI has always seen the writing on the wall and has prioritised its focus on biological control, but is increasing this further in response to market pressure and grower requests.

In the first year (2018) of implementation of the False Codling Moth risk management system (the FMS) for citrus exports to the EU being applied, nine interceptions of live FCM in South African citrus were reported in the EU. Although this can be considered an overwhelming success for the programme, considering the export of 800 000 tons of citrus to Europe that year, it was nonetheless necessary to introduce more stringent requirements into the FMS to further reduce the possibility of live FCM being intercepted in consignments. This was done, but unfortunately the outcome was not as intended, as interceptions increased to 19 during the 2019 export year. Consequently, further stringency was added to the FMS for the 2019/20 season, which will hopefully positively affect the outcome in the market in the ensuing export season. This was supported by significant improvements in the documented FMS, as published in a scientific paper and submitted to the European Commission early in 2020.

Consequently, FCM remained as the top entomological and market access research priority during the last research cycle. Within the FCM programme, 17 different projects were conducted, of which 12 addressed pre-harvest issues and five were postharvest projects. Research within this programme focussed on improvement of SIT and quality control testing for SIT, improvement of efficacy of insect viruses against FCM, the role of orchard nets in FCM management, synergism between compounds and microbes for improved FCM control, reasons for reduced FCM pressue in organic farming, and FCM ecology in the warm northern regions. Some of the highlights of the research conducted within this programme were the successful selection for a CrleGV virus isolate resistant to UV-irradiation, development of a new spermatophore transfer-based QC method for SIT, demonstration of synergy between yeast and virus for improved virulence against FCM, the efficacy of a postharvest combination treatment of CO_2 fumigation followed by a partial cold treatment, and the sensitivity of the SIFT-MS device for potential postharvest detection of infested fruit.

Along with the FMS, for importation of fruit into the EU, the FFMS (Fruit fly management system for export of fresh citrus produced in South Africa) was implemented for the first time, due to the recent regulation of non-European fruit fly species as phytosanitary organisms. There were only three interceptions of live fruit fly larvae in South African citrus in Europe during the 2019 export season. Nonetheless, due to there being zero tolerance for phytosanitary pests and due to the presence of fruit fly species in South Africa, that do not occur in our export markets, research within this programme remained of high priority. Prehaverst projects were aimed at improvements in monitoring, efficacy and optimisation of bait stations and mass trapping, and more ecological projects, such as the relative distribution and abundance of species such as Natal fly, Cape fly and marula fly. Postharvest projects focussed on cold treatments for the different fruit fly species. Highlights in the programme included the finding that Medfly was more cold-tolerant than the other fruit fly pests on citrus, potentially allowing future cold treatment trials to only be conducted with Medfly; and the finding that one of the warmer temperatures used in the FMS, 3.5°C, was adequately effective for use within the FFMS. One of the key challenges within this programme is the difficulty in mass rearing the Cape fly, in order to conduct laboratory research, most importantly, cold treatment studies.

Probably the two most important pests covered under the Other Pests programme were Asian Citrus Psyllid and mealybugs. However, within this programme, research projects also covered control of flat mite, as a vector of Leprosis, control of phytosanitary mites on imported budwood, surveys for oleander scale, the effect of nets on pest management, and environmental limitations of entomopathogenic fungi and potential solutions to these. Highlights within this programme were the discovery that UV-irradiation is the biggest impediment to the efficacy of entompathogenic fungi for arboreal pests, and demonstration of the effect of netting on various citrus pests, probably most importantly, the discovery in two separate projects that citrus thrips damage is significantly lower under nets. This might indicate that although the levels of certain other pests are elevated under nets, netting may be beneficial for implementation of IPM, as the key IPM pest is less of a problem.

It is also important to highlight that the research reported on here was not only conducted by the CRI IPM research team, but was made possible through several collaborations, such as with Rhodes University, Pretoria University, Stellenbosch University and some international collaborators.

During the past year, CRI research entomologists were also very active in transferring technology to growers, in the form of 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, the FFMS, 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 local and international scientific meetings, such as the Congress of the Entomological Society of Southern Africa, the International Invertebrate Pathology and Microbial Control Congress, and the Postharvest Measures Research Group. Additionally, several key papers were published in scientific peer-reviewed journals.

The IPM Portfolio team is confident and prepared to dramatically grow their research capacity and impact in an environment of increased funding in 2021, thanks to the foresightedness of the citrus industry leadership and support of all citrus growers. This will be strongly influential in helping the South African citrus industry to remain at the forefront of IPM research and implementation within the global citrus industry.

PORTEFEULJE OPSOMMING

Die Geïntegreerde Plaag Beheer (IPM) Portefeulje bestaan uit drie programme: Valskodlingmot (VKM), Vrugtevlieë en Ander Plae. Hulle word onderskeidelik gekoördineer deur Sean Moore, Aruna Manrakhan en Tim Grout. Ten minste elke tweede jaar vra CRI se voorligtingspan die sitrusprodusente se opinies oor hul navorsingsbehoeftes en prioriteite, ŉ proses wat streek vir streek uitgevoer word. Dit word met CRI se kennis oor mark toegang en dreigende biosekuriteit bedreigings gekombineer. Navorsing voorstelle word finaal gekeur deur spesialis navorsing komitees met sterk CGA en sitrus tegniese verteenwoordiging. Deur hierdie proses is die hoof navorsingsprioriteite binne die IPM Portefeulje steeds VKM en vrugtevlieë. Die belangrikheid van witluis navorsing het egter oor die laaste paar seisoene toegeneem, veral met verhoogde vlakke in die veld en verhoogde onderskeppings van besmette vrugte bestem vir die Suid Korea mark. Daarbenewens het fokus op die dreigende aankoms van die Asiatiese sitrus bladvlooi, en die vermoë daarvan om die verwoestende vergroening siekte te vektor, die aandag tot hierdie plaag geskuif in die laaste paar jaar. Bo oor hierdie drukkende navorsingsprioriteite is die verhoogde eise, veral deur Europese markte en kleinhandelaars, vir verlaagde vlakke en aantal residue. Begrip vir die potensiële konflik tussen toenemende onverdraagsaamheid vir fitosanitêre plae en siektes en geskende vrugte aan die een kant, en toenemende mark onverdraagsaamheid vir chemiese residue aan die ander kant, was die aanvraag deur die sitrus bedryf vir ŉ sterk fokus op navorsing vir biologiese alternatiewe groter in 2019 as ooit vantevore. CRI het altyd die skrif aan die muur gesien en het die fokus op biologiese beheer geprioritiseer, maar verhoog dit verder in reaksie tot die mark druk en produsent versoeke.

In die eerste jaar (2018) van die implementering van die Valskodlingmot risiko bestuur stelsel (die FMS) vir sitrus uitvoere tot die EU se toepassing, is nege onderskeppings van lewendige VKM in Suid-Afrikaanse sitrus aangemeld in die EU. Alhoewel dit as ŉ oorweldigende sukses van die program beskou kan word, met in ag name van die 800 000 ton sitrus uitgevoer na Europa daardie jaar, was dit nogsteeds nodig om strenger vereistes tot die FMS aan te bring om die moontlikheid vir die onderskepping van lewendige VKM in besendings te verlaag. Dit is gedoen, maar ongelukkig was die uitkoms nie tot wense is nie, aangesien onderskeppings toegeneem het tot 19 gedurende die 2019 uitvoer jaar. Gevolglik is verdere strengheid tot die FMS gevoeg vir die 2019/20 seisoen, wat hopelik ‘n positiewe effek in die mark sal hê in die daaropvolgende uitvoer seisoen. Dit is ondersteun deur betekenisvolle verbeteringe in die gedokumenteerde FMS, soos gepubliseer in ŉ wetenskaplike artikel en voorgelê tot die Europese Kommissie vroeg in 2020.

Gevolglik het VKM die top entomologiese en mark toegang prioriteit gebly gedurende die laaste navorsingsiklus. Binne die VKM program is 17 verskillende projekte gedoen, waarvan 12 vooroes kwessies geadresseer het, en vyf na-oes projekte was. Navorsing binne die program het gefokus op die verbetering van SIT en kwaliteitsbeheer proewe vir SIT, verbetering van doeltreffendheid van insek virusse teen VKM, die rol van boord nette in VKM beheer, sinergisme tussen verbindings en mikrobes vir verbeterde VKM beheer, redes vir verminderde VKM druk in organiese boerdery, en VKM ekologie in die warm noordelike dele. Sommige van die hoogtepunte van die navorsing gedoen binne die program is die suksesvolle seleksie vir ŉ CrleGV virus isolaat wat bestand is teen UV-betraling, ontwikkeling van ŉ nuwe spermatofoor oordrag-gebaseerde gehalte beheer (QC) metode vir SIT, die aantoon van sinergisme tussen gis en virus vir verbeterde virulensie teen VKM, die doeltreffendheid van ŉ na-oes kombinasie behandeling van CO_2 ontsmetting gevolg deur ŉ gedeeltelike koue behandeling, en die sensitiwiteit van die SIFT-MS toestel vir potensiële na-oes opsporing van besmette vrugte.

Saam met die FMS, vir die invoer van vrugte tot die EU, is die FFMS (Vrugtevlieg beheer stelsel vir die uitvoer van vars sitrus geproduseer in Suid-Afrika) geïmplementeer vir die eerste keer, as gevolg van die onlangse regulasie van nie-Europese vrugtevlieg spesies as fitosanitêre organismes. Daar was slegs drie onderskeppings van lewendige vrugtevlieg larwes in Suid-Afrikaanse sitrus in Europa gedurende die 2019 uitvoer seisoen. Aangesien daar geen verdraagsaamheid vir fitosanitêre plae is en as gevolg van die teenwoordigheid van vrugtevlieg spesies in Suid-Afrika wat nie in die uitvoer marke voorkom nie, het navorsing binne die program ŉ hoë prioriteit gebly. Vooroes projekte is gerig tot die verbetering van monitering, doeltreffendheid en optimalisering van lokstasies en massa vangste lokvalle, en meer ekologiese projekte, soos die relatiewe verspreiding en voorkoms van spesies soos die Natal-vlieg, Kaapsevlieg en die maroelavlieg. Na-oes projekte het gefokus op koue behandelings vir die verskillende vrugtevlieg spesies.

Hoogtepunte in die program sluit die bevinding dat Medvlieg meer koud verdraagsaam is as die ander vrugtevlieg plae op sitrus, wat moontlik dui daarop dat toekomstige koue behandelings slegs met Medvlieg gedoen kan word; en die ander bevinding is dat een van die warmer temperature wat in die FMS gebruik is, 3.5°C, voldoende is vir die gebruik in die FFMS. Een van die sleutel uitdagings binne die program is die moeilikheid met massateling van die Kaapsevlieg, om sodoende laboratorium navorsing te doen, maar heel belangrikste, koue behandeling studies.

Waarskynlik twee van die mees belangrikste plae gedek onder die Ander Plae program was die Asiatiese sitrus bladvlooi (ACP) en witluis. Navorsingsprojekte binne die program het egter ook die volgende ingesluit: beheer van platmyt gedek het, as ŉ vektor van Leprose, beheer van fitosanitêre myte ingevoer op knophout, opnames vir oleander dopluis, die effek van nette op plaagbeheer, en omgewingsbeperkings van entomopatogeniese swamme en potensiële oplossings daarvoor. Hoogtepunte binne die program was die ontdekking dat UV-bestraling die grootste hindernis is tot die effektiwiteit van entomopatogeniese swamme vir bogrondse plae, bewyse van die effek van nette op verskeie sitrus plae, waarskynlik die mees belangrikste, die ontdekking in twee verskillende projekte dat sitrus blaaspootjie skade betekenisvol minder onder nette is. Dit kan aandui dat alhoewel die vlakke van verskeie plae hoër is onder nette, kan nette voordelig wees vir die implementering van IPM, aangesien die sleutel IPM plae minder van ŉ probleem is.

Dit is ook belangrik om te beklemtoon dat die navorsing wat in hierdie verslag gerapporteer word, nie net deur die CRI IPM navorsing span uitgevoer is nie, maar was ook moontlik gemaak deur verskeie medewerkers, soos Rhodes Universiteit, Pretoria Universiteit, Stellenbosch Universiteit en sommige internasionale medewerkers.

Gedurende die afgelope jaar was CRI se navorsingsentomoloë ook baie aktief in die oordrag van tegnologie tot die produsente, in die vorm van IPM en siektebestuur lente werkswinkels, die voorverpakkingseisoen na-oes wekswinkels en verskeie ander kleiner forums. Dit was uiters belangrik, veral vir die kommunikasie van kritiese boodskappe, soos die FMS, die FFMS, Leprose beheer en die bedreiging van ACP en HLB. Verskeie Snykante op belangrike onderwerpe is aan produsente vrygestel, so ook artikels in die SA Vrugte Joernaal. Daarbenewens het CRI navorsingsentomoloë deelgeneem aan sekere plaaslike en internasionale wetenskaplike byeenkomste, soos die Kongres van die Entomologiese Vereniging van Suider Afrika, die International Invertebrate Pathology and Microbial Control Congress en die Postharvest Measures Research Group. Daarbenewens is verskeie artikels gepubliseer in wetenskaplike eweknie-beoordeelde joernale.

Die IPM Portefeulje span is vol vertroue en voorbereid om hulle navorsing kapasiteit en impak drasties te vergroot in ŉ omgewing van verhoogde befondsing in 2021, te danke aan die voorsorg van die sitrusbedryf leierskap en ondersteuning van alle sitrus produsente. Dit sal ŉ sterk invloed hê op die Suid-Afrikaanse sitrusbedryf se vermoeë om aan die voorpunt te bly van IPM navorsing en die implementering daarvan in die sitrus bedryf wêreldwyd.

3.2 PROGRAMME: FALSE CODLING MOTH

Programme coordinator: Sean D Moore (CRI)

3.2.1 Programme summary

False codling moth (FCM) remains the most important phytosanitary and market access pest in the citrus industry in South Africa. With the regulation of this pest by the European Union (EU) a couple of years ago, around 75% of South Africa's export volumes are now affected by such regulations and need to comply with stringent export protocols. Consequently, 17 research projects were dedicated to improving our understanding and management of FCM in the last research cycle. Of these, 12 investigated factors associated with preharvest management of FCM, whereas five investigated postharvest issues.

One of the mainstays of the FCM control programme in the field, for many years now, is the use of granulovirus. Three of the research projects specifically investigated means to improve the efficacy of virus products. The first successfully selected for a UV-resistant isolate of CrleGV, with approximately 1000-fold improved virulence relative to the wild-type virus, after five selection cycles (3.2.2). The genetic changes associated with this UV-resistance were also identified. The second project studied the synergism between mutualistic yeasts, found to be naturally associated with FCM larvae, and CrleGV, showing not only elevated virulence, but also that the yeasts may act as female attractants and oviposition stimulants (3.2.17). Lastly, in the virus-related projects, a relatively new project sought to improve the efficacy of virus through two mechanisms: serial passage of a virus (in this case, CrpeNPV) through a heterologous host (FCM) to select for more virulent genotypes; and synergism between two viruses i.e. CrleGV and CrpeNPV (3.2.18). Both approaches are showing early potential.

One of the cornerstones of FCM management are the area-wide techniques for control, namely the sterile insect technique (SIT) and mating disruption. Two projects specifically investigated means for the improvement of the quality of moths used in the SIT programme and for quality control testing of these moths. The first study demonstrated that the addition of a cryoprotectant to the larval diet can significantly improve recaptures of sterile moths in a large field trial during winter (3.2.11). This finding was confirmed by a significantly reduced critical thermal minimum (CTmin) for these moths. Additionally, a significant regression was found between the transfer of spermatophores to females in laboratory cages and mating incidents in field cages, indicating that spermatophore transfer could be used as a quality control measure for sterile male moths. In the second SIT project, anoxia was show to not be a viable alternative to cooling for immobilisation or recovery of sterile moths (3.2.12). Sterility trials are also being conducted to determine if premating of moths prior to irradiation has a negative impact on the efficacy of irradiation, and also to determine whether activity duration irradiation has a similar effect.

Remaining on the theme of semiochemical technologies, one of the FCM projects investigated attractiveness and mating compatibility between males and females from different regional origins (3.2.5). When given a choice, males were indeed significantly more attracted to females from their own populations. This carried over into mating preference. However, when not given a choice, this difference disappeared. Implications for semiochemical based technologies, such as SIT, mating disruption, attract and kill and monitoring, must be investigated. Another semiochemical oriented project, investigated attractants for FCM adult females (3.2.10). To date, no notable attractant has been identified.

Four of the projects in the FCM programme were largely field based. The first was the investigation of the effect that shade netting over citrus orchards would have on FCM pressure (3.2.3). Contrary to hypothesis, it was found that FCM pressure may actually be higher under nets. However, SIT seemed to be more effective under nets, meaning that FCM suppression under nets may be superior to outside of nets over time, potentially leading to better control of FCM under nets. All other pests found in the netted and open orchards were also monitored. The second field project, investigated FCM control in the field, using a new semi-field bioassay protocol (3.2.13). Results were still pending. The third field project entails monitoring of FCM in the field in the warmer northern regions of Limpopo, along with temperature monitoring (3.2.15). It has thus far been determined that temperatures in this region begin to warm up from early July, leading to an increase in FCM activity from that time. Consequently, it may be beneficial to initiate control measures, particularly mating disruption, much earlier in this area. The final, largely field-based project, is investigating reasons for differences in FCM pressure in organic versus conventional farming scenarios (3.2.16). The following comparisons are being made: FCM activity, egg parasitism, entomopathogenic fungi and nematodes in the soil, soil predators using pitfall traps, and fruit preference and susceptibility tests. As this is the first year of the project, results are still pending.

Of the postharvest projects, two investigated detection techniques for identifying infested fruit, whereas the other three evaluated the efficacy of different postharvest disinfestation strategies. The first detection project used the ability of a sniffer dog to identify infested fruit (3.2.6). This followed on from a successful first phase, where the dog was demonstrated to be approximately 98% effective in identifying infested fruit. The objective of this second phase was to add a remote component to the detection. Unfortunately, the project was cancelled after one year, due to circumstances out of our hands and thus little further progress was made. The second detection project analysed headspace volatiles from infested fruit relative to healthy fruit and identified some significantly elevated volatiles and particularly ratios of volatiles (3.2.9). A Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) unit was shown to be able to very rapidly detect damaged fruit within 24 h of being damaged.

The first postharvest disinfestation project used cold treatment, either for complete disinfestation or partial efficacy as part of a systems approach (3.2.7). Trials at 4.5 and 5°C for different durations were completed. Another project looked at combining such partial cold treatments with prior fumigation of infested fruit with CO_2 (3.2.4). A post-fumigation cold treatment of 14 days at 2°C led to 99.92% mortality of FCM fifth instars, which increased to 99.99% when one included post-cold mortality. The final postharvest treatment project was to have examined the efficacy of hot air treatments for disinfestation of fruit (3.2.8). However, progress on this project was unfortunately not possible, due to other ad hoc priorities.

Programopsomming

Valskodlingmot (VKM) bly een van die mees belangrike fitosanitêre en marktoegang plae in die sitrusbedryf in Suid-Afrika. Met die regulering van die plaag deur die Europese Unie (EU) ʼn paar jaar gelede, word ongeveer 75% van Suid-Afrika se uitvoer volumes geaffekteer deur sulke regulasies en moet voldoen aan streng uitvoer protokolle. Gevolglik is 17 navorsingsprojekte in die laaste navorsingsiklus toegewy om ons begrip en bestuur van VKM te verbeter. Twaalf van hierdie het faktore ondersoek wat verwant is met vooroes bestuur van VKM, terwyl vyf na-oes kwessies ondersoek het.

Een van die staatmakers van die VKM beheer program in die veld, vir baie jare, is die gebruik van ʼn granulovirus. Drie van die navorsingsprojekte het spesifiek maniere om die doeltreffendheid van virus produkte te verbeter ondersoek. Die eerste een het ʼn UV-weerstandbiedende isolaat van CrleGV suksesvol geselekteer, met ongeveer 1000-voud verbeterde virulensie relatief tot die wilde-tipe virus, na vyf seleksie siklusse (3.2.2). Die genetiese veranderinge geassosieer met UV-weerstand is ook geïdentifiseer. Die tweede projek het sinergisme tussen mutualistiese gisse, wat natuurlik met VKM larwes geassosieer word, en CrleGV bestudeer, wat nie net verhoogde virulensie getoon het nie, maar ook dat gisse kan optree as ʼn wyfie lokmiddel en eierlegging stimulant (3.2.17). Laastens in die virus verwante projekte, het ʼn betreklike nuwe projek gepoog om die doeltreffendheid van virusse deur twee meganismes te verbeter: reeks deurgang van ʼn virus (in hierdie geval, CrpeNPV) deur ʼn heteroloë gasheer (VKM) om vir meer virulente genotipes te selekteer; en sinergisme tussen twee virusse, byvoorbeeld CrleGV en CrpeNPV (3.2.18). Beide benaderinge toon vroeë potensiaal.

Een van die hoekstene van VKM bestuur is die area wye tegnieke vir beheer, naamlik die steriele insek tegniek (SIT) en paringsontwrigting. Twee projekte het spesifieke maniere ondersoek vir die verbetering van die kwaliteit van motte wat in die SIT program gebruik word, en vir kwaliteit beheer toetse van hierdie motte. Die eerste studie het getoon dat die byvoeging van ʼn kouebeskermer tot die larwe dieet kon die hervangs van steriele motte in ʼn groot veld proef gedurende winter betekenisvol verbeter (3.2.11). Die bevinding is bevestig deur ʼn betekenisvolle verlaagde kritiese termiese minimum (CTmin) vir hierdie motte. Daarbenewens is ʼn betekenisvolle regressie gevind tussen die oordrag van spermatofore tot wyfies in laboratorium hokke en parings gevalle in veld hokke, wat aandui dat spermatofoor oordrag gebruik kan word as ʼn kwaliteit kontrole maatreël vir steriele mannetjie motte. In die tweede SIT projek het anoksie aangetoon dat dit nie ʼn geskikte alternatief is vir verkoeling vir immobilisasie of vir herstel van steriele motte nie (3.2.12). Steriliteit proewe word ook uitgevoer om vas te stel of paring van motte voor bestraling ʼn negatiewe impak op die doeltreffendheid van bestraling het, en ook om vas te stel of aktiwiteit gedurende bestraling ʼn soortgelyke effek het.

Blywend op die tema van semiochemiese tegnologieë, het een van die VKM projekte die aanloklikheid en parings-verenigbaarheid tussen mannetjies en wyfies van verskeie plaaslike oorspronge ondersoek (3.2.5). Wanneer daar ʼn keuse is, was die mannetjies inderdaad betekenisvol meer aangetrokke tot wyfies van hul eie populasie. Dit word oorgedra tot hul parings voorkeur. Alhoewel, wanneer daar nie ʼn keuse is nie, verdwyn die verskil. Implikasies vir semiochemies gebaseerde tegnologieë, soos SIT, parings ontwrigting, lok-en-vrek en monitering moet ondersoek word. Nog ʼn semiochemies georiënteerde projek het lokmiddels vir VKM volwasse wyfies ondersoek (3.2.10). Tot op datum is nog geen noemenswaardige lokmiddels geïdentifiseer nie.

Vier van die projekte in die VKM program was grootliks veld-gebaseer. Die eerste was die ondersoek van die effek wat skadu net oor sitrus boorde sal hê op VKM druk (3.2.3). Inteendeel tot die hipotese, is daar gevind dat VKM druk eintlik hoër onder nette mag wees. Alhoewel, het SIT meer doeltreffend geblyk onder nette, wat beteken dat VKM onderdrukking onder nette oor tyd beter mag wees as buite die nette, wat potensieel tot beter beheer van VKM onder nette kan lei. Alle ander plae gevind onder die nette en oop boorde is ook gemonitor. Die tweede veld projek het VKM beheer in die veld ondersoek deur gebruik van ’n nuwe semi-veld biotoets protokol (3.2.13). Resultate is steeds hangend. Die derde veld projek behels die monitering van VKM in die veld in die warmer noordelike gebiede van Limpopo, saam met temperatuur monitering (3.2.15). Daar is sover vasgestel dat temperature in die gebiede begin opwarm vanaf vroeg Julie, wat lei tot ’n toename in VKM aktiwiteite vanaf daardie tyd. Daarbenewens, kan dit voordelig wees om beheer maatreëls, veral parings ontwrigting, baie vroeër in die gebied te begin. Die finale, grootliks veld gebaseerde projek ondersoek die redes vir verskille in VKM druk in organies vs. konvensionele plaas situasies (3.2.16). Die volgende vergelykings word gemaak: VKM aktiwiteit, eier parasitisme, entomopatogeniese swamme en nematodes in die grond, grond roofdiere deur gebruik van slaggate, en vrugte voorkeur en vatbaarheidstoetse. Aangesien dit die eerste jaar van die projek is, is resultate steeds hangend.

Van die na-oes projekte, ondersoek twee van hulle opsporings tegnieke vir die identifisering van besmette vrugte, waar die ander drie die effektiwiteit van verskillende na-oes ontsmetting strategieë evalueer. Die eerste opsporings projek gebruik die vermoë van ’n snuif hond om besmette vrugte te identifiseer (3.2.6). Dit was ’n opvolg van die suksesvolle eerste fase, waar die hond gedemonstreer het om 98% effektief te wees om besmette vrugte te identifiseer. Die doel van die tweede fase was om ’n afgesonderde komponent tot die opsporing te voeg. Ongelukkig is die projek na een jaar gekanselleer as gevolg van omstandighede buite ons beheer, en is daar min vordering gemaak. Die tweede opsporing projek het die kopruimte vlugtige stowwe van besmette vrugte relatief tot gesonde vrugte geanaliseer en betekenisvolle verhoogde vlugtige stowwe en veral verhoudings van vlugtige stowwe geïdentifiseer (3.2.9). ’n Geselekteerde Ioon Vloei Buis Massa Spektrometrie (SIFT-MS) eenheid het getoon dat dit baie vinnig beskadigde vrugte kan opspoor binne 24 h nadat dit beskadig is.

Die eerste na-oes ontsmetting projek gebruik koue behandeling, of vir volkome ontsmetting of vir gedeeltelike effektiwiteit as deel van ’n stelselsbenadering (3.2.7). Proewe by 4.5°C en 5°C vir verskeie tydperke is voltooi. Nog ’n projek het gekyk na die kombinering van gedeeltelike koue behandelings met voorafgaande ontsmetting van besmette vrugte met CO_2 (3.2.4). ’n Na-ontsmetting koue behandeling vir 14 dae by 2°C het gelei tot 99.92% mortaliteit van VKM vyfde instars, wat toegeneem het tot 99.99% wanneer na-koue mortaliteit ingesluit word. Die finale na-oes behandeling projek was om die effektiwiteit van warm lug behandelings vir vrugte te ondersoek (3.2.8). Vordering op die projek was egter ongelukkig nie moontlik nie, as gevolg van ander ad hoc prioriteite.

3.2.2 FINAL 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

Baculoviruses are susceptible to the ultraviolet (UV) radiation component of sunlight and lose their activity within hours to a few days, after exposure to UV. Several substances have been tested as UV protectants to improve the persistence of baculovirus biopesticides in the field. These include optical brighteners, UV absorbers and anti-oxidants. While very promising in the laboratory, UV-protectants have not been as successful in the field. A few published reports have reported that UV-tolerant baculoviruses could be isolated from a population by repeatedly exposing and re-exposing the virus to UV irradiation with a propagation step in insect host fourth or fifth instars between each exposure cycle. In this study, the South African isolate of Cryptophlebia leucotreta granulovirus (CrleGV-SA) was exposed to UV irradiation for 5 exposure cycles in a Q-Sun Xe-3 HC test chamber (Q-lab, USA) with parameters set to mimic a typical summer day in the Sundays River Valley, Eastern Cape Province. In between exposures the virus survivors were allowed to multiply in FCM fifth instars. Surface dose bioassays were also conducted to determine the LC_{50} of the virus after each exposure cycle. Samples from exposure Cycle 1 and Cycle 5 (UV-tolerant) irradiated for 72 h were prepared for Next Generation Sequencing (NGS) of DNA. The resultant sequence data were analysed using the Geneious R11 software and compared with the unexposed CrleGV-SA sequence. In-silico restriction enzyme analysis (REN) with several enzymes was also carried out on both the Cycle 1 and Cycle 5 exposed samples

and the resulting digestion patterns were compared with the original CrleGV-SA digestion patterns. The same samples were also analysed by transmission electron microscopy (TEM) and Attenuated Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) to evaluate the effect of UV irradiation on the structure of the CrleGV-SA OB. In addition, three UV protectants, lignin sulphate, Break-Thru OE446 (OE446) and Uvinul Easy were prepared with CrleGV-SA to give final protectant concentrations of 0.09%, 0.9% and 9%. The protectant-virus suspensions were exposed to UV for 24 h in the Q-Sun test chamber and bioassays conducted to determine the protective effect of each protectant concentration. The most successful protectants were then combined with the UV-tolerant CrleGV-SA and exposed to UV for 24 h in the Q-Sun test chamber and surface dose bioassays conducted afterwards. Samples exposed to UV in Cycle 5 had lower LC_{50} values compared to samples in the early cycles. With each re-exposure cycle the LC_{50} values moved closer to that of the unexposed control. The LC_{50} of virus samples decreased from 2.89 x 10^8 OBs/ml after 24 h UV-exposure in Cycle 1 to 2.16 x 10^5 OBs/ml after the same duration of exposure in Cycle 5; and from 2.11 x 10^9 OBs/ml in Cycle 1 after 72 h UV-exposure to 1.73 x 10^6 OBs/ml after the same duration of exposure. This represented a 1338-fold difference and a 1220-fold difference, respectively. When the UV-tolerant samples were sequenced seven SNPs were identified in Cycle 1, which were thought to help establish UV tolerance, while a further seven SNPs were identified in Cycle 5 samples; these were thought to further establish and maintain the UV-tolerance. Additionally, REN analysis with EcoR1 for both test samples yielded digestion patterns that were different from those of the original CrleGV-SA. TEM data showed that UV damages the virion as well as the crystalline structure of the OB. This is the first time visual evidence for UV damage to baculoviruses has been published. Comparison of Cycle 1 and Cycle 5 UV exposed OBs revealed that the Cycle 5 OBs were significantly larger than the Cycle 1 OBs ($P<0.05$). In addition, several peaks in the fingerprint region were shown to have either appeared or disappeared from the ATR-FTIR spectra after UV irradiation. However, there was no difference in the spectra of the Cycle 1 and Cycle 5 virus samples. The tests with potential UV-protectants revealed that the 0.9% lignin, 9% OE446 and 9% Uvinul Easy were the most effective in protecting the virus from UV. However, there was no significant difference in their protection of UV tolerant CrleGV-SA and wild type CrleGV-SA. Going forward, it is recommended that the 0.9% lignin, 9% OE446 and 9% Uvinul Easy combinations be explored further in future studies, particulary in the field. This study therefore forms an important foundation for the development of UV-tolerant baculovirus that will last longer in the field.

Opsomming

Bakulovirusse is vatbaar vir die ultraviolet (UV) bestraling komponent van sonlig en verloor hul aktiwiteit binne ure tot ŉ paar dae na blootstelling tot UV. Verskeie stowwe is getoets as UV beskermers om die volharding van bakulovirus bio-insekdoders in die veld te verbeter. Dit sluit optiese verhelderers, UV absorbeerders en anti-oksidante in. Alhoewel hierdie baie belowend in die laboratorium was, was UV beskermers nie in die veld suksesvol nie. ŉ Paar gepubliseerde verslae het berig dat UV bestande bakulovirusse geïsoleer kan word vanaf ŉ populasie deur die herhaaldelike blootstelling en herblootstelling van die virus aan UV bestraling met ŉ voortplantings stap in die vierde en vyfde instar insek gasheer tussen elke blootstelling siklus. In hierdie studie is die Suid-Afrikaanse isolaat van Cryptophlebia leucotreta granulovirus (CrleGV-SA) blootgestel aan UV bestraling vir 5 blootstelling siklusse in ŉ Q-SUN Xe-3HC Xenon Test Chamber (Q-lab, USA) met parameters gestel om ŉ tipiese somers dag in die Sondagsrivier Vallei, Oos-Kaap, na te boots. Tussen blootstellings is die virus oorlewendes toegelaat om te vermeerder in VKM vyfde instars. Oppervlak dosis biotoetse is ook gedoen om vas te stel wat die LC_{50} van die virus is na elke blootstelling siklus. Monsters van blootstelling Siklus 1 en Siklus 5 (UV bestand), bestraal vir 72 ure, is voorberei vir Next Generation Sequencing (NGS) van DNS. Die gevolglike nukleotied volgorde data is ontleed deur die Geneious R11 sagteware te gebruik en te vergelyk met die nie blootgestelde CrleGV-SA nukleotied volgorde. In-silico restriksie ensiem analise (REN) met verskeie ensieme is ook uitgevoer op beide die Siklus 1 en Siklus 5 blootgestelde monsters en die gevolglike spysverterings patrone is vergelyk met die oorspronklike CrleGV-SA spysverterings patrone. Dieselfde monsters is ook geanaliseer deur transmissie elektron mikroskopie (TEM) en Verswakte Weerkaatsing Fourier Transformeer Infrarooi Spektroskopie (ATR-FTIR) om die effek van UV bestraling op die struktuur van die CrleGV-SA okklusie liggame (OB) te evalueer. Daarbenewens, is drie UV beskermers, lignien sulfaat, Break-Thru OE446 en Uvinul Easy voorberei saam met CrleGV-SA om ŉ finale beskermer konsentrasie van 0.09%, 0.9% en 9% te gee. Die beskermer-virus suspensie is blootgestel aan UV vir 24 ure in die Q-Sun toets kamer en biotoetse gedoen om die beskermings effek van elke beskermer konsentrasie te bepaal. Die mees suksesvolle beskermers is gekombineer met die UV-bestande CrleGV-SA en blootgestel

aan UV vir 24 ure in die Q-Sun toets kamer en oppervlak dosis biotoetse daarna gedoen. Monsters blootgestel tot UV in Siklus 5 het ŉ laer LC_{50} waarde gehad in vergelyking met monsters in vroeër siklusse. Met elke herblootstelling siklus het die LC_{50} waarde nader aan die van die nie-blootgestelde kontrole beweeg. Die LC_{50} van die virus monster het afgeneem van 2.89 x 10^8 OBs/ml na 24 ure UV blootstelling in Siklus 1 tot 2.16 x 10^5 OBs/ml na dieselfde blootstellings tydperk in Siklus 5; en van 2.11 x 10^9 OBs/ml in Siklus 1 na 72 ure UV blootstelling tot 1.73 x 10^6 OBs/ml na dieselfde blootstellings tydperk. Dit verteenwoordig ŉ 1338-voud verskil en ŉ 1220-voud verskil, onderskeidelik. Toe die UV bestande monsters se nukleotied volgordes bepaal is, is sewe SNPs geïdentifiseer in Siklus 1 wat vermoedelik die UV bestandheid help vestig. Terwyl ŉ verdere sewe SNPs geïdentifiseer is in Siklus 5 monsters, vermoedelik om die UV bestandheid verder te vestig en te handhaaf. Daarbenewens het REN analises met EcoR1 vir beide die toets monsters spysverterings patrone opgelewer wat anders is as die oorspronklike CrleGV-SA. TEM data het getoon dat UV beskadig die virion asook die kristallyne struktuur van die OB. Dit is die eerste keer dat visuele bewyse vir UV skade tot bakulovirusse gepubliseer is. Vergelyking tussen Siklus 1 en Siklus 5 UV blootgestelde OBs het getoon dat Siklus 5 OBs aansienlik groter is as die Siklus 1 OBs ($P<0.05$). Boonop is getoon dat verskeie pieke in die vingerafdruk streek of verskyn of verdwyn vanaf die ATR-FTIR spektrum na UV bestraling. Daar was geen verskil in die spektrum van die Siklus 1 en Siklus 5 virus monsters nie. Die proewe met die potensiële UV-beskermers het getoon dat die 0.9% lignien, 9% OE446 en 9% Uvinul Easy die mees effektief was om die virus te beskerm teen UV. Daar was geen beduidende verskil in hul beskerming van UV-bestande CrleGV-SA en wilde tipe CrleGV-SA. Dit word aanbeveel dat die 0.9% lignien, 9% OE446 en 9% Uvinul Easy kombinasies verder ondersoek word in toekomstige studies, veral in die veld. Hierdie studie vorm dus ŉ belangrike fondasie vir die ontwikkeling van UV tolerante bakulovirus wat langer in die veld sal hou.

Introduction

The use of baculoviruses as biopesticides continues to gain popularity as they provide a more advantageous approach to combat crop pests than chemical pesticides. As such they have become important in the agricultural economy. The Cryptophlebia leucotreta granulovirus (CrleGV) has been formulated as a biocontrol agent against the false codling moth (FCM), and is registered for use as a viral biopesticide on citrus crops in South Africa (Moore 2002, Moore *et al*., 2015). The virus is ingested by the moth larva and replicates within the larva, killing it. A defined minimum concentration of the viral biopesticide must be maintained on the trees to kill the moth larvae and prevent fruit damage (Moore *et al*, 2010).

One of the major setbacks with the use of baculoviruses as biopesticides is their susceptibility to ultraviolet radiation (UVA and UVB) from the sun. Exposure to UV light damages the virus DNA and thus kills the virus. The effect of sunlight increases with duration of exposure (Mwanza, 2015). However, UV susceptibility varies with virus species.

In his CRI-funded MSc project (Mwanza, 2015), and his PhD research, Patrick Mwanza has found that CrleGV has reduced infectivity following exposure to UVA, UVB and combined UVA and UVB in laboratory experiments simulating field conditions, and following sunlight exposure in field studies. He observed in his masters' study that despite the length of UV exposure there was always residual virus activity. It was hypothesized that UV-resistant CrleGV spontaneously arises following prolonged UV exposure. As a result, this study investigated the existence of UV-resistant CrleGV-SA. It has been previously reported that it is possible to select for UV-resistant baculoviruses by repeatedly exposing them to UV and propagating the survivors in larvae (Brassel & Benz 1979; Shapiro & Bell, 1984; Jeyarani *et al*., 2013). A selection process, involving repeated exposure to UV light and selection of those viruses that retain infectivity, was carried out in the laboratory. The isolated UV-resistant CrleGV-SA was tested for increased infectivity compared to the original population, to determine the extent of this resistance. In addition to dose response bioassays we investigated the potential mechanisms of UV-resistance by genomic sequencing and aligning the sequences of both the wild type and the resistant CrleGV-SA to identify affected genes that either confer structural protection against UV or improved repair of UV-generated DNA damage. In addition to investigating the UV-resistant virus population, we tested three selected UV-protectants for efficacy in maintaining the CrleGV-SA virulence after UV exposure.

Data obtained in this study will be crucial in the development of a new commercial UV-resistant CrleGV-SA biopesticide. Such a biological control agent would have improved and prolonged activity, reducing reapplication costs. This will be a benefit to current users of CrleGV biopesticides. In addition, it will encourage more citrus farmers to use the biopesticide alongside chemical pesticides, with benefits for the environment and for export of produce to markets with stringent environmental requirements.

This project was completed as a PhD study and the full thesis is available on request for full details of the study.

Stated objectives

A. Isolation of UV-resistant CrleGV from a laboratory source of CrleGV: Generation of UV-resistant CrleGV by exposure of purified virus to UVA and UVB light under laboratory conditions and analysis of structural changes in the resistant virus.

B. Genomic sequencing of UV-resistant CrleGV and comparison with original virus isolate, wild strains of CrleGV identified in South Africa and the commercial Cryptogran strain of CrleGV to identify possible mutations to the virus DNA associated with UV-resistance.

C. Testing and comparison of potential UV protectants: Determination of the efficacy of selected UV protectants.

D. Identification of the optimal combination of UV-resistant virus and UV protectant for commercial use.

Materials and methods

<u>Isolation of UV-resistant CrleGV-SA</u>

CrleGV-SA was purified and samples exposed to daylight equivalent UVA and UVB Irradiance (300 Wm^{-2}), with a Daylight-Q filter in a Q-SUN Xe-3HC Xenon Test Chamber (Q-LAB), which uses Xe-3 xenon arc lamps within a constant climate chamber to reproduce the damage caused by full-spectrum sunlight. The following conditions, which replicate those of citrus orchards during spraying season, were used: temperature of 30°C, relative humidity of 42%. Samples were exposed for time intervals from 60 minutes to 72 hours. This exposure cycle was repeated 5 times. The samples were exposed to UV, propagated in *T. leucotreta* fifth instars and re-exposed to UV. After each cycle surface dose response bioassays with 1st instar FCM were conducted to determine the change in LC_{50} and LC_{90} within each cycle and across different cycles (Fig. 3.2.2.1).

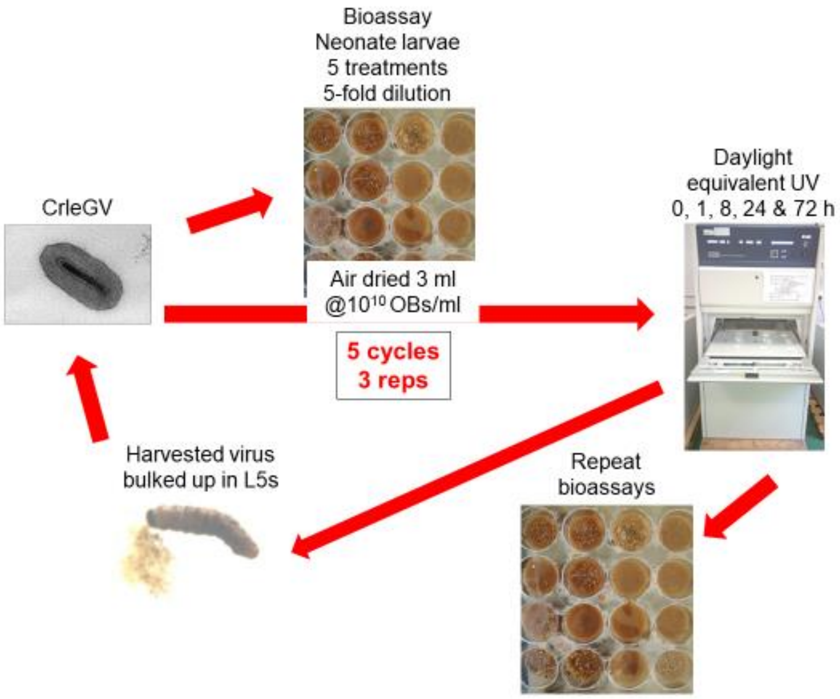

Figure 3.2.2.1. Protocol for isolation of UV-resistant CrleGV-SA.

Genetic changes in UV-resistant CrleGV-SA

Samples from exposure cycle 1 and cycle 5 (UV-tolerant) irradiated for 72 h were prepared for Next Generation Sequencing (NGS) of DNA. The resultant sequence data were analysed using the Geneious R11 software (New Zealand) and compared with the unexposed CrleGV-SA sequence. In-silico restriction enzyme analysis (REN) with several enzymes was also carried out on both the cycle 1 and cycle 5 exposed samples and the resulting digestion patterns were compared with the original CrleGV-SA digestion patterns.

Transmission Electron Microscopy

Analysis of structural changes to the UV-resistant isolates of CrleGV has been conducted using TEM: Samples exposed to UV in the test chamber have been analysed by TEM. Viral pellets were fixed in a Karnovsky fixative (2.4 % glutaraldehyde, 2% paraformaldehyde in 0.05 M, pH 7.2 phosphate buffer and 0.001M $CaCl_2$) for 2 hours, then in 0.5% OsO_4 in double distilled water for 1 hour and stained with 0.5% aqueous Uranyless, dehydrated in acetone, was embedded in Spur's low viscosity embedding medium. Sectioning was carried out in a Leica Ultra cut microtome (Leica, Germany). Post staining was done with 3% aqueous Uranyless and Reynold's lead citrate. The prepared sections were then examined in a JOEL JEM-2100 TEM (JOEL, Japan). Attenuated Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) was also used to evaluate the effect of UV irradiation on the structure of the CrleGV-SA OB.

UV Protectants

Three potential UV protectants were tested. These were combined individually with CrleGV-SA and irradiated with UV for 24 h in the Q-SUN Xe-3HC Xenon Test Chamber. Surface dose bioassays were conducted to determine if they provided protection to CrleGV-SA.

Results and discussion

Table 3.2.2.1. Task table

Objective / Milestone	Achievement
A.1. Isolation of UV-resistant CrleGV from a laboratory source of CrleGV	Five UV exposure cycles have been completed with their accompanying bioassays.
A.2. Analysis of structural changes to UV-resistant isolates	TEM analysis has been completed. Raman was not possible due to breakdown of equipment. However, Attenuated Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) was conducted instead.
B.1. Genomic sequencing of UV-resistant CrleGV and comparison with original virus isolates and wild isolates.	Genome sequencing and restriction enzyme analysis after Cycles 1 and 5 have been completed.
B.2. Bioinformatic comparison to identify any changes in DNA sequence that could confer UV resistance.	7 and 14 SNPs were identified in surviving CrleGV OBs after Cycle 1 and Cycle 5., respectively, in comparison with wild type CrleGV.
D. Testing and comparison of potential UV protectants and determination of the efficacy of selected UV protectants	3 different concentrations of three potential UV protectants have been exposed to UV in the Q-SUN Xe-3HC Xenon Test Chamber and bioassays were conducted to test their protective ability.

Bioassays were conducted on samples exposed to UV over various cycles. It was recorded that the LC_{50} values generally decreased from Cycle 1 to Cycle 5 after 8 h, 24 h and 72 h UV exposure (Table 3.2.2.2; Fig. 3.2.2.2). LC_{90} values recorded at 72 h also show a similar trend. This suggests successful isolation of UV-resistant virus. Probit line analysis shows that there is a significant difference between lines from Cycle 1 to Cycle 5.

Table 3.2.2.2. LC_{50} values determined from Probit analysis after bioassays

Cycle	Control (no UV)	8 h	24 h	72 h
1	2.37×10^4	4.73×10^7	2.89×10^8	2.11×10^9
2	2.37×10^4	4.93×10^7	1.19×10^8	1.50×10^9
3	2.37×10^4	4.26×10^7	1.15×10^5	8.18×10^6
4	2.37×10^4	1.22×10^7	4.13×10^5	6.12×10^6
5	2.37×10^4	6.38×10^6	2.16×10^5	1.72×10^6

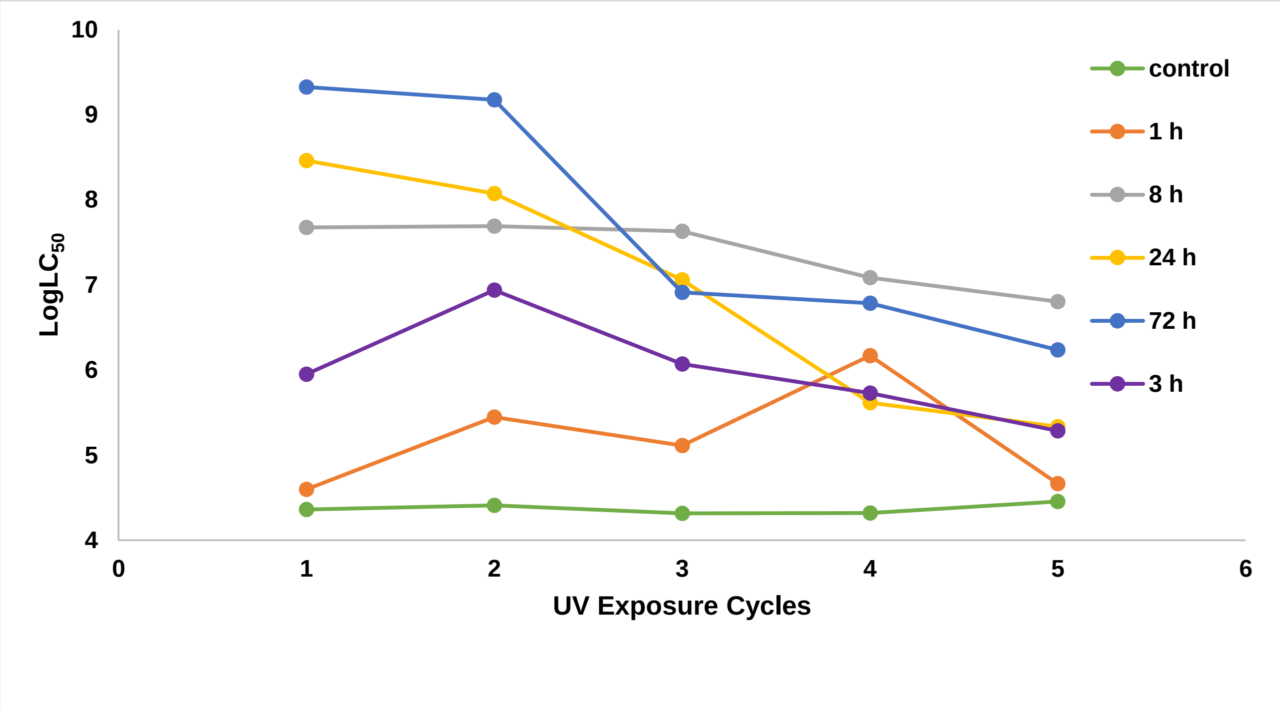

Figure 3.2.2.2. Change in LC_{50} at each UV exposure time point over five UV exposure cycles

Genetic changes in UV-resistant CrleGV-SA

In the genome sequence, seven SNPs were identified in Cycle 1 virus, which were thought to help establish UV tolerance, while a further seven SNPs were identified in Cycle 5 samples (Fig. 3.2.2.3; Table 3.2.2.3); these were thought to further establish and maintain the UV-tolerance. Additionally, REN analysis with EcoR1 for both test samples yielded digestion patterns that were different from those of the original CrleGV-SA.

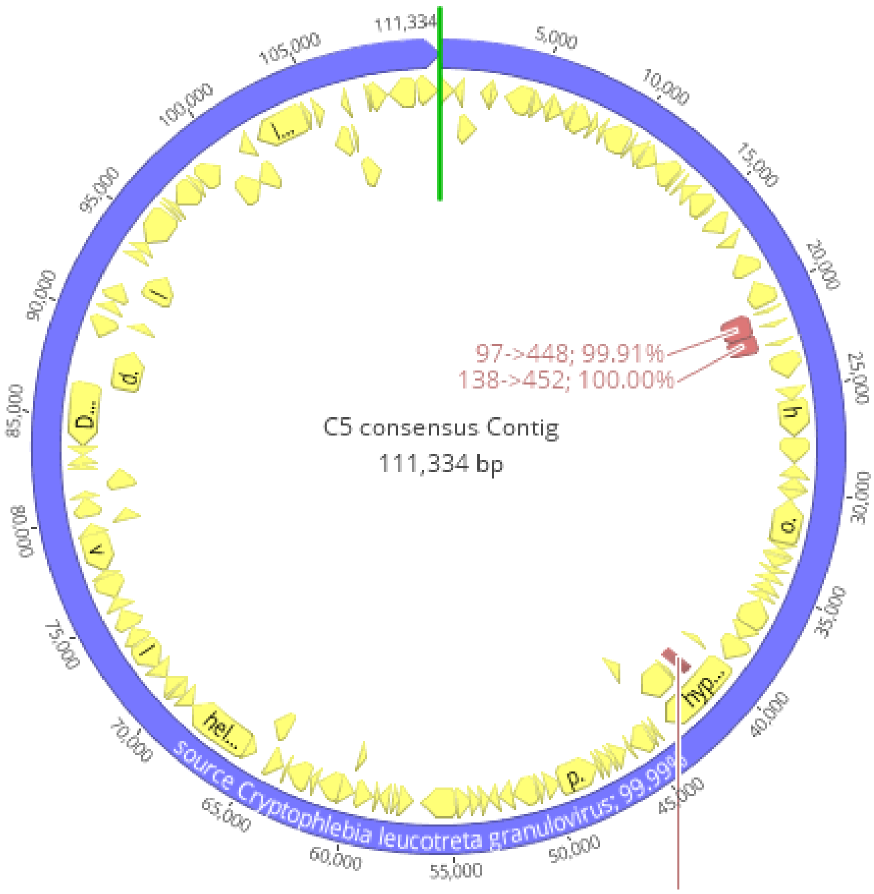

Figure 3.2.2.3. CrleGV-SA genome from UV exposure cycle 5 (C5) after annotation using the published CrleGV-SA sequence as the reference.

Table 3.2.2.3. Common SNPs detected in both of CrleGV-SA genomes from virus exposed to UV in cycle 1 and cycle 5

Name	Nucleotide position	Amino Acid Change	Change	Codon Change	Poly-morphism Type	Protein Effect	Protein
A	78522	S -> F	G -> A	TCT -> TTT	SNP (transition)	Substitution	Hypothetical CDS
C	59752	H -> Q	A -> C	CAT -> CAG	SNP(transversion)	Substitution	Hypothetical CDS
C	59752		A -> C		SNP(transversion)	Extension	Hypothetical CDS
C	59779	S -> G	T -> C	AGT -> GGT	SNP (transition)	Substitution	Hypothetical CDS
CC	59709	LI -> LV	TT -> CC	TTA,ATT -> TTG,GTT	Substitution	Substitution	DNA binding protein
T	13168	A -> V	C -> T	GCT -> GTT	SNP (transition)	Substitution	Hypothetical CDS
T	59734	D -> E	A -> T	GAT -> GAA	SNP(transversion)	Substitution	DNA binding protein

Transmission Electron Microscopy

TEM data showed that UV damages the virion as well as the crystalline structure of the OB. This is the first time visual evidence for UV damage to baculoviruses has been published (Fig. 3.2.2.4). Comparison of Cycle 1 and Cycle 5 UV exposed OBs revealed that the Cycle 5 OBs were significantly larger than the Cycle 1 OBs ($P<0.05$). In addition, several peaks in the fingerprint region were shown to have either appeared or disappeared from the ATR-FTIR spectra after UV irradiation. However, there was no difference in the spectra of the Cycle 1 and Cycle 5 virus samples.

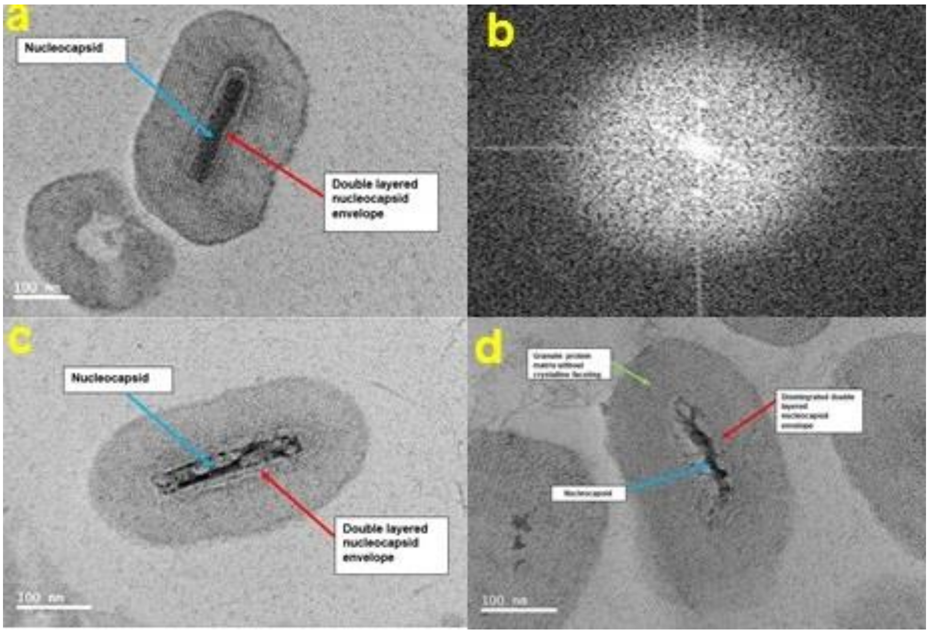

Figure 3.2.2.4. TEM SE section of CrleGV-SA OBs exposed to UV for 72 h in UV exposure cycle 5 indicating a) OB crystalline structure intact even after UV exposure and nucleocapsid (blue arrow) also intact as well as nucleocapsid envelope (red arrow); b) Damaged CrleGV-SA OB with the nuclear envelope (red arrow) still visible; the nucleocapsid (blue arrow) appears damaged; c) Damaged CrleGV-SA OB with the nuclear envelope (red arrow) still visible; the nucleocapsid (blue arrow) appears damaged and d) damaged OB with the nuclear envelope and virion damaged.

UV protectants

The tests with potential UV-protectants revealed that the 0.9% lignin, 9% OE446 and 9% Uvinul Easy were the most effective in protecting the virus from UV (Table 3.2.2.4). However, there was no significant difference in their protection of UV tolerant CrleGV-SA and wild type CrleGV-SA. Going forward, it is recommended that the 0.9% lignin, 9% OE446 and 9% Uvinul Easy combinations be explored further in future studies, particularly in the field. This study therefore forms an important foundation for the development of UV-tolerant baculovirus that will last longer in the field.

Table 3.2.2.4. The effect of selected UV-protectants on the LC_{50} of UV-resistant CrleGV-SA after UV-irradiation for 24 h

Time (h)	**LC_{50} (OBs/ml)**	**95% Fiducial limits**		**X^2**	**P**
		Lower	**Upper**		
Unirradiated CrleGV-SA C5	5.50×10^4	2.23×10^0	6.08×10^5	0.135	0.982
Irradiated CrleGV-SA C5	4.68×10^5	5.79×10^3	2.89×10^6	1.620	0.659

0.9 % lignin	6.45 x10^4	3.21 x 10^1	7.01 x 10^5	0.255	0.963
9 % Uvinul Easy	5.85 x 10^4	3.04 x 10^1	6.34 x 10^5	0.214	0.970
9 % OE446	8.57 x 10^4	1.65 x 10^1	1.03 x 10^6	0.148	0.980

Conclusion to date

In this study, a UV-resistant isolate of CrleGV-SA was successfully selected through repeated exposure to UV-irradiation and serial passaging of the virus through FCM larvae. The selection of UV-resistant virus was confirmed by identifying the specific genetic changes associated with this resistance.

Technology transfer

Conferences where data have been presented

- Microscopy Society of Southern Africa, 2016 (poster), 2017 (oral)
- CRI Symposium, 2016 (poster), 2018 (oral)
- Society for Invertebrate Pathology 2018

Future Research

This study is being taken further under a separate CRI-funded project (1263: Genetic analysis and applied use of a selected UV-resistant strain of CrleGV for improved control of FCM). The objectives of the study are to further study the genetic changes that have been recorded, to bulk up the UV-resistant virus *in vivo*, to determine whether the genetic integrity of the UV-resistance has been maintained, and to conduct field trials to compare efficacy and persistence with the wild type CrleGV-SA.

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3.2.3. **FINAL REPORT: FCM control under nets – Is pest-freedom possible?**

Project 1189 (Apr 2018 – March 2020) by Sean Moore, Wayne Kirkman, Paul Cronje, Mellissa Peyper (CRI), and Tamryn Marsberg (RU)

Summary

Netting of citrus orchards is thought to provide protection from various abiotic factors, but the effect on biotic factors, particularly insect pests requires further study. This study focused on the effect nets have on key pests, residue levels and various horticultural aspects. Orchards of similar cultivars were monitored inside and outside of nets in several orchards at three sites throughout the Sundays River Valley. Pest management programmes inside and outside of nets were similar, including the release of sterile moths, in both environments at two of the sites. Data trees inside and outside of the nets were monitored weekly for fruit infestation during the 2017/2018 and 2018/2019 season. During the 2017/2018 season, FCM infestation was not lower under nets. This could be due to nets been erected over orchards with previously high FCM infestation. During the 2018/2019 season, no FCM infestation was recorded inside or outside of nets. This was due to an overall low number of FCM present in the Sundays River Valley and thus no final conclusion can be made yet on the effect that nets have on FCM infestation. Pheromone traps were also monitored for sterile and wild moth catches. Wild moth catches were higher under nets; however, so too were sterile moth catches, and consequently, a higher ratio of sterile moths was recaptured under nets in both seasons, indicating that SIT may indeed be more effective under nets. These same orchards were used to scout for key citrus pests: red scale, mealybug, thrips, brown and green leafhopper, bud mite and fruit fly. Red scale and mealybug were significantly higher under nets in both seasons. Thrips damage was significantly higher outside of nets. This may be due to significantly higher numbers of predatory mites and spiders under nets. There was no significant difference between bud mite and leafhopper damage inside and outside nets. Further research under nets, particularly on mealybug and red scale, would be valuable. Although this project is terminated, there are several other projects investigating IPM under nets.

Opsomming

Die benetting van sitrusboorde bied vermoedelik beskerming teen verskeie abiotiese faktore, maar die effek van biotiese faktore, veral insekplae vereis verdere studies. Hierdie studie het gefokus op die effek wat nette het op sleutel plae, residu vlakke en verskeie tuinboukundige aspekte. Boorde met soortgelyke kultivars is binne en buite nette in verskeie boorde by drie persele regoor Sondagsrivier Vallei gemonitor. Plaagbestuursprogramme binne en buite die nette was soortgelyk, insluitende die loslating van steriele motte, in beide gebiede by twee van die persele. Data bome binne en buite die nette is weekliks gemoniteer vir vrug-besmetting gedurende die 2017/2018 en 2018/2019 seisoen. Gedurende die 2017/2018 seisoen was VKM besmetting nie laer onder die nette nie. Dit kan wees as gevolg van die nette wat oor boorde opgerig is met vorige hoë VKM besmetting. Gedurende die 2018/2019 seisoen is geen besmetting binne of buite die nette aangeteken nie. Dit was as gevolg van ‘n algehele lae vlak van VKM druk in die Sondagsrivier Vallei en daarom kon geen finale gevolgtrekking gemaak word oor die effek van nette op VKM besmetting nie. Feromoon lokvalle is ook gemonitor vir steriele en wilde mot vangste. Wilde mot vangste was hoër onder nette, maar so ook die steriele mot vangste, en daarom, is ‘n hoër verhouding van steriele motte onder nette gevang in beide seisoen, wat dui dat SIT wel onder nette meer doeltreffend mag wees. Dieselfde boorde is gebruik om ondersoek te doen vir verskeie sleutel plae: rooidopluis, witluis, blaaspootjie, bruin en groen bladspringers, knopmyt en vrugtevlieg. Rooidopluis en witluis was aansienlik hoër onder die nette in beide seisoene. Blaaspootjie skade was aansienlik hoër buite die nette. Dit kan wees as gevolg van die aansienlike hoër aantal predatoriese myte en spinnekoppe onder die nette. Daar was geen aansienlike verskil tussen knopmyt en bladspringer skade binne en buite die nette nie. Verdere navorsing onder nette, veral op witluis en rooidopluis, sal waardevol wees. Alhoewel hierdie projek beëindig is, is daar verskeie ander projekte wat IPM onder nette ondersoek.

Introduction

The use of nets for protecting trees and fruit against various abiotic factors and for creating a more favourable microclimate for better tree growth and fruit production, is rapidly becoming more popular in the southern

African citrus industry. Although the main justifications for using nets are protection against sun, wind and hail, improved irrigation management, and improved tree growth, protection against biotic factors could also be an advantage, which up to this point is relatively untested. Although reports of elevated levels of certain pests under nets have been made, particularly mealybug, it is possible that control of certain other pests could be improved. This may pertain particularly to those pests which are active fliers, such as moth and fruit fly pests. Although limited data exist to indicate that FCM cannot be excluded from nets, a net could be considered as an island-type environment, where dramatic suppression or even eradication, albeit temporary, of an organism becomes more likely than in a continental environment. Geographically isolated areas, such as islands, typically have lower rates of re-invasion, making eradication particularly economically feasible, especially when weighed against the typically large impacts of invasive species' establishment on island biodiversity (Liebhold & Tobin, 2009). An example of this is the successful eradication of three gypsy moth species from New Zealand, using a programme based on *Bacillus thuringiensis* (Glare, 2009). This is mainly because immigration pressure of organisms should be far lower in an island environment, due to the physical barrier or divide between the island and the external environment from which the foreign organisms could enter.

In addition to the main objective of measuring FCM control under nets, other pests present were also monitored and compared within and outside of nets. Finally, horticultural aspects were also compared i.e. yield, fruit size and variation, colour, internal fruit quality (TSS, acid, ratio), rind quality and thickness, navel-end splitting, general blemishes. This was conducted within the CRI Citriculture Portfolio.

Stated objectives

- To compare the residual efficacy of FCM control measures under and outside of nets.
- To determine whether FCM can be eradicated (albeit temporarily) under nets.
- To compare any other pests.
- To compare horticultural aspects.

Materials and methods

The trials were conducted in 2017/2018 and 2018/2019 on three different farms in the Sundays River Valley, Eastern Cape (Table 3.2.3.1). Nets providing 20% shading on top and 40% shading on the sides were erected at the end of the 2017 citrus harvesting season. Monitoring of trial sites was then initiated in the first week of January 2018. On each farm, two to three orchards were chosen inside nets and two or three orchards of the same or very similar citrus cultivar, and of similar age, were chosen outside the nets. Pest control practices for all orchards at each site were identical. FCM in these orchards was monitored weekly and scouting for key citrus pests was conducted periodically, until harvest.

Table 3.2.3.1. Details of the orchards monitored during the trial.

Region	Farm	In/Out	Orchard number	Cultivar	Co-ordinates
Sundays River Valley, Eastern Cape	Olifantsbos	Inside	18	Cambria	33°37'26.25"S 25°41'21.11"E
		Inside	22	Cambria	33°37'31.75"S 25°41'23.05"E
		Inside	24	Cambria	33°37'33.28"S 25°41'17.85"E
		Outside	1	Cambria	33°37'36.17"S 25°41'1.20"E
		Outside	2	Cambria	33°37'41.97"S 25°40'55.46"E
		Outside	3	Cambria	33°37'40.66"S 25°41'9.04"E
	Douglas	Inside	54	Washington	33°26'22.19"S

					25°31'38.06"E
		Inside	52	Washington	33°26'26.40"S 25°31'41.73"E
		Outside	47A	Washington	33°26'22.45"S 25°31'45.33"E
		Outside	47B	Washington	33°26'22.36"S 25°31'46.86"E
	Sur Le Son	Inside	23 B	New Hall Navel	33°26'32.42"S 25°29'38.45"E
		Inside	23 A	New Hall Navel	33°26'30.42"S 25°29'32.16"E
		Outside	22 B	Fukumoto	33°26'26.77"S 25°29'40.06"E
		Outside	22 A	Fukumoto	33°26'26.03"S 25°29'33.40"E

Residue analysis

Residues were analysed for both seasons. During the 2018 season a once-off collection of 30 random fruit at the end of the season were collected from ten data trees mentioned below in the FCM monitoring. These fruit were collected while wearing gloves and packaged into breathable plastic bags. Samples were then frozen and sent to Hearshaw and Kinnes Analytical laboratories for residue analysis. This was again repeated in the 2019 season, however, fruit samples were taken at the beginning of the season and at the end of the season.

Temperature and humidity

Temperature and humidity were also monitored inside and outside of nets at all trial sites. A single DS1923L-F5 Maxim Temperature and Humidity iButton (Cold Chain Thermodynamics Software, Fairbridge Technologies, Gauteng, South Africa) was placed inside and outside netted orchards. The iButtons recorded the temperature and humidity at hourly intervals for the duration of the study.

Monitoring of false codling moth

a) Fruit infestation

FCM infestation of fruit was monitored inside and outside of netted orchards. Ten data trees were selected in the middle row and in the middle of the orchard, for each block monitored. Fallen fruit was then collected separately from each of these data trees on a weekly basis until harvest. Each tree's fallen fruit was analysed separately. Infestation was recorded by dissecting fruit with a sharp knife and determining the presence of FCM larvae. Presence of FCM larvae was recorded by observing a larva in the fruit, and signs of tunnelling and frass within the fruit (Moore *et al.* 2015). This monitoring continued until harvest for both of the seasons.

b) Moth population

FCM male moth populations were also monitored by placing yellow delta traps lined with a sticky lining and Chempac FCM lure (Chempac (Pty) Ltd, South Paarl, South Africa) to attract males. A single trap was placed inside and outside of netted orchards. Traps were placed in an orchard, in the fifth row and on the tenth tree. These traps were also used to monitor the sterile moths released at two of the three sites. Sterile moths were only monitored at two of the sites as they were only released under the nets at these two sites. The delta traps were monitored weekly by XSit (Pty) Ltd (South Africa). XSit also distinguished between wild and sterile moths captured. Sterile moths were distinguished by the addition of Calco Oil Red® (Royce International, Sarasota, Florida, USA) to the artificial diet. When sterile moths were squashed the inner parts were pink in colour whereas wild moths were cream-coloured.

Monitoring of fruit fly

Mediterranean fruit fly populations were only monitored in the 2019 season by placing a single Sensus trap with Capilure® (River Bioscience (Pty) Ltd, South Africa) and a Sensus trap with Questlure® (River Bioscience (Pty) Ltd, South Africa) into each orchard at all three sites inside and outside of nets. Capilure® is an attractant used for males, whereas Questlure® lure is a female attractant. The Sensus traps contain a Dichlorvos tablet which kills the adult flies once they have entered the trap. Traps were placed in the same row as the ten data trees used for the fallen fruit analysis. The Capilure® trap was placed 5 trees before the first data tree and the Questlure® was placed 5 trees after the tenth data tree. These traps were placed in the orchard six weeks before harvest. Traps were examined and emptied weekly and the number of Medfly male and females were counted.

Monitoring for other key citrus pests

Scouting for red scale, mealybug and thrips was conducted as a once-off scout at the end of the season in 2018 and once a month in the 2019 season. The same ten data trees that were used to collect fallen fruit for FCM (mentioned above) were used to scout for the three key pests. Ten fruit on either side of the ten trees were examined for the presence or absence of the pests (Grout 2019). Findings were then recorded on a datasheet, which was then used for the analysis.

During the 2019 season, while scouting for the three key pests, an increased level of leafhopper damage was observed, this was characterised by chlorotic yellow marks on the fruit. A once-off scout for leafhopper damage was completed, again by using the same ten data trees as mentioned above. Ten fruit on either side of the tree were examined for leafhopper damage. Adult green and brown leafhopper were also monitored by placing three sticky yellow card traps diagonally across all orchards monitored inside and outside of the nets. Traps were left out for 7 days and removed. The number of green and brown leafhoppers were recorded (Moore 2013).

A once-off scout for bud mite was also completed during the 2019 season as an increase in damage was observed during the weekly monitoring. Again, using the same ten data trees as mentioned above, ten fruit were selected and examined for the typical signs of bud mite which included the malformation of fruit and elongated navel end (Grout 2011).

Scouting for predatory mites and spiders

Predatory mites, the main predators of citrus thrips, were scouted once at the end of the 2019 season. The decision to scout for predatory mites was to determine if they had an effect on the thrips populations inside and outside of nets. The same ten data trees, as mentioned previously were used for scouting. Five leaves on either side of the tree were examined for the presence or absence of predatory mites.

During the various scouting for pests, an increased presence of spiders nesting amongst the fruit was noticed. A once-off scout for spiders was completed. Again, the same ten data trees as mentioned previously were used. Ten fruit on either side of the tree were examined for the presence or absence of spiders. Samples of spiders were collected, placed in ethanol and sent to be morphologically identified by Professor Charles Haddad at the University of Free State, Bloemfontein, South Africa.

Statistical analysis

Statistical analyses were performed using STATISTICA version 13.4.0.14 (TIBCO Software Inc. 2018). Data from all three sites were combined for statistical analysis to allow for comparison to be made between inside and outside of nets and not between sites. FCM infestation, fruit fly and temperature and humidity data were compared using a t-test for two independent samples. The scouting results for various other key pests of citrus and natural enemies were compared using a Mann-Whitney U test. Significant differences were indicated by a p-value of $p < 0.05$ and n values indicate the number of fruit examined.

Results and discussion

Objective / Milestone	Achievement
A. Compare the residual efficacy of FCM control measures under and outside of nets	Residue samples for both seasons have been completed
B. Determine whether FCM can be eradicated (albeit temporarily) under nets	FCM has been monitored under nets for two seasons
C. Scout for other key pests	Surveys for red scale and mealybug infestation, thrips infestation and damage, bud mite damage, brown and green leaf hoppers and fruit flies have been conducted for all orchards. Results are similar between 2018 and 2019 season.
D. Compare horticultural aspects	This is being conducted in a separate project (PHI-4-12) within the Citriculture Portfolio, by the University of Fort Hare.

Residue analysis

Residue results for both seasons are shown in the tables below (Table 3.2.3.2 and 3.2.3.3). Residues were not notably higher under nets and no residues exceeded any MRLs for exports.

Table 3.2.3.2. Residual efficacy of various control options used in all of the orchards in 2017/2018.

	ORCHARD													
	Sur Le Son				**Douglasdale**				**Olifantsbos**					
PRODUCT	**IN A**	**IN B**	**OUT A**	**OUT B**	**IN 52**	**IN 54**	**OUT A**	**OUT B**	**IN 18**	**IN 22**	**IN 24**	**OUT 1**	**OUT 2**	**OUT 3**
2,4D (free acid)														
Azoxystrobin									0.012	0.013	0.016	<0.01	<0.01	
Buprofezin														
Carbendazim						<0.01	<0.01						0.055	
Chlorfenapyr													<0.01	
Chlorpyrifos											<0.01			
Fenbutatin-Oxide	0.068	0.097							0.029	0.039	0.049			
Formetanate														
Mercaptothion											<0.01			
Methoxyfenozide	0.18	0.13	0.15	0.12	0.19	0.16	0.28	0.37						
Pyraclostrobin		<0.01			<0.01		0.011	0.022	0.036	0.021	0.024	0.018	0.028	0.014
Pyriproxyfen					0.013				0.012	0.011	0.024	0.018	0.023	0.017

Table 3.2.3.3: Residual efficacy of various control options used in all of the orchards in 2018/2019.

	ORCHARD													
	Sur Le Son				**Douglasdale**				**Olifantsbos**					
PRODUCT	IN A	IN B	OUT A	OUT B	IN 52	IN 54	OUT A	OUT B	IN 18	IN 22	IN 24	OUT 1	OUT 2	OUT 3
2,4D (free acid)														
Azoxystrobin														
Buprofezin														
Carbendazim														
Chlorfenapyr														
Chlorpyrifos									0.028	0.014	0.017	<0.01	0.025	0.022
Fenbutatin-Oxide														

Formetanate														
Mercaptothion														
Methoxyfenozide														
Pyraclostrobin	<0.01	<0.01	<0.01	<0.01	<0.01									
Pyriproxyfen	<0.01	0.01	<0.01	<0.01	<0.01	0.013			<0.01	<0.01		<0.01	<0.01	<0.01
Spirotetramat	<0.01	<0.01												
Imidacloprid			0.015	0.011										

Temperature and humidity

Temperature for the 2018 season was recorded from October 2017 to July 2018. The temperature data were then converted into effective heat units for comparison between inside and outside. There was no significant difference between effective heat units inside and outside nets ($T_{18, 10}$ = 0.01, P = 0.99) (Figure 3.2.3.1). The mean effective heat units for inside was 479.30 EHU and outside was 614.53 EHU.

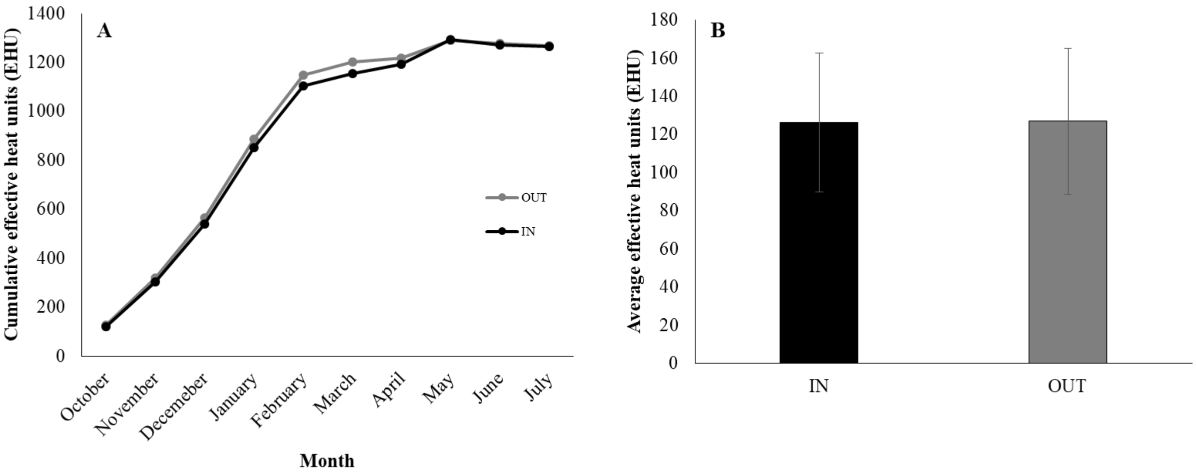

Figure 3.2.3.1. The comparison of effective heat units inside and outside netted orchards during the 2018 season in the Sundays River Valley.
(A) Cumulative effective heat units over the season and (B) overall mean effective heat units inside and outside nets (t-test, P = 0.99).

Relative humidity (RH) data were recorded at the same time as temperature throughout the 2018 season. The RH data were converted to vapour pressure deficit (VPD) to compare inside and outside nets. The VPD was not significantly different between inside and outside of nets ($T_{18, 10}$ = -0.56, P = 0.58) (Figure 3.2.3.2). The mean VPD inside of nets was 0.22 kPa and outside of the nets was -0.13 kPa.

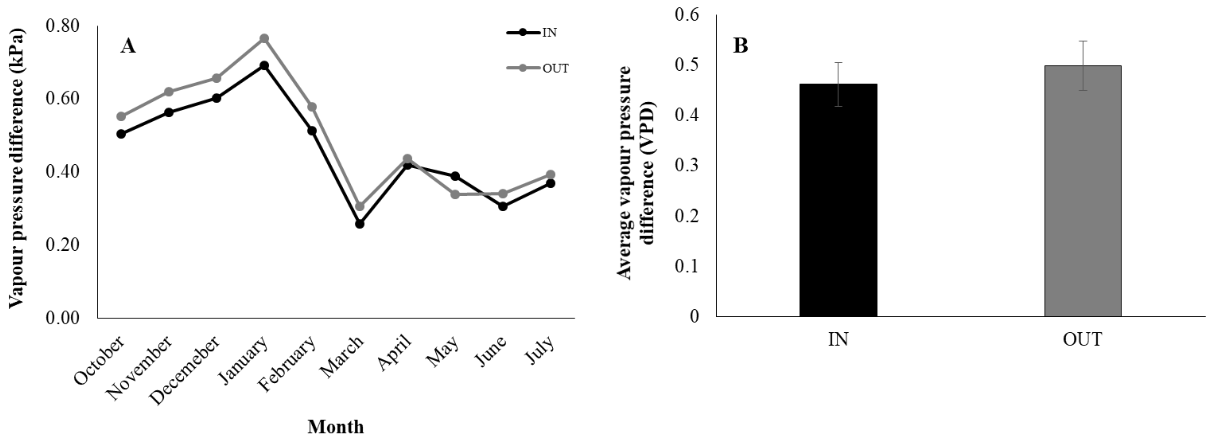

Figure 3.2.3.2. The comparison of vapour pressure deficit (relative humidity) inside and outside of netted orchards during the 2018 season in the Sundays River Valley. (A) Vapour pressure units throughout the season and (B) overall mean vapour pressure difference inside and outside nets (t-test, P = 0.58).

Temperature for the 2019 season was recorded from September 2018 to May 2019. Effective heat units for each month were then calculated from the temperature recordings. No significant difference in EHU was recorded between inside and outside the net ($T_{16, 9}$ = -0.08, P = 0.94) (Figure 3.2.3.3). The mean effective heat units for inside was 202.03 EHU and outside was 198.04 EHU

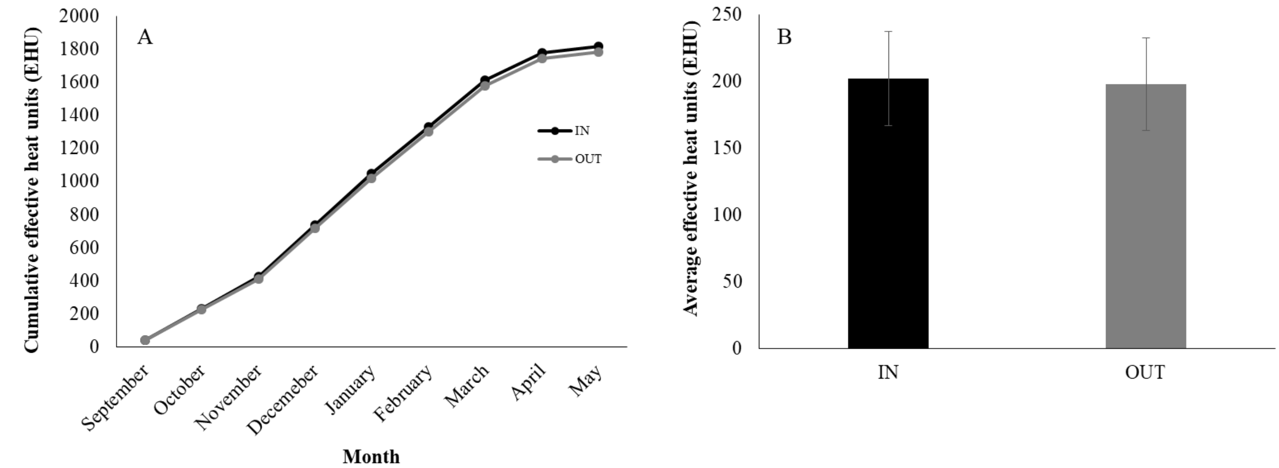

Figure 3.2.3.3. The comparison of effective heat units inside and outside netted orchards during the 2019 season in the Sundays River Valley. (A) Cumulative effective heat units over the season and (B) overall mean effective heat units inside and outside nets (t-test, $P = 0.94$).

Relative humidity (RH) data was recorded at the same time as temperature throughout the 2019 season. The RH data was converted to VPD to compare inside and outside of nets. No significant difference was recorded between inside and outside of nets ($T_{16, 9} = -0.17$, $P = 0.86$) (Figure 3.2.3.4). The mean VPD inside of nets was 0.54 kPa and outside of the nets was 0.56 kPa.

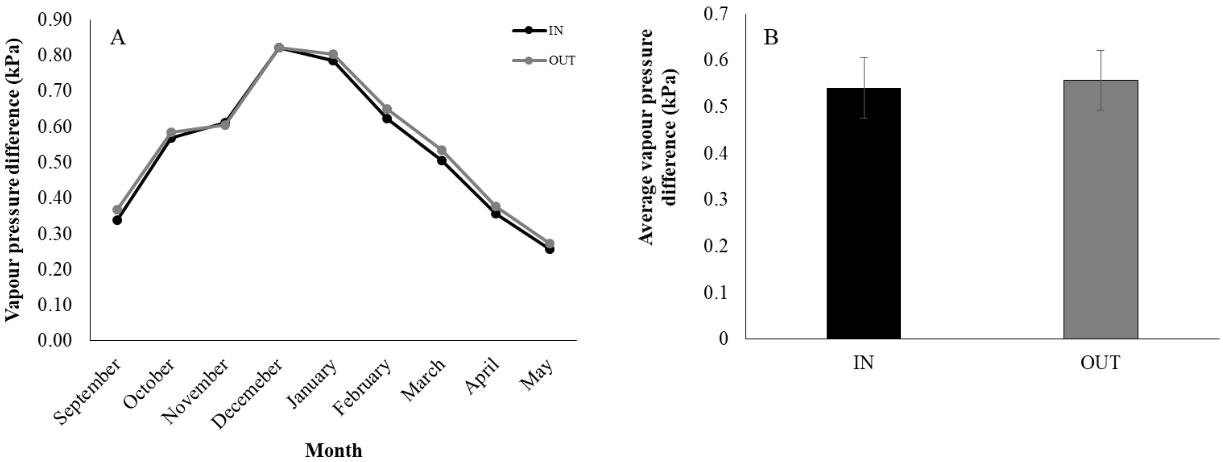

Figure 3.2.3.4. The comparison of vapour pressure deficit (relative humidity) inside and outside of netted orchards during the 2019 season in the Sundays River Valley. (A) Vapour pressure units throughout the season and (B) overall mean vapour pressure difference inside and outside nets (t-test, $P = 0.86$).

Monitoring of false codling moth

FCM evaluations for all sites have been completed for a second consecutive season. During the 2017/2018 season FCM was not lower under nets (Figure 3.2.3.5). In 2018/2019, no FCM was found inside or outside of nets. Thus, no final conclusion could be made yet on the effect that nets have on FCM infestation. FCM was extremely low throughout the Sundays River Valley, which could potentially be due to the stringent FMS in place as well as good farming practices. With regard to sterile and wild moth catches, a higher number of sterile moths were recaptured under nets compared to outside. During the 2018 season evaluations a ratio of 9.52:1 (sterile:wild) moths were recaptured under nets, whereas a ratio of 6.44:1 (sterile:wild) moths were recaptured outside of nets. The number of sterile moths recaptured inside nets in 2018/2019 was almost double that recorded in 2017/2018 (Figure 3.2.3.6 & 3.2.3.7). This can be seen by the ratio of moths recaptured. During the 2019 evaluations a ratio of 237.1:1 (sterile:wild)moths were recaptured outside of nets and 212.15:1 (sterile:wild) moths were recaptured inside of nets.

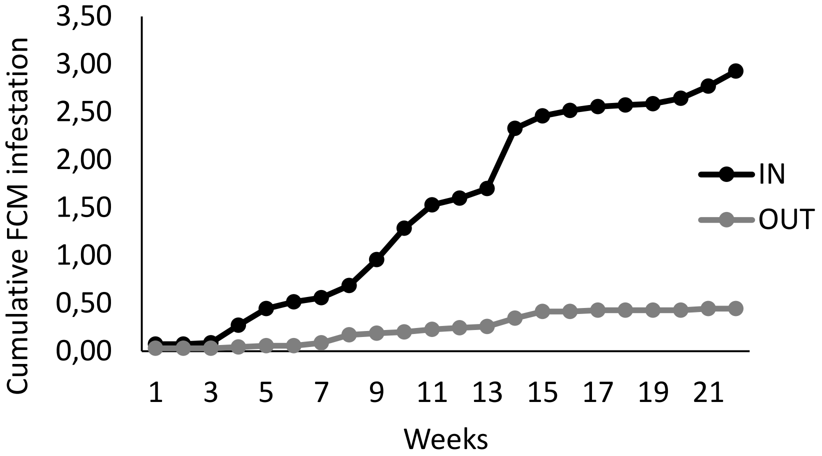

Figure 3.2.3.5. Cumulative FCM infestation inside and outside of nets for all three sites monitored in the Sundays River Valley in 2018 (T-test = 6.75, P = 0.0001).

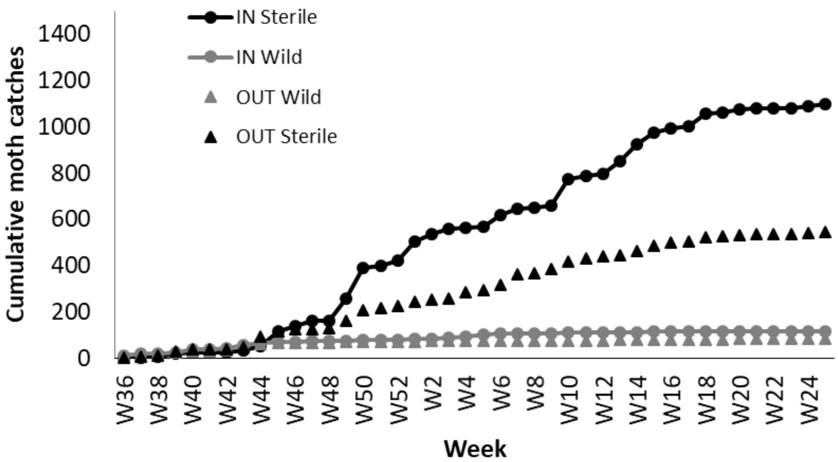

Figure 3.2.3.6. Cumulative sterile and wild moth catches inside and outside of nets for two of the sites monitored in the Sundays River Valley in 2018 (Sterile: T-test=7.98, P=0.001; Wild: T-test = 10.79, P = 0.0001).

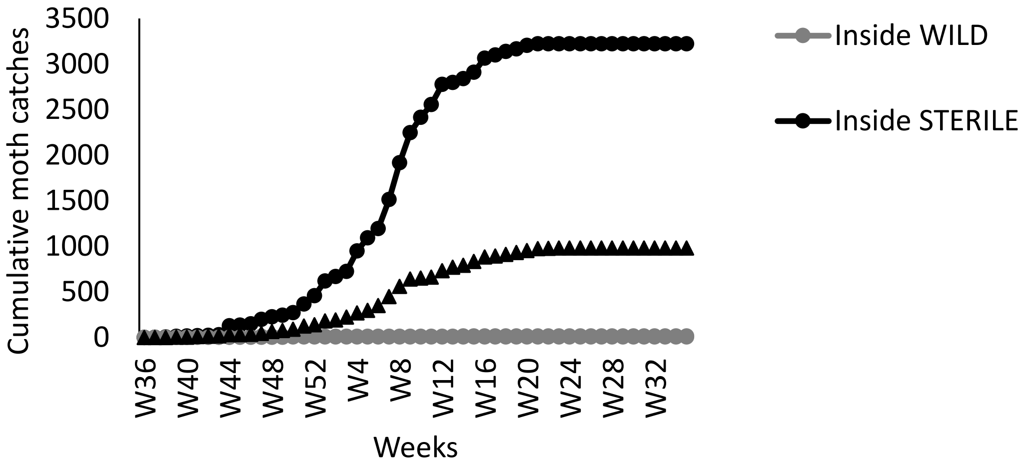

Figure 3.2.3.7. Cumulative sterile and wild moth catches inside and outside of nets for two of the sites monitored in the Sundays River Valley in 2019 (Sterile: T-test = 10.84, P = 0.0001; Wild: T-test = 16.99, P = 0.0001).

Monitoring for fruit fly

A significantly higher number of Mediterranean fruit flies was captured outside of nets (T-test = -3.20, P = 0.01) (Figure 3.2.3.8).

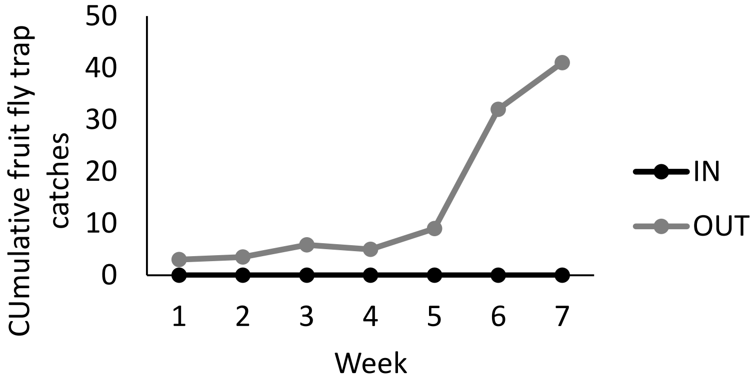

Figure 3.2.3.8. Cumulative fruit fly catches for inside and outside of netted orchards in the Sundays River Valley in 2019.

Monitoring of key citrus pests

During the 2018 season red scale and mealybug infestation and thrips damage were scouted for several weeks before harvest. Red scale and mealybug were found to be significantly higher inside the nets (Red scale: Mann-Whitney U Z = -2.19, P =0.028 (n=70); Mealybug: Mann-Whitney U Z = -1.99, P = 0.046 (n=70)). Thrips damage was significantly higher outside of the nets (Figure 3.2.3.9).

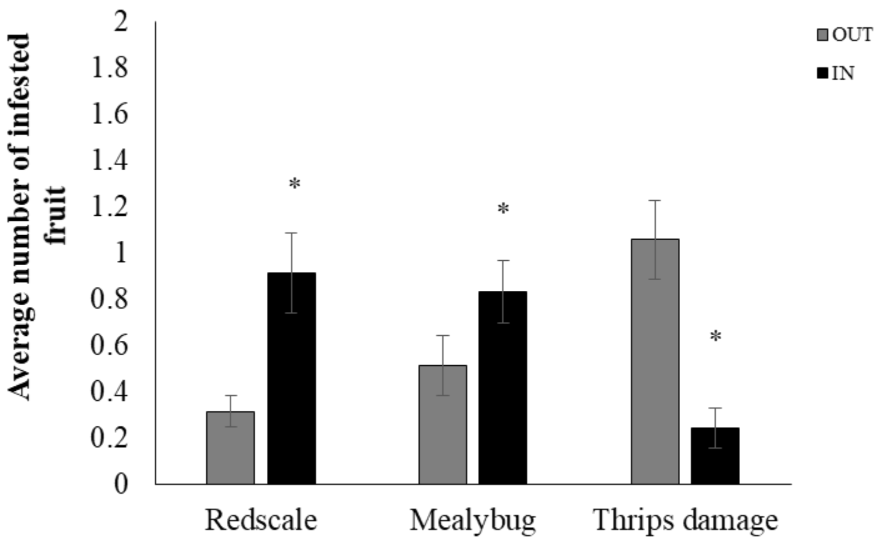

Figure 3.2.3.9. Mean number of red scale and mealybug infested and thrips damaged fruit for all three sites monitored in the Sundays River Valley in 2018 (* indicates significance, P < 0.05).

During the 2018/2019 season, red scale, mealybug and thrips infestation was scouted for once a month until harvest. Thrips damage was only scouted for at the end of the season. Figure 3.2.3.10 shows the infestation results from the beginning of the season (February). There was no significant difference between infestation of the three pests inside and outside of nets.

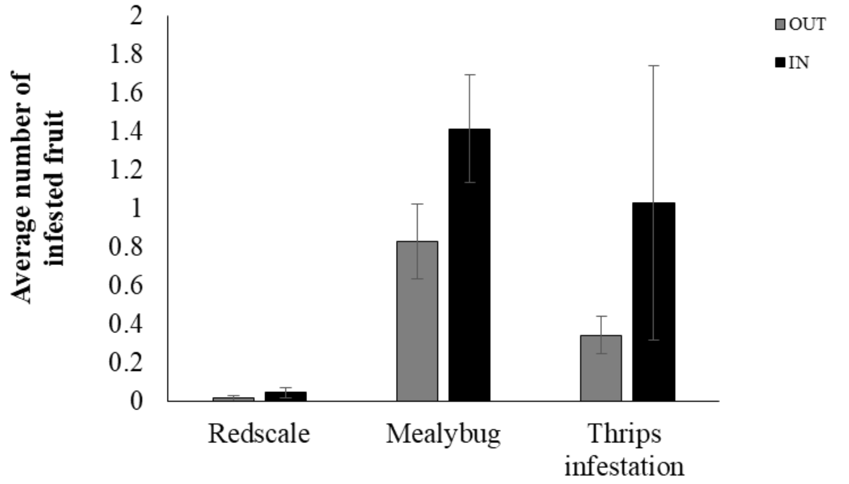

Figure 3.2.3.10. Mean number of red scale and mealybug infested and thrips damaged fruit for all three sites monitored in the Sundays River Valley during February 2019.

Figure 3.2.3.11 shows scouting results from the end of the season (May). Red scale and mealybug infestation was significantly higher inside the nets compared to outside (Red scale: Mann-Whitney U Z = -3.19, P = 0.001; Mealybug: Mann-Whitney U Z = -2.48, P = 0.01). Thrips damage was significantly higher outside of nets compared to inside (Mann-Whitney U Z = 3.95, P = 0.000).

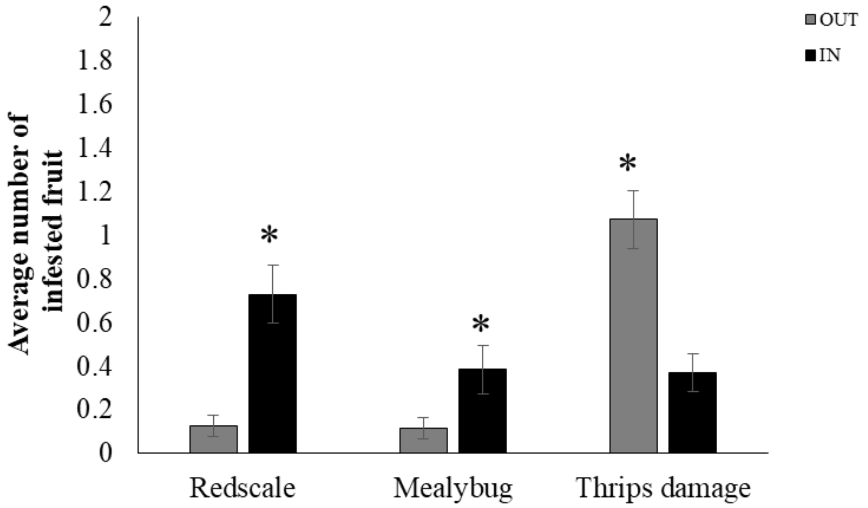

Figure 3.2.3.11. Mean number of red scale and mealybug infested and thrips damaged fruit for all three sites monitored in the Sundays River Valley during May 2019 (*indicates significance, P <0.05).

During the 2019 season, an increase in bud mite damage was detected. A once-off scout was completed and it was recorded that there was no significant difference between bud mite damage inside and outside of nets (Mann-Whitney U Z = -0.62, P = 0.533 (n = 70)) (Figure 3.2.3.12).

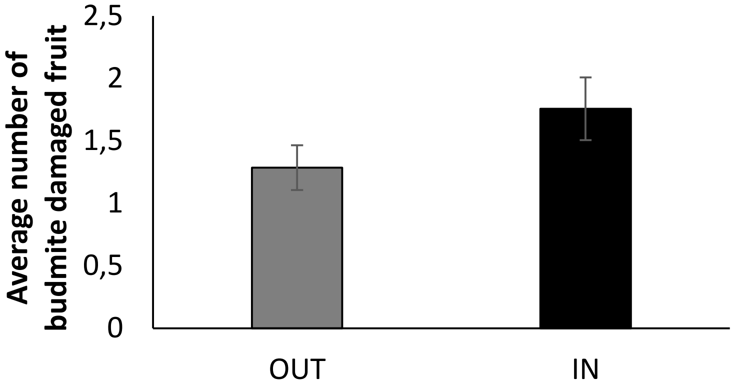

Figure 3.2.3.12. Mean number of bud mite damaged fruit for all three sites monitored in the Sundays River Valley in 2019.

No significant difference was recorded in trap catches between inside and outside of nets for both species of leafhoppers (Green leafhopper: Mann-Whitney U Z = 0.35, P = 0.72 and brown leafhopper: Mann-Whitney U Z = -0.79, P = 0.43 (n = 70)) (Figure 3.2.3.13A). Also, no significant difference was recorded for leafhopper damage inside and outside of nets (Mann-Whitney U Z = -0.44, P = 0.66 (n = 70)) (Figure 3.2.3.13B).

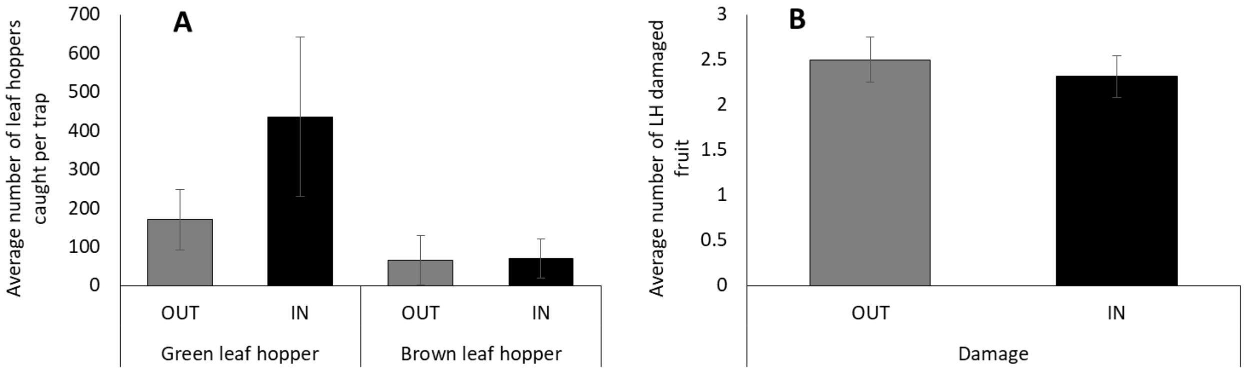

Figure 3.2.3.13. Mean number of green and brown leafhoppers per trap (A) and mean number of damaged fruit (B) for all three sites monitored in the Sundays River Valley in 2019.

Scouting for predatory mites and spiders

A significantly higher number of predatory mites were recorded inside of nets compared to outside (Mann-Whitney U Z = 5.053, P = 0.000 (n=70)) (Figure 3.2.3.14).

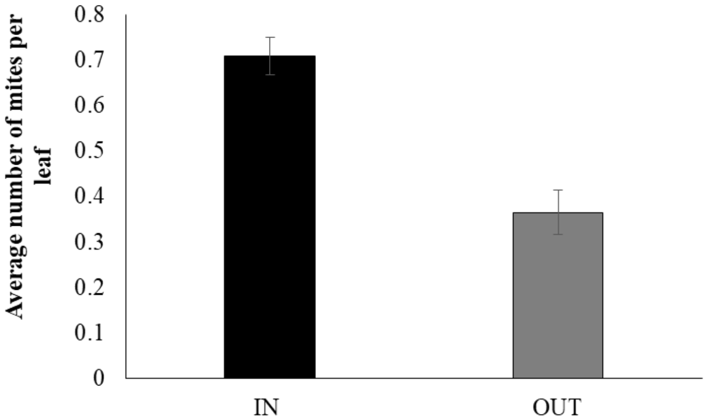

Figure 3.2.3.14. Mean number of predatory mites recorded per leaf for all three sites monitored in the Sundays River Valley during the 2019 season.

Results recorded from a once-off scout for spiders, found that spiders were significantly higher inside of nets compared to outside (Mann-Whitney U Z = -2.661, P = 0.008 (n = 30)) (Figure 3.2.3.15). Two species of spiders were identified by Prof Charles Haddad, *Baduman longinqua* (Araneae: Desidae) (Koch 1867) and *Chresiona invalida* (Araneae: Amaurobiidae) (Simon 1898) (Figure 3.2.3.16). *Baduman longinqua* is an introduced species from Australia and *C. invalida* is endemic to South Africa. These higher levels of spiders under nets, may have contributed to the lower levels of thrips damage under nets, through predation (or even the non-consumptive effect) on thrips.

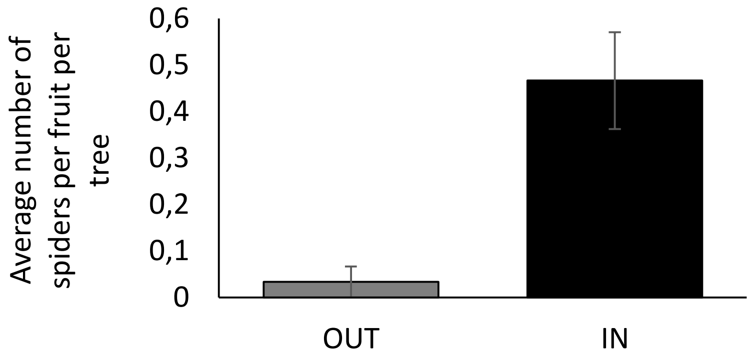

Figure 3.2.3.15. Mean number of spiders inside and outside of netted orchards in the Sundays River Valley in 2019.

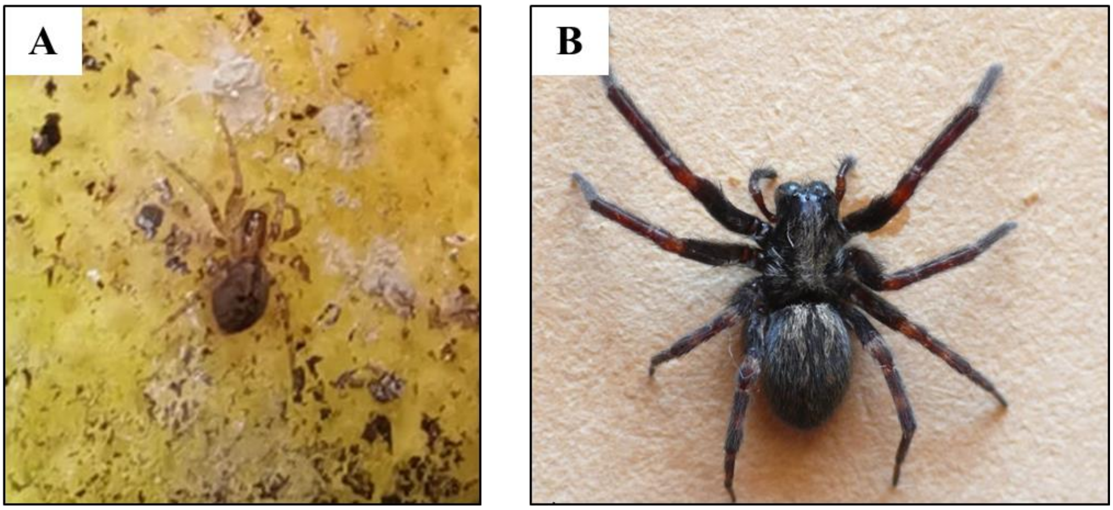

Figure 3.2.3.16. Two different species of spiders collected inside of netted orchards and morphologically identified. A: *Chresiona invalida* and B: *Badumna longinqua*.

A. Compare horticultural aspects

This is being conducted in a separate project (PHI-4-12) within the Citriculture Portfolio, by the University of Fort Hare.

Conclusion

Orchards inside and outside of nets were monitored over two seasons and various findings were recorded. During the first season of monitoring, FCM infestation was not lower under nets compared to outside, however this could be due to nets been erected over orchards with previously high FCM populations. During the second season of monitoring, no FCM infestation was recorded. The FCM population throughout the Sundays River Valley was extremely low, thus no clear conclusion can be made yet as to the effect of nets on the level of FCM infestation. A positive effect of nets with regard to FCM, was that a higher number of sterile moths was recaptured inside of nets compared to outside, and consequently a higher sterile to wild moth ratio was achieved under nets. Consequently, nets may improve the effectiveness of SIT.

A similar trend for the three key pests was recorded for both seasons. Red scale and mealybug infestations were significantly higher inside of nets compared to outside. This could be due to nets creating a favourable climate for the pests to thrive in. Thrips infestation was only monitored during the 2019 season; no significant difference was recorded between inside and outside of the nets. However, for both seasons thrips damage was significantly higher outside of the nets. This could be a result of the higher number of predatory mites recorded inside. It has been reported that if there is more than one predatory mite per leaf, successful biological control of thrips is achieved (Grout & Richards 1992). Another factor that could have an effect on the thrips population inside of nets, is the significantly higher number of spiders found under nets. These spiders are known as generalist predators and could potentially feed on thrips. The two spider species were also found amongst the mealybug and sooty mould.

No significant difference was recorded between the number of adult leafhoppers and leafhopper damage inside and outside of nets. No significant difference was also recorded for bud mite damage inside and outside of nets.

Fruit fly was only recorded outside of nets and all specimens were Medfly. It is important to continue to monitor these netted environments to determine if similar patterns are observed. Further research is also required to determine the effect nets have on the increased mealybug and red scale populations.

In conclusion, it appears that nets do not have a dramatic effect on FCM levels, but it is possible that where SIT is used, populations under nets may be suppressed to a greater extent over time. It is also not yet clear whether nets are advantageous or detrimental to successful implementation of IPM. Certain key pests are elevated under nets, but the most important pest, influencing the ability to implement a true IPM programme i.e. thrips, is less problematic under nets.

Future research

This project has now terminated. However, there are currently several other CRI funded projects that are investigating various aspects of IPM under nets.

Technology transfer

Presentation at 2018 Citrus Symposium.
Presentation at 2019 Entomological Society of Southern Africa Congress
Presentations at 5 regional citrus study groups during the 2019 IPM and DM roadshow

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3.2.4 FINAL REPORT: Semi-commercial control of FCM using sequential CO_2 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 14 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 benefit of the prior fumigation appears to be lost. The commercially used FCM diet was unsuitable for fumigation trials so the more porous fruit fly diet was also evaluated 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 CO_2 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 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 larval 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%. Further research using simultaneous fumigation of Valencia oranges infested with fifth instar FCM, either packed in export cartons, or loose in crates, followed by 14 days at 2°C, gave very similar results. This showed that fruit could be packed then treated at another facility, if necessary. Other research with second instar Medfly in oranges confirmed that 14 days at 2°C after CO_2 fumigation was equally effective against this fruit fly. No significant difference in taste or internal quality could be found after treating various citrus types and cultivars with carbon dioxide followed by 14 days at 2°C. No further research on this topic is planned.

Opsomming

Hierdie navorsing is beplan as ŉ verifikasie van vroeër resultate met koolstofdioksied beroking, gevolg deur 14 dae teen 2°C. Die *IPM* navorsingskomitee het egter die insluiting van warmer temperature versoek. Hierdie warmer temperatuur koue-periodes van 3°C vir 13 dae en 4°C vir 17 dae het nie die vereiste mate van beheer van VKM vyfde instars gegee nie, en die voordeel van die voorige beroking blyk verlore te wees. Die kommersieel-gebruikte VKM dieet was nie geskik vir berokingsproewe nie, en dus is die meer poreuse vrugtevliegvoeding ook geëvalueer, maar is gevind om nie veel beter te wees nie. Die mortaliteit vir vyfde instar VKM in Valencia vrugte, gestoor teen 4°C vir 17 dae sonder beroking, was 100%, maar ent 98.2% vir dieselfde koue-periode ná CO_2 beroking teen 70%. In vergelyking, het dieselfde behandelings vir vyfde instar VKM in vrugtevliegmedium mortaliteite van onderskeidelik 41.0% en 66.1% gegee. Verdere navorsing moes dus in vrugte uitgevoer word, wat die getal larwes wat per behandeling blootgestel kon word, beperk het. Ná drie proewe met ŉ na-beroking koue-behandeling van 12 dae teen 2°C, wat die behandeling van 5 785 vyfde instars behels het, is larwe mortaliteit van 99.20% verkry, gebaseer op beweging wanneer hulle gesteur is. ŉ Verdere sewe herhalings is met ŉ na-beroking koue-behandeling van 14 dae teen 2°C uitgevoer. Hierdie proewe het die behandeling van ŉ totaal van 15 829 vyfde instars behels, en die larwe mortaliteit wat op grond van versteuring verkry is, was 99.92%. Larwes wat ná behandeling beweging getoon het, is op vrugtevliegmedium geplaas ten einde vas te stel of enige in ŉ papie sou ontwikkel en tot volwassenheid oorleef. Slegs een lewendige volwassene is verkry, wat ŉ finale mortaliteit van 99.99% gegee het. Verdere navorsing,

wat die gelyktydige beroking van Valencia lemoene, besmet met vyfde instar VKM, óf gepak in uitvoerkartonne, óf los in kratte, gevolg deur 14 dae teen 2°C, behels het, het soortgelyke resultate gelewer. Dit het getoon dat vrugte gepak kan word en by ʼn ander fasiliteit behandel kan word, indien nodig. Ander navorsing met tweede instar Medvlieg in lemoene het bevestig dat 14 dae teen 2°C ná CO_2 beroking, net so effektief teen hierdie vrugtevlieg was. Geen betekenisvolle verskil in smaak of interne kwaliteit kon gevind word nie ná behandeling van verskeie sitrus tipes en kultivars met koolstofdioksied, gevolg deur 14 dae teen 2°C. Geen verdere navorsing word op hierdie onderwerp beplan nie.

Introduction

The only post-harvest disinfestation treatment currently in commercial use for false codling moth (FCM) *Thaumatotibia leucotreta* in citrus, is a 22 or 24 d cold treatment at a temperature of -0.6°C. This treatment has detrimental effects on fruit quality, resulting in colour loss in Clementine mandarins and unacceptably high levels of chilling injury in lemons and Marsh grapefruit. Although it has been recently shown that the required cold treatment for FCM can be reduced by a few days (Moore et al. 2017) and a systems approach is now being used for citrus exports to the European Union, a quicker postharvest treatment that is more acceptable than irradiation (Hofmeyr et al. 2016) would be valuable in case the systems approach is rejected. A high concentration of carbon dioxide as a short-duration shock treatment, followed by reduced intensity cold treatment, has been suggested as a potential disinfestation treatment for Medfly *Ceratitis capitata* larvae in citrus fruit (Alonso et al. 2005, Palou et al. 2008). Physiological research on FCM has also shown that high concentrations of carbon dioxide can increase the susceptibility of FCM to subsequent cold treatments that can then be shortened (Boardman et al. 2017). High concentrations of carbon dioxide in air are known to generally cause more arthropod mortality than low concentrations of oxygen in atmospheres (Mitcham et al. 2006) and low oxygen levels can result in off-tastes in citrus (Tate and Hattingh 2000 unpublished). Carbon dioxide is also less influenced by temperatures below 25°C than when high concentrations of nitrogen are used to lower oxygen levels (Adler 1995). Carbon dioxide shock treatments may even have a beneficial effect in reducing subsequent chilling injury in citrus (Hatton and Cubbedge 1982, Bertolini et al. 1991). So whereas a major shortcoming of most potential disinfestation treatments is that insecticidal efficacy and fruit damage are in most cases closely associated, this sequential treatment of carbon dioxide and a short cold treatment may actually combine increased insecticidal efficacy with a reduction in the sensitivity of fruit to chilling injury.

In the PHI-3 Project 60/2014 we conducted a series of experiments to evaluate the impact of carbon dioxide fumigation and Vapormate (ethyl formate) on various internal and external phytosanitary citrus pests. Our research with Vapormate on the external pest grain chinch bug (*Macchiademus diplopterus*) was published (Grout and Stoltz 2016) and resulted in the registration of Vapormate in Jan 2018 for grain chinch bug on citrus and pears at 240 g/m^3 for 4 h. However, we did not find Vapormate to be reliably efficacious against FCM larvae within citrus fruit, but found that high doses of carbon dioxide had a more consistent effect, although not providing adequate mortality alone. We therefore pursued the use of a carbon dioxide shock treatment followed by a short cold period during 2017 as a means of controlling FCM (Grout and Stoltz 2020) and Medfly larvae in citrus fruit. We are now in a position to finalise protocols for commercial use where 70% CO_2 for 24 h will be followed by short cold treatments at 3, 4 or 5°C pulp temperature which are temperature options with relevance to the FCM systems approach. We hope to verify that periods of 13, 14 and 15 days at these respective temperatures will provide total control of the most cold-tolerant fifth instar FCM. All these periods are shorter than the shortest journey to an EU port so could be done on board a vessel or partially completed in a container on route to the harbour. Once a suitable combination of treatments is determined for commercial use, replicates in a shipping container with FCM-infested fruit in plastic crates and packed for export in cartons will be conducted to verify that this protocol can work in commercial use. Finally, the optimal commercial treatment will be used to see whether Medfly larvae in fruit packed in export cartons is equally susceptible to this treatment.

Stated objectives

1. Compare the susceptibility of FCM 5th instars in media and in oranges to sequential combinations of CO_2 at 70% for 24 h and 5 d cold treatment at 3°C

2. Conduct validation trials with 5th instars in media and oranges using CO_2 at 70% for 24 h followed by 13 days at 3°C, or 14 days at 4°C or 15 days at 5°C.
3. Can FCM 5th instars be controlled in fruit packed in export cartons that is fumigated and cold treated as effectively as in fruit in plastic crates?
4. Is the treatment that is shown to be efficacious against FCM larvae equally effective against Medfly larvae?
5. Determine whether different types of citrus picked just prior to the optimal picking time is distinguishable after fumigation and cold treatment from fruit picked at the optimal time and only receiving the same cold treatment.

Materials and methods

1. Compare susceptibility of FCM larvae in media with those in fruit

Valencia oranges that had been kept in cold storage allowed for this research to be conducted in February 2018. FCM eggs and media ingredients were obtained from River Bioscience via courier. Sufficient media was used per 350 ml jar to produce 150-200 FCM larvae. Pieces of egg sheet (10 x 10 mm) placed on each fruit result in approximately 10 larvae per fruit. More than this leads to cannibalism and the destruction of the fruit before evaluation. Inoculation was under a laminar flow bench. Jars of media were autoclaved beforehand and fruit dipped in a combination of three fungicides. Infested fruit and media were then incubated at 25°C until most larvae were in the fifth instar, then they were fumigated with CO_2 at 70% in air for 24 h at ambient temperature, the fruit was waxed by hand, packed in export cartons and moved to a cold room where the fruit pulp and media temperature were maintained at 3°C for 5 days. Fruit and media were then moved back to 25°C for 48 h before larval mortality was determined. An untreated control for both fruit and media was used to determine natural mortality without fumigation or cold. Fruit and media temperatures were monitored with multiple thermocouples.

This experiment was repeated twice. Due to poor results with the FCM medium similar comparisons were made using carrot-based fruit fly medium. Cold treatments were 13 days at 3°C and 17 days at 4°C, with or without prior fumigation with CO_2 at 70% in air for 24 h. Evaluation was as above after being held at 25°C for 48 h after the cold period.

2. Conduct validation trials with 5th instars in oranges using CO_2 at 70% for 24 h followed by 12, 13 or 17 days at 2, 3 or 4°C

The comparisons with fruit and rearing media showed that media could not be used as a substitute for fruit, therefore 150 fruit were treated per replicate and 10 left untreated. Infested fruit were fumigated with CO_2 at 70% in air for 24 h at 25°C, then fruit were waxed by hand and packed in cartons before being moved into the cold room. Only one cold room was available, so two staggered replicates were conducted at each temperature, being sure to finish the cold treatment of the first replicate before the cold treatment of the second replicate started. The same approach was used for each temperature-time combination and the same cultivar of Valencia oranges was used for the two replicates at the three temperatures. After removal of fruit from the cold rooms they were held at 25°C for 48 h before determining mortality. Trained casual labourers assisted in searching for any larvae that showed movement on prodding and all live and dead larvae recovered were counted.

3. Can FCM 5th instars be controlled in fruit packed in export cartons that is fumigated and cold treated as effectively as in fruit in plastic crates?

Growers who do not have a degreening facility or the means to fumigate fruit in bins as it arrives at the packhouse may prefer to pack the fruit, then send the fruit to another facility for fumigation and cold storage or loading onto refrigerated containers. At Nelspruit we have a quarter-sized shipping container (Fig. 3.2.4.1) that can take at least two pallets of fruit. We used this to simultaneously fumigate unwaxed, infested Benny Valencias in crates and waxed, infested Benny Valencias packed in export cartons (Fig. 3.2.4.2). The crates were stacked adjacent to one another on a pallet with a gap between the pallet loads of fruit in cartons. A fan was placed in the container to circulate air (Fig. 3.2.4.3). Six crates or cartons in each pallet contained approximately 100 Valencias that had been infested with fifth instar FCM. These crates or cartons were stacked at three different heights and at each height one crate or carton was on the outside and one in the centre of the pallet. The fruit was fumigated with CO_2 at 70% in air for 24 h at ambient temperature, then the infested fruit in the crates were waxed and packed in cartons and moved with the infested fruit that was previously in cartons into a cold room where the pulp temperature was maintained at 2°C for 14 days. In the meantime, 30 infested, untreated fruit were evaluated for natural larval mortality. When the cold treatment was complete, the fruit was removed and held at 25°C for 48 h before being cut and larval mortality determined by prodding and looking for movement. The first replicate was conducted in February 2020 but due to frequent load shedding was scrapped. Three further replicates were conducted in March and April.

Figure 3.2.4.1. Shipping container with shade roof and CO_2 containers outside.

Figure 3.2.4.2. Preparing the crates and cartons for placement in the container

Figure 3.2.4.3. Placement of pallets within shipping container.

4. <u>Is the treatment that is shown to be efficacious against FCM larvae equally effective against Medfly larvae?</u>

Earlier research for objectives 2 and 3 showed that a mortality of more than 99.9% of 19 212 fifth instar FCM was achieved in Valencia oranges placed in a cold room in export cartons at 2°C for 14 days after fumigation with 70% CO_2 in air. However, this treatment must also be equally efficacious against Medfly *Ceratitis capitata* larvae that is known to be the most widespread of the fruit flies in South Africa that attack citrus fruit (De Villiers et al. 2013). Valencia oranges were inoculated with Medfly eggs inserted into a hole drilled under the removed calyx of each fruit, then plugged with cotton wool and sealed with wax. Fruit were then incubated at 26°C for 6 days until the majority of larvae were in the second instar. Fruit other than the control fruit were then fumigated with carbon dioxide at 70% in the air at 26°C for 24 h. These fruit were then placed in export cartons and transferred to the cold room at 2°C while the control fruit remained at 26°C for 48 h before dissecting and determining mortality based on larval movement (Grout et al. 2011). Thermoprobes in the pulp of the fruit in the cold room were monitored until half of them reached the required temperature, then the 14-day treatment was deemed to have started. After 14 days, the fruit was moved to the 26°C room for 48 h before being cut and larval mortality determined. In order to have 10 000 treated larvae we planned to conduct four replicates with 250 treated fruit and 50 control fruit per replicate.

5. <u>Determine whether different types of citrus picked just prior to the optimal picking time is distinguishable after fumigation and cold treatment from fruit picked at the optimal time and only receiving the same cold treatment.</u>

Early citrus cultivars evaluated in the period April to June were: Clementine or Nova, a Navel orange, and Star Ruby grapefruit. Late cultivars evaluated in July to September were Nadorcott mandarin, Midknight Valencia and Valencia. Twenty fruit from each cultivar were picked 1 week before optimal harvest time from the same side of 4 trees in an orchard (5 per tree) and fumigated with CO_2 at 70% in air for 24 h at 25°C, waxed, then stored for 13 days at 3°C. When the cold storage period was complete the fruit were moved to a cold room at 7°C for a week until another 20 fruit picked from the same trees a week later in the optimal picking window that was not fumigated but was waxed and stored for 13 days at 3°C were ready. All fruit were brought to ambient temperature before comparing taste and internal quality. The internal quality of fruit from both harvest times were tested by splitting 15 of the fruit from each harvest time into three replicates of 5 each and providing a separate result for Brix and acid for each replicate. The five remaining fruit from each harvest time were cut up and 5 different people with experience in tasting citrus were asked whether they could tell any difference between the two harvest times.

Results and discussion

1. <u>Compare susceptibility of FCM larvae in media with those in fruit</u>

Results with the commercial FCM medium from River Bioscience were disappointing as the fumigation for 24 h with CO_2 at 70% in air had a negligible effect (Table 3.2.4.1), presumably due to poor penetration.

Table 3.2.4.1. Mean percentage mortalities achieved with 5th instars in FCM media and fruit after fumigation and or cold at 3°C for 5 days

Treatment	Media	N-media	Fruit	N-fruit
Control	2.5	1868	7.9	507
CO_2 70% only	5.8	3348	38.7	398
Cold only	10.2	4080	12.1	436
CO_2 70% + cold	12.9	4003	66.9	1343

Fruit fly carrot-based media was also evaluated at 4°C with and without fumigation, but even though the cold period was as long as 17 days the mean mortality for cold alone was 41% and with prior fumigation, 66%. Mortalities for the same treatments in fruit were close to 100% (Table 3.2.4.2). We therefore could not use media to evaluate these sequential treatments as a substitute for fruit.

Table 3.2.4.2. Mean percentage mortalities achieved with 5th instar FCM in fruit fly media and Valencia fruit after fumigation and or cold

Treatments	Media	N-media	Fruit	N-fruit
Control			4.4	905
Cold only at 3°C for 13 days			98.6	1 025
CO_2 70% + Cold at 3°C for 13 days			97.5	3 637
Control			1.5	1 062
Cold only at 4°C for 17 days	41.0	1 479	100	520
CO_2 70% + Cold at 4°C for 17 days	66.1	2 976	98.2	1 900

2. Conduct validation trials with 5th instars in oranges using CO_2 at 70% for 24 h followed by 12, 13 or 17 days at 2, 3 or 4°C

The fruit results from comparisons with media are included in Table 3.2.4.3 with further treatments conducted with cold treatments at 2°C for 12 days and 4°C for 17 days. It was clear with these numbers of larvae per treatment that the cold alone at 4°C for 17 days or 2°C for 14 days provided 100% mortality. The best treatment with both CO_2 and cold was the fumigation followed by 2°C for 14 days with mean corrected mortality of 99.918% using 15 829 larvae in Valencias. A total of 13 larvae were found to move on prodding with this treatment, but only one larva survived to adulthood giving an adult mortality of 99.994%. This treatment was then used to compare infested fruit in open crates with export cartons when fumigated in a shipping container.

Table 3.2.4.3. Corrected mortalities of 5th instar FCM in Valencia fruit after cold alone or fumigation plus cold

Treatments	Mortality (%)	No. larvae
Cold only at 3°C for 13 days	98.571	1 025
CO_2 70% + Cold at 3°C for 13 days	97.382	3 637
Cold only at 4°C for 17 days	100.00	520
CO_2 70% + Cold at 4°C for 17 days	99.128	1 503
Cold only at 2°C for 12 days	99.589	2 199
CO_2 70% + Cold at 2°C for 12 days	99.205	5 785
Cold only at 2°C for 14 days	100.00	6 109
CO_2 70% + Cold at 2°C for 14 days	99.918	15 829

3. Can FCM 5th instars be controlled in fruit packed in export cartons that is fumigated and cold treated as effectively as in fruit in plastic crates?

The shipping container was not quite gas tight so the carbon dioxide had to be topped up in order to maintain levels close to 70% in air. Over the three replicates, after fumigation and the cold treatment, no larvae survived in the crates and only one larva survived in the export cartons (Table 3.2.4.4). However, after placing that larva on fruit fly medium it did not survive to the pupal stage. Treatments were not compared statistically because they were almost identical. It was encouraging to find that waxed, infested fruit packed in export cartons and stacked on a pallet were virtually as susceptible to the combined treatment as fruit that were fumigated before waxing in plastic crates. This means that the treatments could be applied after packing at another facility, if necessary.

Table 3.2.4.4. Results from three replicates to compare the susceptibility of fifth instar FCM in fruit that was waxed and packed in export cartons with larvae that were in unwaxed fruit, fumigated in plastic crates.

Replicate	Untreated control		Plastic crates		Export cartons	
	No. larvae	Mortality (%)	No. larvae	Mortality (%)	No. larvae	Mortality (%)

1	389	0.00	1 018	100.00	678	99.85
2	234	6.41	1 101	100.00	849	100.00
3	308	0.97	1 744	100.00	1 856	100.00
Totals & means	931	1.93	3 863	100.00	3 383	99.97

4. Is the treatment that is shown to be efficacious against FCM larvae equally effective against Medfly larvae?

Over four replicates the overall mortality of 26 320 second instar Medflies was 99.87% (Table 3.2.4.5), a similar result to the fifth instar FCM mortality of over 99.9%. These results verified that the combined treatment would be suitable for controlling both quarantine pests at a level similar to that of the current systems approach used for citrus going to Europe (Hattingh et al. 2020).

Table 3.2.4.5. Results from a trial to verify that the combined treatment of CO_2 fumigation followed by a cold treatment is efficacious for *Ceratitis capitata* second instars.

Replicate	**Untreated control**		**CO_2 70% followed by 2°C for 14 days**	
	No. larvae	**Mortality (%)**	**No. larvae**	**Mortality (%)**
1	1 366	4.39	7 617	99.82
2	2 570	0.00	5 755	99.83
3	3 662	1.50	8 196	99.89
4	2 890	1.52	4 752	99.98
Totals & means	10 488	1.52	26 320	99.87

5. Determine whether different types of citrus picked just prior to the optimal picking time is distinguishable after fumigation and cold treatment from fruit picked at the optimal time and only receiving the same cold treatment.

Very little difference in taste has been found between fruit picked one or two weeks earlier and fumigated, compared with fruit picked later and not fumigated, and few differences have been found in internal quality. It therefore appears that we can recommend that fruit to be fumigated is picked at the beginning of the picking window.

Conclusion

Although the fumigation of FCM larvae in growth medium could not be used as an alternative to fruit infestation, we were able to use adequate numbers of larvae in fruit to show that the combined efficacy of fumigation and cold treatment dropped with higher temperatures. Carbon dioxide fumigation followed by 14 days at 2°C pulp temperature gave mortality of fifth instar FCM in excess of 99.9%, whether oranges were fumigated and cold treated in export cartons or in plastic crates. This combined treatment gave similar efficacy of 99.87% mortality against second instar Medflies in Valencia oranges. There did not appear to be any distinguishable detrimental effect on internal fruit quality or taste with these treatments so it is likely that the treatment could be used on fruit early in the picking window.

Future research

No research is planned on this topic in the future. There is sufficient knowledge now to implement these treatments on a commercial scale in the event that the current systems approach is rejected.

Technology transfer

A talk was presented at the 2018 CRI citrus research symposium and a brief résumé provided of the most recent work in lieu of the 2020 CRI citrus research symposium.

Earlier research in this project was published as:

Grout, T.G., K.C. Stoltz. 2020. Carbon Dioxide Fumigation to Shorten Cold Disinfestation Treatments for *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) in Citrus Fruit. J. Econ. Entomol. 113 (1): 144-151.

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3.2.5 FINAL REPORT: Sexual attraction and mating compatibility between *Thaumatotibia leucotreta* populations and implications for semiochemical dependent technologies

Project 1202 (Jan 2018 – Dec 2019) by Jennifer Upfold, Candice Coombes, Martin Hill (RU) and Sean Moore (CRI)

Summary

The indigenous false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick), is a regulated citrus pest of many international markets for phytosanitary concerns. Considerable research efforts have been invested in the past decades to develop semiochemcial technologies, such as monitoring with sex pheromones, attract-and-kill, mating disruption and the sterile insect technique. One of the potential obstacles identified with semiochemical control is the differences in the ratio of the compounds comprising the sex pheromone at different geographical locations, resulting in what is known as regional attraction. This has been identified in FCM populations from three different countries, however, regional attraction within South African FCM populations was unknown. Therefore, the study identified five genetically distinct FCM populations from laboratory-reared colonies, originating from geographically isolated populations in South Africa. The FCM populations were named after their origin of Addo, Citrusdal, Marble Hall and Nelspruit. The fifth laboratory-reared population was found to be a mix of Addo and Citrusdal, called the “Old” colony. These five genetically distinct populations as well as a population from Xsit (Pty) Ltd, used for the sterile insect technique (SIT), were used in regional attractiveness and mating trials. The results showed that males were significantly (P <0.05) more attracted to females originating from the same population. Furthermore, regional attractiveness was assessed through choice/no-choice mating compatibility trials. Significant sexual isolation (ISI**) occurred between mating combinations Addo × Nelspruit (ISI = 0,13; $t2$ = 6.23; p = 0.02), Addo × Marble Hall (ISI = 0,11; $t2$ = 4.72; p = 0.04), Citrusdal × Nelspruit (ISI = 0,11; $t2$ = 4.95; p = 0.04), and Citrusdal × Marble Hall (ISI = 0,12; $t2$ = 4.31; p = 0.04). In these combinations, Addo and Citrusdal males were found to have outcompeted Nelspruit and Marble Hall males for more mating events. Significant sexual isolation was also recorded for Xsit × Marble Hall (ISI = 0.12; t2 = 4.98; p = 0.01) and Xsit × Citrusdal (ISI = 0.13; t2 = 3.96; p = 0.01) populations. The male relative performance index was significant in both combinations, indicating that other laboratory-reared males outcompeted the Xsit males in these two combinations. When given no choice, evaluated as spermatophore transfer/female/48 h, all males (including Xsit) were successful in transferring spermatophores to all FCM populations, with no significant differences. These results indicate that there may be incipient pre-isolation mechanisms affected by local natural selection, resulting in localised sexual attraction via differences in the sex pheromone isomer ratios. These findings provide important information for semiochemical technologies and the implication of these results with regard to monitoring with sex pheromones, attract-and-kill, mating disruption and sterile insect technique are discussed.

**ISI: The Index of Sexual Isolation (ISI) accounts for the number of pairs obtained for each possible mating combination, and ranges from -1, where most couples consisted of the second population listed, to 1 where most couples consist of the first population listed. A value of 0 represents random mating.

Opsomming

Die inheemse valskodlingmot (VKM), *Thaumatotibia leucotreta* (Meyrick), is 'n gereguleerde sitrusplaag vir baie internasionale markte as gevolg van fitosanitêre kommernisse. Die afgelope dekades is aansienlike navorsingspogings belê om semio-chemiese tegnologieë te ontwikkel, soos monitering met seksferomone, lok-en-doodmaak, paringsontwrigting en die steriele insekstegniek. Een van die moontlike struikelblokke wat met semiochemiese beheer geïdentifiseer is, is verskille in die verhouding van die verbindings wat die seksferomoon bevat in populasies van verskillende streke, wat lei tot wat bekend is as streeksaantrekking. Dit is geïdentifiseer in VKM -bevolkings uit drie verskillende lande, maar die moontlike verskille in streeksaantrekking tussen Suid-Afrikaanse VKM-bevolkings was tot nou toe onbekend. Daarom het die studie vyf geneties onderskeide VKM-bevolkings uit laboratorium-geteelde kolonies geïdentifiseer, afkomstig van geografies geïsoleerde bevolkings in Suid-Afrika. Die VKM-bevolkings is vernoem na hul oorsprong van Addo, Citrusdal, Marble Hall en Nelspruit. Die vyfde laboratorium geteelde kolonie was 'n mengsel van Addo en Citrusdal, en is bekend as die 'Ou' kolonie. Hierdie vyf geneties onderskeibare bevolkings sowel as 'n populasie van Xsit (Pty) Ltd, wat gebruik word vir die steriele insekstegniek (SIT), is in streeks-aantreklikheid en paringsproewe gebruik. Die resultate het getoon dat mannetjies beduidend (P = <0,05) meer aangetrokke was tot wyfies van dieselfde populasie. Verder is streeksaantreklikheid beoordeel deur keuse/nie-keuse-paring-verenigbaarheidsproewe. Betekenisvolle seksuele isolasie (ISI**) het plaasgevind tussen paringskombinasies Addo × Nelspruit (ISI = 0,13; t2 = 6.23; p = 0,02), Addo × Marble Hall (ISI = 0,11; t2 = 4,72; p = 0,04), Citrusdal × Nelspruit (ISI = 0,11; t2 = 4,95; p = 0,04), en Citrusdal × Marble Hall (ISI = 0,12; t2 = 4,31; p = 0,04). In hierdie kombinasies is daar gevind dat Addo- en Citrusdal-mannetjies meer meedingend was as mannetjies van Nelspruit en Marble Hall vir paringsgeleenthede. Beduidende seksuele isolasie is ook aangeteken vir Xsit × Marble Hall (ISI = 0.12; t2 = 4.98; p = 0.01) en Xsit × Citrusdal (ISI = 0.13; t2 = 3.96; p = 0.01). Die mannetjie relatiewe prestasie-indeks was in albei kombinasies beduidend, wat daarop dui dat mannetjies van die ander laboratorium kolonies meer mededingend was as die Xsit-mannetjies in albei hierdie twee kombinasies. As geen keuse gegee is nie, gemeet as spermatofooroordrag/wyfie/48 uur, was alle mans (Xsit ingesluit) suksesvol met die oordrag van spermatofore na alle VKM-populasies, met geen noemenswaardige verskille

nie. Hierdie resultate dui aan dat daar aanvanklike voor-isoleringsmeganismes kan wees wat deur plaaslike natuurlike seleksie beïnvloed kan word, wat kan lei tot gelokaliseerde seksuele aantrekking, deur verskille in die seksferomoon isomeer verhoudinge. Hierdie bevindings bied belangrike inligting vir semiochemiese tegnologieë en die implikasie van hierdie resultate met betrekking tot monitering met geslagsferomone, lok en doodmaak, paringsontwrigting en steriele insekstegnieke word bespreek.

** ISI: die Index of seksuele isolasie (ISI) is verantwoordelik vir die aantal pare wat vir elke moontlike paringskombinasie verkry is, en wissel van -1, waar die meeste paartjies uit die tweede bevolking bestaan, tot 1 waar die meeste paartjies uit die eerste bestaan bevolking gelys. 'n Waarde van 0 verteenwoordig ewekansige paring.

Introduction

FCM threatens the citrus industry, which is worth over twenty billion ZAR (CGA 2017). International markets expect high quality fruit, free of insect pests and diseases but more importantly the phytosanitary pest, FCM. Many of South Africa's current export partners have a zero tolerance for FCM, as the pest is not present in those countries. Therefore, if the pest is detected in an export consignment, the entire consignment can be rejected, resulting in substantial monetary loss. Multiple management options have thus been developed to protect the industry. Many of these rely on pheromones, namely monitoring, mating disruption, attract and kill and the sterile insect technique (SIT). The most recent sex pheromone analysis for female FCM indicates four major isomers comprising the pheromone: (E)-8-dodecenyl acetate, (E)-7-dodecenyl acetate, (Z)-8-dodecenyl acetate and dodecyl acetate (Levi-Zada et al. 2019). However, the relative composition of these isomers may change regionally (Angelini et al. 1981; Hall et al. 1984; Attygalle et al. 1986; Levi-Zada et al. 2019).

Further evidence of regionally diverse sex pheromones was recorded by Joubert (2017) who completed a study which found clear favouring by males for females from the same region. Another example of this phenomenon is displayed by male FCM from Mpumalanga, South Africa, that are not readily attracted by the commercial synthetic pheromone used in delta traps to monitor FCM population in the Western Cape Province (Mgocheki & Addison 2016). This could be problematic for control strategies that rely on the sex pheromone, such as SIT, where the sterile moths already have a disadvantage in reduced fitness over wild males. Reduced fitness from irradiation and transport, coupled with reduced sexual communication, may result in a considerable decrease in SIT efficacy. However, due to its success to date, SIT has been rolled out elsewhere in the country, including the Eastern Cape (Sundays River Valley and Gamtoos River Valley) and the Northern Cape, with potential to expand into Mpumalanga and Limpopo.

Due to the current control technologies utilising the sex pheromone, which offer unprecedented reduction in infestation, with no negative secondary environmental effects; it is important to identify if regional compatibility between geographically distinct populations of FCM occurs in South Africa. Additionally, the regional suitability of sterile moths must be determined, to avoid potential loss in efficacy of SIT if used in other locations across the country. Therefore, the primary aim of the research was to use genetically distinct FCM populations with differences in the ratio of the sex pheromone, in attraction and mating compatibility trials. If regional attractiveness was found to be an issue, strategies to overcome its effect need to be devised. However, a protocol for the extraction and analysis of the sex pheromone was trialled but optimisation of the technique was not achieved. Therefore, using genetic analysis, the laboratory-reared FCM populations were found to be genetically distinct and were used for the mating and compatibility trials. Further work on the differences in the ratio of the sex pheromone needs to be completed.

Stated objectives

i) To establish if five laboratory-reared populations, originating from different geographic locations are still genetically isolated using AFLP technique.

ii) To analyse ratios of pheromone compounds from genetically distinct populations (DID NOT COMPLETE)

iii) To determine regional attractiveness between males and females from different populations.

iv) To determine whether differences in pheromone compounds and preferences in regional attractiveness translate into differences in mating compatibility.

Materials and methods

i) AFLP technique developed by Vos et al. (1995), protocol used and followed with minor modifications as described by Paun & Schönswetter (2012). DNA extraction via salt extraction developed by Hunt (1997) (Pages 21-25, thesis).

iii) Mating attraction trials followed the same protocol as Joubert (2017) with minor modifications. Six delta traps were placed in a temperature controlled (>20°C) wind tunnel, 5 m apart and 1.5 m from the ground. Two females from the same population were housed in a plastic vial and placed in a trap. All five different populations were represented, including a control, comprising the sixth trap. Males from one population were released and recaptured on the sticky floor of the delta traps housing the females. The trial was repeated four times for each population (Pages 39-41, thesis).

iv) To determine mating compatibility, choice and no-choice trials were conducted (Pages 49-52, thesis).

Results and discussion

i) The results showed genetically distinct populations of laboratory-reared FCM originating from geographically separate locations in South Africa. These laboratory-reared populations may thus be used as a model to test differences in regional sexual attraction and mating compatibility. The “Old” population will be presented as a fifth population and not a mixed group as originally intended, as the genetics revealed it is not genetically similar to all South African FCM populations. If differences in attraction between regions are found in the genetically distinct laboratory populations, this will likely be occurring in genetically distinct wild populations as well and the implications for IPM strategies involving semiochemical control will need to be assessed. (Pages 26-34, thesis)

iii) Differences in population level attraction were observed. A significant attraction by males for females from their own population was found in the laboratory-reared moths. No significant preference was found by the sterile moths for any of the females of different origins (Addo, MarbleHall, Citrusdal, Nelspruit and “Old”). However, recapture rates were very low. These findings suggest geographic variation of the sex pheromone is highly likely in FCM populations across South Africa. In previous studies where behavioural bioassays have found a significant attraction of males and females from the same population, chemical analysis revealed differences in the sex pheromone ratio, and likewise where behavioural bioassays have shown no significant preference from males to females of the same population, no variations in the sex pheromones were found (Tòth et al. 1992; Watanabe & Shimizu 2017) (Pages 41-45, thesis).

iv) The following conclusions can be drawn from the sexual compatibility study results. Firstly, when given the choice between mating within their own population or with a different population, sexual isolation was observed between Addo × Nelspruit, Addo × Marble Hall, Citrusdal × Nelspruit and Citrusdal × Marble Hall. In these combinations, Nelspruit and Marble Hall males displayed reduced mating compatibility compared to Addo and Citrusdal. Secondly, sexual isolation also occurred between the sterile population when crossed with Marble Hall and Citrusdal, however, overall, the relative sterility index for these two populations remained close to the theoretical value for equal competitiveness between Xsit and non-Xsit males. Thirdly, Xsit males had consistently lower recorded spermatophore transfer, and when comparing the overall spermatophores transferred of all Xsit males and all non-Xsit males, more spermatophores were present in females paired with non-sterile males, albeit not significantly. Over all, the results demonstrate that mating compatibility via spermatophore transfer is successful for all FCM populations as well as the Xsit population. The Xsit males were outcompeted by males from Marble hall and Citrusdal resulting in a significant sexual isolation index. Further sexual isolation occurred between mating combinations of laboratory populations, Addo × Nelspruit, Addo × Marble Hall, Citrusdal × Nelspruit and Citrusdal × Marble Hall indicating that these populations have incipient pre-isolation mechanisms affected by local natural selection. These findings provide important information for semiochemical control technologies, especially the SIT programme against FCM and support the potential to use and release anywhere in the country (Pages 52-58, thesis).

Conclusion

Assortative mating by geographical regions of false codling moth was found, which could be due to different environmental conditions leading to genetic differentiation. This indicates an unstable sexual communication system for FCM, which is subject to variation between populations, as has already been shown in FCM populations from other countries (Angelini et al. 1981; Hall et al. 1984; Attygalle et al. 1986). However, the primary reasons for this have not yet been identified. Evidence of mating disruption and interference with other closely related sympatrically occurring species has driven sex pheromone variation in other Lepidoptera, such as codling moth (Duménil et al. 2014). However, available information on intraspecific variation in the sex pheromone is limited, with most studies considering interspecific pheromone variation, due to the general assumption that lepidopteran sex pheromones have very low variation because of their importance as species-recognition signals (Duménil et al. 2014; Groot 2014). Overall, the fragmented agricultural environment that leads to sedentary populations of FCM, increases the chances of developing different sex pheromone signals and developing resistance against mating disruption and other semiochemical-based control techniques. Therefore, it is important to monitor these changes between populations and alter pest management strategies for optimised control.

Future research

Overall, this study would have benefited from clearer recorded information on laboratory-reared population origins, in particular from the Xsit colony, as the origins for this population are unclear. Aside from the origins, information on the period of time maintained in the laboratory, as well as population crashes and sex ratios would be beneficial to further research conducted on these populations in any capacity, whether it be genetics, behavioural or physiological. Analysis of the female sex pheromones using sample enrichment probes (SEP) and analysed through gas chromatography coupled to mass spectrometry (GC-MS) was investigated but results could unfortunately not be generated. Due to the sensitivity of the SEP, the experimental design still needs to be optimised to reduce 'noise' in the mass spectrometry analysis. The method was developed for the extraction of the female *Margarodes prieskaensis* Jakubski (Coccoidea: Margarodidae) sex pheromone and was chosen as it will be able to indicate exactly what, how much and in what quantities the sex pheromone is released by the female FCM (Burger *et al.* 2016). Although the extraction of the entire gland has previously been conducted, modern methods using GC-MS will be able to identify the compounds that are released and therefore important in sexual attraction rather than identifying all compounds present in the gland (Angelini et al. 1981; Hall et al. 1984; Attygalle et al. 1986; Hofmeyr & Calitz 1991; Burger et al. 2016). The experiment still needs to be conducted to ensure that it is the variation in the sex pheromone that is causing regional attractiveness, and no other potential factors such as differences in peak pheromone release time and diet. However, since variation in the sex pheromone has already been found in geographically isolated populations (Angelini et al. 1981; Hall et al. 1984; Attygalle et al. 1986; Hofmeyr & Calitz 1991), it is reasonable to conclude that it is the primary reason for regional attractiveness.

Another aspect not discussed, but still important to emphasise, is the limited knowledge of intraspecific deviation in sex pheromone blends of female Lepidoptera and differences in pheromone blends within populations, which have occurred in other lepidopteran species, such as codling moth (Duménil et al. 2014). Therefore, it may be beneficial to investigate the within population variation in the sex pheromone of FCM, as well as the males broadening response, which would contribute to a decrease in the efficacy of all established semiochemical-based management technologies.

Technology transfer

Citrus Research Symposium 2018 – Poster presentation
Entomological Society of Southern Africa – 20 min presentation

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3.2.6 **FINAL 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. In the first year of the

study, 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. Our intention was that this method would be implemented when training resumed in April, when ripe fruit would again be available. Unfortunately, the two co-workers on the project from Citrus Pest Detection Dogs experienced irreconcilable differences, making it impossible for the project to continue. Consequently, the project was terminated at the start of the financial year, before any expenditure on the project was incurred.

Opsomming

Die vermoeë van ŉ snufferhond om VKM-besmette vrugte is in ŉ vorige geregistreerde navorsingsproef met ŉ 98.9% akuraatheid bewys. Hierdie is beduidend meer akuraat as die huidige menslike inspeksie sisteem wat gebruik word wat ‘n maksimum vermoeë van 77.8% het. Daar is egter twee potensiële struikelblokke tot die suksesvolle toepassing hiervan in sitruspakhuise. Eerstens kan inspeksie van kartonne of pallette in die pakhuis uiters arbeids intensifef wees en mag dalk verskeie honde en hondehandteerders benodig, veral in ŉ 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 ŉ afgeleë vlugtigestof opsporingsstelsel oorkom word, wat ŉ vakuum-pomp en absorberenede 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 ŉ snufferhond in enige geskikte perseel voorgestel word. Tot op hede is verskeie opsies vir die versameling van vlugtigestowwe ondersoek, soos onderdrukte lug met ŉ verstelbare drukklep wat die onderdrukte lug kan insuig en ook die druk kan beheer. ŉ Battery-krag vakuumpomp, met voldoende suigings vermoë, is vir hierdie doeleinde ontwikkel. Geskikte filters is geselekteer vir absorbsie van vlugtigestowwe wat van besmette en gesonde vrugte geëkstrakteer word, en die snufferhond, Max, is op die eersgenoemde ingeprent. Sean Moore het ŉ besoek gemaak aan die honde hanteerders en het ŉ 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. Ons doel was dat hierdie metode geïmplementeersou word wanneer opleiding in April 2019 hervat moes word, wanneer ryp vrugte weer beskikbaar sou wees. Ongelukkig het die twee mede-werkers op die projek, van Citrus Pest Detection Dogs, onversoenbare verskille gehad, wat dit onmoontlik gemaak het om met die projek voort te gaan. Gevolglik is die projek aan die begin van die finansiele jaar opgeskort, voor voordat enige uitgawes aan die projek aangegaan is.

Introduction

The ability of a sniffer dog has been demonstrated and proven within a previously registered research project (Moore et al. 2016a). A series of trials were conducted where the dog was offered 10 cartons of oranges, of which usually only one contained an infested fruit. From a total of 140 runs over 6 replications, the dog achieved 98.9% true positive detections, of which 81.2% were on the first passing. This is substantially more accurate than the currently used human inspection system, which has a maximum potential of 77.8% (Moore et al. 2016b). Therefore, there is potential for using dogs for postharvest detection of FCM infested fruit in cartons. However, there are a couple of potential hurdles to the successful implementation of this. 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. The speed and ease with which this can be conducted and the reduction in labour ought to be significant, thus also reducing costs. Such a system was developed by Joynt (2003) in 1993 for detecting explosives and was termed the Remote Explosive Scent Tracing (REST) (Hayter 2003). Stan Gillham was instrumental in assisting Dr Joynt with development of the system for Mechem.

Stated objective

To develop a remote vapour detection system for sniffer dog detection of FCM infested fruit packed for export.

Materials and methods

A device for collection and retention of volatiles was built. Filters were obtained from the USA and housed in custom-made plastic holders, which fitted on the vacuum device. Healthy citrus fruit were infested with neonate FCM larvae by placing larvae onto fruit immediately after hatching and allowing them to penetrate. Within a few days and as soon as successful penetration of fruit could be confirmed, these fruit were sent to Citrus Pest Detection Dogs for exposure of both infested and healthy fruit to absorbent filters.

Four to five healthy fruit and the same number of infested fruit were placed into two separate cardboard boxes. Each box had a small hole, just sufficient for the point of the vacuum device, with a maximum sealed suction of 3.9 kPa. Headspace was suctioned from each box for 60 s per filter, with seven filters from the healthy fruit and one fruit the infested fruit on each occasion. Filters in holders were then placed in random order on stands at dog-head height, positioned in a straight line approximately 1.5 m apart. The sniffer dog was then instructed to sniff the filters in order and would sit when he detected the filter used for the infested fruit. After each run, the position of the infested filter was changed. Several runs were conducted before the dog was rested and fresh volatiles were suctioned for further runs.

Healthy and infested fruit were couriered to the dog handlers on a weekly basis, so that there was always recently infested non-decaying fruit available for imprinting.

Once a thorough imprinting process had taken place, Sean Moore visited the dog handling process and assessed the performance of the dog. Filters of various ages and filter holders were also taken back to Rhodes University, where GCMS was conducted with each to determine retention of fruit volatiles.

Results and discussion

In observing the performance of the sniffer dog, it was clear that he was not able to detect volatiles associated with fruit infestation as easily as he was recorded to do directly from the fruit. Successful detection of the correct filter was sporadic and thus no statistical recording of performance was conducted.

Through GCMS, no volatiles were detected in any of the filters or filter holders. This indicated that it was likely necessary that the filters should be frozen immediately after suctioning of volatiles in order to retain their volatiles.

Our intention was to resume the study in April when ripe Navel oranges were once again available. Unfortunately, a disagreement occurred between the two external collaborators, and they could not be reconciled. Consequently, the project was terminated and the funding withdrawn.

Conclusion

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. Unfortunately, it has been necessary to prematurely terminate this project due to irreconcilable differences between our two collaborators.

Future research

It has been irrefutably proven that a dog has the ability to detect FCM infested fruit, at an early stage of infestaton, and to differentiate this from healthy fruit, at a higher level of accuracy than any other technology tested to date. If appropriate collaborators are identified in the future, this research can be resumed.

Technology transfer

None.

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

Project 1039 (April 2012 – March 2020) by Sean Moore, Wayne Kirkman, Peter Stephen, Sean Thackeray, Mellissa Peyper, Kim Stolz, Tim Grout and Vaughan Hattingh (CRI)

Summary

As of 1 January 2018, FCM was regulated as a phytosanitary organism by the European Union. The regulation requires citrus 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. The mortalities of the most cold-tolerant larval stages of FCM, using several time-temperature combinations, have been determined. However, results at 4.5°C and 5°C showed a high degree of variability, probably because insecticidal efficacy was significantly reduced at these temperatures, and it was thus necessary to conduct further replicates at these regimes. Trials were completed, resulting in a clear trend in mortality at 4.5°C for different durations, ranging from 88.12 to 97.91% at durations of 16 to 26 days. Results at 5°C were improved, but the mortality trend at durations from 16 to 26 days was not as neat, with a couple of outliers still recorded. Nonetheless, mortality ranged from 48.82 to 93.74% at these durations. Results from these time-temperature combinations have been included in the FCM systems approach for chilling sensitive cultivars. Currently trials to compare the cold susceptibility of seven regional cultures is underway. These cultures will be exposed to 3°C at durations from 16 to 26 days. Five of the seven cultures are reared at Rhodes University, Eastern Cape, they are Addo, Nelspruit, Marble Hall, Old and Citrusdal. One culture each is reared at River Bioscience in Hermitage, Eastern Cape, and the last at XSit in Citrusdal, Western Cape.

Opsomming

Vanaf 1 Januarie 2018 is VKM gereguleer as 'n fitosanitêre organisme deur die Europese Unie, Die regulasie vereis dat sitrus vrugte vanaf 'n VKM vrye-area of plek van produksie verkry word, of 'n koue behandeling ontvang of enige ander behandeling wat kan verseker dat die versending vry van VKM is. Koue ontsmetting van sitrus vrugte vanaf Suid-Afrika na Europa in nie haalbaar nie, as gevolg van die relatiewe kort verskepingstydperk, veral vanaf die Kaapstad hawe, maar dit is ook onmoontlik as gevolg van die hoë volumes vrugte wat uitgevoer word na Europa en die onvoldoende infrastruktuur om die koue behandelings te fasiliteer van hierdie groot volumes. 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. Resultate by 4.5° en 5°C het egter 'n hoë graad van veranderlikheid getoon, waarskynlik omdat die insekdodende effek aansienlik verlaag is by hierdie temperature, en dit was daarom nodig om verdere herhalings te doen by hierdie regimes. Proewe is voltooi, wat 'n duidelike tendens in mortaliteit toon vir 4.5°C vir verskeie tydperke, wat wissel vanaf 88.12 tot 97.91% by tydperke van 16 tot 26 dae. Resultate vir 5°C is verbeter, maar die mortaliteit tendens vir die tydperk 16 tot 26 dae was nie so netjies nie, met 'n paar uitskieters steeds aangeteken. Nietemin het mortaliteit gewissel vanaf 48.82 tot 93.74% by hierdie tydperke. Resultate van hierdie nuwe tyd-temperatuur kombinasies is nou ook in die VKM stelselsbenadering ingesluit vir koue gevoelige kultivars. Tans is proewe onderweg om die koue vatbaarheid van sewe streeks kulture te vergelyk. Hierdie kulture word blootgestel aan 3°C vir tydperke van 16 tot 26 dae. Vyf van hierdie kulture word geteel by Rhodes Universiteit, Oos-Kaap, en hulle is die Addo, Nelspruit, Marble Hall, Ou en Citrusdal kulture. Een kultuur elk word geteel by River Bioscience in Hermitage, Oos-Kaap en XSit in Citrusdal, Wes-Kaap.

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

Project 1060 (2013/4, 2015/6-2019/20) by T G Grout and P R Stephen (CRI)

Summary

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

Opsomming

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

3.2.9 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 these same trends were not observed, mainly due to variability in D-limonene levels as a result of extremely unusual climatic conditions in the Eastern Cape, which resulted in excessive splitting, fruit drop and scorching of fruit and trees. In the trials conducted in 2018 on infested Washington and Witkrans Navel oranges, as well as Midknight 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. In 2019, GCMS trials were repeated on all the same cultivars as the previous year. In all cases, except for Clementine mandarins, D-Limonene levels decreased 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, as in 2018, there was a significant increase in beta-Ocimene levels with time after infestation. Discussions were held with Nukor, who use an online X-ray tomography unit, supplied by Biometic, to detect defects inside logs for the forestry industry. This technology could prove useful in postharvest FCM detection in citrus, and collaborative research is planned. Opportunities to explore Selected Ion Flow Tube Mass Spectrometry (Sift-MS) in collaboration with the University of Leuven in Belgium are also being explored.

Opsomming

Vorige studies het getoon dat ŉ Soliede Fase Mikro-ekstraksie (SPME) bo-damp vlugtige stowwe wat skoon vrugte omring, effektief kan opvang en konsentreer. Opsporing van vlugtige verbindings is met ŉ Gas Chromatografie-Massaspektrometrie (GCMS) sisteem gedoen. GCMS-analises is op vyf belangrike vlugtige verbindings van sitrus gedoen: D-limonien, 3,7-dimetiel-1,3,6-oktatrieen, (E)-4,8-dimetiel-1,3,7-nonatrieen, cariofeleen 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 ŉ beduidende verskil in die verhouding van hierdie verbindings tussen gesonde en besmette vrugte. In soortgelyke proewe in 2017 is hierdie tendense nie waargeneem nie, hoofsaaklik weens die variasie in die D-limonien vlakke, wat veroorsaak is deur uiters ongewone klimaatstoestande wat gelei het tot grootskaalse vrugbars, vrugval en skroei van vrugte en bome. In proewe wat in 2018 op besmette Washington en Witkrans Navellemoene, asook Midknight 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 ŉ betekenisvolle toename in beta-osimeen vlakke met tyd na besmetting met onopspoorbare vlakke in die kontroles. In 2019 is GCMS proewe herhaal op dieselfde kultivars as die vorige jaar. In alle gevalle, behalwe Clementine mandaryne, het die D-limonien vlakke met tyd na besmetting afgeneem, terwyl vlakke van naftaleen toegeneem het. Daar was ten alle tye ŉ beduidende verskil in die verhouding van hierdie verbindings in gesonde en besmette vrugte. In Clementine mandaryne was daar weereens ŉ betekenisvolle toename in beta-osimeen vlakke met tyd na besmetting. Besprekings is gehou met Nukor, ŉ maatskapy wat ŉ X-straal tomografie eenheid, verskaf deur Biometic, gebruik om defekte in hout op te spoor vir die bosboubedryf. Die tegnologie kan nuttig wees vir die na-oes opsporing van VKM in sitrusvrugte, en samewerking is beplan. Moontlikhede vir verdere samewerking met die Universiteit van Leuven in België om die vermoe van hulle Geselekteerde Ioon-vloei Buis-massaspektrometrie" (Sift-MS) eenheid om VKM in sitrusvrugte op te spoor is ondersoek.

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

Project 1162 (April 2017 – March 2020) 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 field trial conducted 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. Butyl sulphide, glacial acetic acid, pear ester and combinations of the above were tested, as well as a 4K blend which consists of pear ester, linalool oxide pyranoid (LOX), acetic acid and nonatriene. None of the volatiles tested thus far have demonstrated 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 ŉ lokaas van gesintetiseerde wyfie mot seksferomoon, en lok dus net mannetjie motte. So ŉ 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 die area nie. Identifikasie van ŉ lokmiddel vir wyfies sal ŉ meer akkurate moniterings tegniek moontlik maak. Vorige werk het ŉ paar vlugtige stowwe en mengsels van verbindings geïdentifiseer wat belofte inhou vir aanlokking van volwasse wyfie VKM. ŉ Veld proef 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 is getoets in die laboratorium. ŉ Reeks hok proewe word tans gedoen deur 50 ongepaarde wyfie motte in die hok vry te laat. ŉ Geel delta lokval met ŉ taai vloer, gelaai met die gevulde vlugtige stof vrysteller word windop van die motte geplaas. Butielsulfied, asynsuur, peer-ester en mengsels van bogenoemde is getoets, sowel as ŉ 4K mengsel wat peer-ester, linalooloksied piranoiëde (LOX), asynsuur en nonatriene bevat. Proewe is tans onderweg, met geen van die vlugtige stowwe wat sover ŉ 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, Mellissa Peyper (CRI), Clarke van Steenderen, Tammy Marsberg, Candice Owen, 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 and 2019, comparing recaptures of moths reared on a trehalose augmented diet with those reared on the normal diet. Recaptures were higher for trehalose moths for both years. Additionally, trap catches indicated that trehalose-fed moths survived longer in the field than control moths. In order to further examine the validity of these results, the CTmin (critical thermal minimum) of moths with and without trehalose was compared and found to be significantly lower with certain doses of trehalose. 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 from sterile males to wild females (River Bioscience laboratory reared moths were used as 'wild') with 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. The conclusion from this trial was that laboratory based spermatophore transfer trials can be used as a reliable quality control measure for sterile males, as there was a statistically significant regression between spermatophore transfer and mating incidents. However, these trials are currently being repeated using 'wild' moths that are exposed to the same storage and transport methods as sterile moths. This is to determine whether the storage and transport has an effect on mating incidence. Data are currently being analysed. 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 a result of F1 steriles. Currently AFLPs have been unsuccessful due to impure DNA from larvae, as larvae contain mouth and gut microbes that disturb the signals in AFLPs. An alternative method was attempted. This involved sequencing the ITS gene region; however, there was not enough variability in the sequences to allow us to determine whether a larva was an offspring of a F1 sterile coupling. Further optimisation of the AFLPs will be tested. If these optimisations do not work, an alternative method will be required.

Opsomming

Die steriele insek tegniek (SIT) vir VKM is kommersieel in sitrus in Suid-Afrika 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, wanneer dit by die larwe dieet bygevoeg word. Gevolglik is 'n proef gedurende herfs en winter van 2018 en 2019 uitgevoer wat die hervangs van motte wat op 'n trehalose dieet geteel is vergelyk is met motte wat op die gewone dieet geteel is. Hervangste was hoër vir die trehalose motte vir albei jare. Daarbenewens, het vangs aangedui dat trehalose motte langer in die veld oorleef as kontrole motte. Om die geldigheid van hierdie resultate verder te ondersoek, is die CTmin (kritieke termiese minimum) van motte met en sonder trehalose vergelyk en dit is gevind dat dit aansienlik laer was by sekere dosisse trehalose. 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 veldhok proef om die parings voorvalle tussen steriele en wilde motte te vergelyk. Wilde mannetjies het aansienlik hoër spermatofoor oordrag getoon 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 met steriele mannetjies in die hokke en spermatofoor oordrag in laboratoriumtoetse. Die gevolgtrekking uit hierdie proef was dat laboratoriumgebaseerde spermatofooroordragproewe as 'n betroubare maatreël vir kwaliteitsbeheer vir steriele mannetjies gebruik kan word, aangesien daar 'n statistiese beduidende regressie tussen spermatofoor oordrag en parings gevalle was. Hierdie proewe word egter tans herhaal deur gebruik te maak van 'wilde' motte wat blootgestel word aan dieselfde bergings- en vervoermetodes as steriele motte. Dit is om vas te stel of die berging en vervoer 'n invloed op die voorkoms van paring het. Data is besig om ontleed te word. Ten slotte word AFLP toetse ontwikkkel om te onderskei tussen wilde larwes en Xsit-larwes, om te bepaal of enige larwes wat vrugte in die veld besmet, van F1-steriele motte afkomstig is. AFLPs is tans nie suksesvol nie as gevolg van onsuiwer DNA van larwes. Aangesien larwes mond en ingewande-mikrobes bevat word die seine in AFLPs versteur. Daar is gepoog om 'n alternatiewe metode te gebruik. Dit behels die volgorde van die ITS-geenstreek, maar daar was nie genoeg variasie in die rye om te bepaal of 'n larwe 'n nakomeling van 'n F1-steriele koppeling was nie. Verdere optimalisering van die AFLP's sal getoets word. As hierdie optimalisering nie werk nie, sal 'n alternatiewe metode benodig word.

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

Project 1221 (April 2018 – Dec 2019) by Marelize de Villiers, Sean Moore, Vaughan Hattingh (CRI), Craig Chambers (River Bioscience), Nevill Boersma, Clarissa Mouton (Xsit)

Summary

Since 2007, the sterile insect technique (SIT) for FCM has been implemented commercially in citrus in South Africa. Despite relatively good success, some shortcomings in moth quality have been identified. These include 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 lower level of sterility of irradiated moths than originally observed. Currently, moths are reared at Xsit in Citrusdal and cold immobilised during transport to release sites in the Western and Eastern Cape. However, due to the negative impact of cold immobilization on fitness and performance of the moths in the field, an alternative to cold immobilization needs to be investigated. Immobilization by anoxia (an absence of oxygen) was tested. 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 anoxia treatments to have a positive effect on longevity, but flight ability was impaired. Due to the latter, anoxia is not considered a viable option to replace cold immobilisation during irradiation. 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. Sterility trials were conducted to determine if premating of moths prior to irradiation has a negative impact on the efficacy of irradiation. More experiments need to be done to draw meaningful conclusions. The effect of cooling of moths during irradiation as opposed to moths being active was also investigated, indicating that cooling may suppress egg hatch. The primary investigator on this project, Marelize de Villiers, has resigned. The project will now be conducted at Xsit in Citrusdal, by the Xsit researcher, Liana de Araujo.

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. Dit sluit in die negatiewe effek van koue-immobilisering op die fiksheid van die motte, 'n hoër temperatuur aktiwiteitsdrempel van laboratorium-geteëlde bestraalde motte in vergelyking met wilde motte, en 'n laer vlak van steriliteit van die bestraalde motte as oorsponklik waargeneem. Huidiglik word motte by Xsit in Citrusdal geteël en met koue geïmmobiliseer gedurende vervoer na vrylatingspunte in die Wes- en Oos-Kaap. Weens die negatiewe impak van koue-immobilisering op fiksheid en gedrag van motte in die veld, moet 'n alternatief vir koue-immobilisering egter ondersoek word. Immobilisering deur anoksie ('n afwesigheid van suurstof) is getoets. 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 aangedui dat anoksie behandling 'n positiewe effek op die langlewendheid van motte gehad het, maar vliegvermoë benadeel het. Weens laasgenoemde word anoksie nie as 'n geskikte opsie om koue-immobilisering tydens bestraling te vervang beskou 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. Steriliteitsproewe is gedoen om te bepaal of die paring van motte voor bestraling 'n negatiewe effek op die effektiwiteit van bestraling het. Meer eksperimente is nodig om betekenisvolle gevolgtrekkings te maak. Die effek van verkoeling van motte gedurende bestraling in stede van motte wat aktief is, is ook ondersoek en het aangedui dat verkoeling die uitbroei van eiers onderdruk. Die hoof navorser op hierdie projek, Marelize de Villiers, het bedank. Navorsing op hierdie projek sal nou deur Xsit te Citrusdal voortgesti word, deur die Xsit navorser, Liana de Araujo.

3.2.13 PROGRESS REPORT: Field trials for control of FCM

Project 1225 (April 2019 – March 2020) by Sean Moore, Wayne Kirkman, Mellissa Peyper (CRI), Tammy Marsberg, Luke Cousins (RU)

Summary

False codling moth (FCM) has recently been declared a regulated pest by the EU. Due to this regulation it has become imperative to develop and test new control options for FCM. These control options include new and experimental chemical and biological products. Orchards with a low level of infestation will be selected in the Sundays River Valley to test the required products. A new protocol was developed for a semi-field trial, where trees were sprayed in the orchard and fruit collected at different intervals and inoculated in the laboratory. Eight adjacent trees were sprayed per treatment, including an untreated control. Thirty fruit were collected from both the northern and southern side of the tree to establish if there was a difference in efficacy. Fruit were collected at the following intervals: 1 day, 1, 2, 4, 6 and 8 weeks. In the laboratory each fruit was inoculated with four neonate FCM larvae. After two weeks the fruit were dissected and the larval instar for each fruit recorded. The first semi-field trial was initiated in January 2020, and a second one in February 2020. These trials evaluated both the efficacy of biological and chemical products for FCM control. In the first trial Delegate, Exirel, Warlock, Runner, Coragen and Cryptogran were evaluated. Runner was the most effective of the

chemicals, with all the others being of similar efficacy. Runner remained effective for 8 weeks, whereas the other products were only effective for 4 or 6 weeks. Cryptogran was effective on the southern (shady) aspect of the tree for 6 weeks but less than 2 weeks on the northern (sunny) aspect. In the second trial the efficacy of a range of experimental biological and chemical treatments was compared, including a synergism combination between two chemical products. Synergism did not appear to be evident in the field. One of the biological products showed good promise.

Opsomming

Valskodlingmot (VKM) is onlangs as ŉ gereguleerde plaag verklaar deur die EU. As gevolg van hierdie regulasie is dit noodsaaklik om nuwe beheer opsies vir VKM te ontwikkel en toets. Hierdie opsies sluit nuwe en eksperimentele chemiese en biologiese beheer produkte in. Boorde met lae vlakke van VKM gaan in die Sondagsrivier Vallei gekies word om hierdie produkte te toets. ŉ Nuwe protokol is ontwikkel vir ŉ semi-veldproef waar bome in die boorde gespuit is en vrugte op verskillende tussenposes gepluk en in die laboratorium ingeënt is. Agt tot 10 aangrensende bome is per behandeling gespuit, insluitende ŉ onbehandelde kontrole. Dertig vrugte word vanaf beide die noordelike en suidelike kant van die boom gepluk om vas te stel of daar ŉ verskil is in die doeltreffendheid van die produk. Vrugte word op die volgende tussenposes gepluk: 1 dag, 1, 2, 4, 6 en 8 weke. In die laboratorium word elke vrug met vier pasuitgebroeide VKM larwes ingeënt. Na twee weke word die vrugte gedissekteer, en die larwe instar aangeteken. Die eerste semi-veldproef is in Januarie 2020 begin, en die tweede een in Februarie 2020. Hierdie proewe het die doeltreffendheid van beide biologiese en chemiese produkte vir die behandeling van VKM getoets. In die eerste proef Delegate, Exirel, Warlock, Runner, Coragen en Cryptogran is geëvalueer. Runner was die mees doeltreffend van die chemiese produkte en die ander se werking was soortgelyk. Runner se werking het vir 8 weke gehou, waar die ander produkte het net vir 4 of 6 weke gewerk. Cryptogran was vir 6 weke op die skadu kant van die boom doeltreffend, maar minder as 2 weke op die sonnige kan van die boom. In die tweede proef is die werking van ŉ reeks eksperimentele biologiese en chemiese produkte vergelyk, insluitend ŉ sinergistiese kombinasie van twee chemiese produkte. Geen duidelike sinergisme kon gemeet word nie. Een van die biologiese produkte se werking was belowend gewees.

3.2.14 PROGRESS REPORT: Synergism between insecticides for improved control of FCM

Project 1226 (April 2019 – March 2021) by Sean Moore and Tamryn Marsberg (CRI)

Summary

An insect growth regulator (IGR), which binds to the ecdysone receptor complex in lepidoteran larvae and mimics insect moulting, resulting in early death, was selected. This was because its mode of action is similar to that achieved by deletion of the ecdysteroid glycosyl transferase (egt) gene in baculoviruses i.e. moulting, suppressed by the egt gene, is restored, resulting in a more rapid death. Consequently, bioassays were conducted against *Thaumatotibia leucotreta* larvae, with the IGR and a baculovirus to test for synergism. Bioassays were analysed using the Tammes-Bakuniak graphical method. During 2019, two surface dose methods were used to determine which approach would be more appropriate for testing for synergism. Variable results were obtained with these two methods. However, the method selected to continue testing synergism was to combine a constant LC value of the IGR, mixed with a serial dilution of the baculovirus. This was then repeated in 2020. Synergism was recorded at the LC_{90} for three of the treatments; the LC_5 of the IGR with the serial dilution of the baculovirus, the LC_{10} of the IGR with the serial dilution of the baculovirus and the LC_{50} of the IGR with the serial dilution of the baculovirus. These LC_{90} treatments will then be used to test lethal times, in order to determine if a faster speed of kill of the baculovirus can be accomplished with the addition of the IGR. Once synergism and improved speed of kill is adequately tested, these combinations will be tested in the field.

Opsomming

ŉ Insekgroeireguleerder (IGR), wat bind aan die ekdisoon reseptor kompleks in Lepidoptera larwes en insek vervelling naboots, wat lei tot vroeë dood, is gekies. Dit was omdat die werking daarvan soortgelyk is aan die wat verkry word deur die verwydering van die ekdysteroïde glukosiel transferase (egt) geen in bakulovirusse,

m.a.w. vervelling, onderdruk deur die egt-geen, word herstel, wat lei tot ŉ vinniger dood. Gevolglik is biotoetse teen *T. leucotreta* larwes uitgevoer, met die IGR en ŉ bakulovirus om te toets vir sinergisme. Biotoetse is met behulp van die Tammes-Bakuniak grafiese metode geanaliseer. Gedurende 2019 is twee oppervlakdosis metodes gebruik om te bepaal watter benadering meer geskik sou wees om vir sinergisme te toets. Met hierdie twee metodes is verskillende resultate verkry. Die metode wat gekies is om sinergisme verder te toets, was om ŉ konstante LC-waarde van die IGR te kombineer, gemeng met ŉ reeksverdunning van die bakulovirus. Dit is daarna in 2020 herhaal. Sinergisme is by die LC_{90} aangeteken vir drie behandelings: die LC_5 van die IGR met die reeksverdunning van die bakulovirus, die LC_{10} van die IGR met die reeksverdunning van die bakulovirus en die LC_{50} van die IGR met die reeksverdunning van die bakulovirus. Hierdie LC_{90} behandelings word dan gebruik om die dodelike tye te toets, om vas te stel of ŉ vinniger dodelike spoed van die bakulovirus bereik kan word met die toevoeging van die IGR. Sodra sinergisme en verbeterde snelheid van doodmaak voldoende getoets is, sal hierdie kombinasies in die veld getoets word.

3.2.15 PROGRESS REPORT: FCM population phenology in the warm northern citrus production regions and implications for management practices

Project 1227 (April 2019 – March 2021) by Hannah Otto (InsecTec), Lezel Beetge (Noordchem) and Sean Moore (CRI)

Summary

Control measures for FCM have traditionally not been initiated earlier than November, an approach which has generally proved successful. However, the bulk of research conducted to determine the effectiveness of these management practices and the timing of applications, have been conducted in the cooler Cape regions. In these regions, FCM activity generally only starts to pick up to meaningful levels during November, when evening temperatures become sufficiently warm for FCM activity to increase. However, in the northern citrus production regions, particularly Limpopo Province, evening temperatures can reach these levels already in August. Consequently, initiation of control measures only in November is a lot later than ideal. This pertains particularly to the use of area-wide population suppression technologies, such as SIT and mating disruption, which need to be initiated very early in the season, while FCM levels are still very low, to achieve optimal efficacy. Consequently, a study is being conducted to determine exactly when evening temperatures in the northern production regions increase to the moth's activity thresholds and when these increases in activity are first detected. Ten orchards have been selected in the Letsitele and Hoedspruit regions and are being monitored weekly for FCM moth catches in pheromone-baited traps. Simultaneously, temperature and humidity in these orchards are being logged. From April to mid-July 2019 very few moths were caught. However, from the second week in July, moth catches escalated. This first peak in FCM catches was a lot earlier than in the Cape regions. Moth activity continued to escalate, with the highest peak in catches recorded thus far, being in the first week of March 2020. By the beginning of April, this had dropped to a similarly low level as at the same time during 2019. However, moth catches during 2020 have so far been a lot higher than during 2019.

Opsomming

Beheermaatreëls is tradisioneel nie vroeër as November vir VKM ingestel nie, 'n benadering wat oor die algemeen sukses getoon het. Die grootste deel van die navorsing wat gedoen is om die doeltreffendheid van hierdie bestuurspraktyke en die tydsberekening van toedienings te bepaal, is egter in die koeler Kaapse streke gedoen. In hierdie streke begin VKM-aktiwiteit meestal eers in November tot die betekenisvolle vlakke toe te neem, wanneer aandtemperature warm genoeg word vir verhoging in VKM-aktiwiteit. In die noordelike sitrusproduksiestreke, veral Limpopo Provinsie, kan die aandtemperature egter reeds in Augustus hierdie vlakke bereik. Gevolglik is die inisiëring van beheermaatreëls eers in November heelwat later as ideaal. Dit is veral belangrik vir die gebruik van areawye populasie onderdrukkings tegnologieë, soos SIT en paringsontwrigting, wat baie vroeg in die seisoen geïnisieer moet word, terwyl die VKM-vlakke nog baie laag is, om optimale effektiwiteit te verkry. Gevolglik word 'n studie tans onderneem om vas te stel presies wanneer die temperature in die noordelike produksiestreke tot die aktiwiteitsdrempelwaardes van die mot toeneem en wanneer die toename in aktiwiteit die eerste keer opgespoor kan word. Tien boorde is in die Letsitele- en Hoedspruit-streke gekies en word weekliks gemonitor vir VKM vangstes in feromone-lokvalle. Terselfdertyd

word temperatuur en humiditeit in hierdie boorde aangeteken. Van April tot middel Julie 2019 is baie min motte gevang. Vanaf die tweede week in Julie het motvangste egter toegeneem. Hierdie eerste piek in die vangste van VKM was heelwat vroeër as in die Kaapse streke. Motaktiwiteit het verder toegeneem, met die hoogste vangste wat tot dusver aangeteken is, in die eerste week van Maart 2020. Teen die begin van April vlakke gedaal tot 'n soortgelyke lae vlak as in dieselfde tyd gedurende 2019, maar mot vangste gedurende 2020 was tot dusver heelwat hoër as gedurende 2019.

3.2.16 PROGRESS REPORT: An assessment of the reasons for lower FCM infestation in organic versus conventional citrus orchards

Project 1253 (2019/8-2021/9) by Luke Cousins, Sean Moore (CRI), Martin Hill (RU), Mellissa Peyper, Candice Coombes (CRI) and Antoinette Malan (SU)

Summary

Significantly lower levels of FCM infestation are recorded in fruit from organic than fruit from conventional orchards, in packhouse assessments in the Eastern Cape. Conventional wisdom might conclude that naturally occurring biological control would be greater on an organic farm. However, this cannot simply be assumed. Consequently, this study is evaluating all of the possible factors that could be contributing to this difference, namely above and below ground biocontrol, fruit biochemistry and farming practices. The progress of the project is on schedule. Analysis of FCM ecology has been underway since October 2019. Numbers of wild FCM caught within study sites have been very low, showing no clear difference between farming practices yet. Due to the low FCM numbers, egg counts have also been very low with no clear differences. Three soil samples have been taken and analysis of the last sample is almost concluded. So far, there have been no clear differences in abundance between EPFs and EPNs in organic and conventional soil. There does seem to be a difference in EPF species between the organic and conventional farms in Kirkwood which will be confirmed by further samples and final genetic identification. The early season fruit susceptibility and oviposition preference studies have been completed. Results are showing no clear difference in susceptibility so far. There was an observed oviposition preference for organic fruit in the choice experiments, however, the no-choice experiments showed no significant difference. (It is expected that later season trials will reveal something). The late season fruit susceptibly trials are currently underway. Pitfall trap specimen collection has been added to the project and two collections have been completed so far. Analysis of these samples is still taking place. Wet analysis of early stage Palmers and Delta Valencias has been completed by Bemlab laboratories, from which we are awaiting a report. Ripe fruit will be harvested in the next month for a second wet analysis by Bemlab. A separate dry fruit analysis of the ripe fruit remains to be conducted by CAL laboratories. Fruit physical measurements and juice analysis has been completed for immature and ripe Palmer navels, with the Deltas to be completed shortly. Results from these experiments are being processed currently.

Opsomming

Aansienlike laer vlakke VKM besmetting word aangeteken in vrugte van organiese as vrugte van konvensionele boorde, in pakhuis beoordelings in die Oos-Kaap. Konvensionele wysheid kan tot die gevolgtrekking kom dat die biologiese beheer wat natuurlik voorkom baie meer op 'n organiese plaas sou wees. Dit kan egter nie bloot aanvaar word nie. Gevolglik evalueer hierdie studie al die moontlike faktore wat tot hierdie verskil kan bydra, naamlik bo- en ondergrondse biologiese beheer, vrug biochemie en boerderypraktyke. Die vordering van die projek is volgens skedule. Analise van die VKM ekologie is sedert Oktober 2019 aan die gang. Die getalle wilde VKM wat op studiepersele gevang is, was baie laag, en toon nog geen duidelike verskil tussen boerderypraktyke nie. Weens die lae VKM getalle was die eiertelling ook baie laag met geen duidelike verskille nie. Drie grondmonsters is geneem en ontleding van die laaste monster is amper voltooi. Daar was tot dusver nog geen duidelike verskille in voorkoms tussen entomopatogeniese swamme en entomoptogeniese nematodes in organiese en konvensionele grond nie. Dit lyk asof daar 'n verskil is in die swam-spesies tussen die organiese en konvensionele boerderye in Kirkwood, wat bevestig sal word deur verdere monsters en finale genetiese identifikasie. Die vrugsensitiwiteit en die voorkeuring van die vrugte in die vroeë seisoen is voltooi. Die resultate toon tot dusver geen duidelike verskil in die vatbaarheid nie. Daar was 'n waarneembare voorkeur vir eierlegging op organiese vrugte in die keuse-proewe, maar die geen-keuse-proewe het geen noemenswaardige verskil getoon nie (daar word verwag dat proewe in die latere seisoen iets

sal oplewer). Die laat seisoen vatbaarheid proewe is tans aan die gang. Die insameling van slaggatvalle is by die projek gevoeg en twee versamelings is tot dusver voltooi. Die ontleding van hierdie monsters vind nog steeds plaas. Nat ontleding van Palmers en Deltas vroeg in die seisoen is deur Bemlab-laboratoriums voltooi, waarna ek op 'n verslag wag. Ryp vrugte word in die volgende maand geoes vir 'n tweede nat ontleding deur Bemlab. 'n Afsonderlike droëvrugte analise van die ryp vrugte moet nog deur CAL-laboratoriums uitgevoer word. Die fisiese metings van vrugte en sap analise is voltooi vir onvolwasse en ryp Palmer Nawels, met die Deltas wat binnekort voltooi sal word. Die resultate van hierdie eksperimente word tans verwerk.

3.2.17 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 demonstrated that *Cydia pomonella* larvae are closely associated with yeast from the genus *Metschnikowia*. The identification of this mutualistic association between *C. pomonella* larvae and yeasts from the genus *Metschnikowia* led to the development of a novel biocontrol technique. By combining the Cydia pomonella granulovirus with yeast, a significant increase in neonate *C. pomonella* larval mortality was observed, both in laboratory assays and field trials, when compared with CpGV being applied alone. This novel approach has only been demonstrated in *C. pomonella* and not in any other lepidopteran pest, including *Thaumatotibia leucotreta*. We proposed to determine which species of yeast occur naturally in *T. leucotreta* larvae and to examine whether any of these yeasts, when combined with Cryptophlebia leucotreta granulovirus, increase larval mortality.

Navel oranges infested with *T. leucotreta* larvae were collected from orchards in Addo, Nelspruit and Stellenbosch, to be analysed for the presence of yeast. A total of six yeast species were isolated and identified from the gut of *T. leucotreta* larvae, namely *Meyerozyma guilliermondii*, *Hanseniaspora opuntiae*, *Clavispora lusitaniae*, *Kluyveromyces marxianus*, *Pichia kudriavzevii* and *Pichia kluyveri*. Oviposition preference and attraction assays have been conducted on *T. leucotreta* females, using the isolated yeast species. It has been demonstrated that their behaviour is influenced by isolated yeast species and that they do prefer specific yeast species over others, namely *Pichia kudriavzevii*. Detached fruit bioassays have also been conducted to firstly determine the optimal yeast to virus ratio and secondly to identify the best performing yeast species when combined with CrleGV-SA. The optimal yeast concentration to use in the yeast/virus treatments was determined to be 10^6 cells/ml with *P. kudriavzevii* and *Saccharomyces cerevisiae* performing best.

Currently, the isolated yeast species are being subjected to gas chromatography-mass spectrometry (GCMS) analysis to identify the volatiles responsible for causing attraction. Detached fruit bioassays are also being conducted with Break-THRU® S240 and molasses to further increase larval mortality rates and semi-field trials with *P. kudriavzevii* and *S. cerevisiae* are underway. Additionally, larval movement, oviposition preference and attraction assays are still being conducted as well.

Opsomming

Dit is voorheen bewys dat *Cydia pomonella* larwes naby verwant is aan gis van die genus *Metschnikowia*. Die identivisering van hierdie mutualistise assosiasie tussen *C. pomonella* larwes en gis van die genus *Metschnikowia* het gelei tot die ontwikkeling van ŉ nuwe biologiese beheer tegniek. Die kombinering van Cydia pomonella granulovirus met gis het gelei tot ŉ noemenswaardige verhoging in pasuitgebroeide *C. pomonella* larwe mortaliteit in beide die labratorium toetse en veldstudies, in vergelyking met wanneer CpGV alleen toegedien word. Hierdie nuwe benadering is slegs gedemostreer in *C. pomonella* en nie in enige van die ander Lepidoptera peste nie, insluitend *Thaumatotibia leucotreta*. Ons doel was om te bepaal watter spesie gis natuurlik in *T. leucotreta* larwes voorkom en om vas te stel of ŉ kombinasie van hierdie giste met Cryptophlebia leucotreta granulovirus, ŉ hoër larwe mortaliteit tot gevolg het. Nawel lemoene wat besmet is met *T. leucotreta* larwes is versamel in boorde op Addo, Nelspruit en Stellenbosch om geanaliseer te word vir die teenwoordigheid van gis. ŉ Totaal van ses gis spesies is gëisoleer en gëidentifiseer van die inhoud van *T.*

leucotreta larwes, naamlik *Meyerozyma guilliermondii*, *Hanseniaspora opuntiae*, *Clavispora lusitaniae*, *Kluyveromyces marxianus*, *Pichia kudriavzevii* and *Pichia kluyveri*.

Eierlegging voorkeur en aantrekking toetse is gedoen met wyfie *T. leucotreta* met die gebruik van die geisoleerde gis spesies. Dit is bewys dat hulle gedrag beinvloed word deur geisoleerde gis spesies en dat hulle spesifieke gis spesies bo ander verkies, naamlik *Pichia kudriavzevii*. Verskeie vrug biotoetse is ook gedoen om eerstens te bepaal wat is die optimale gis tot virus verhouding en om tweedens, die beste presterende gis spesie te identifiseer wanneer dit met CrleGV-SA gekombineer word. Dit is bevind dat die optimale gis konsentrasie om te gebruik in die gis/virus behandeling, 10^6 selle/ml met *P. kudriavzevii* en *Saccharomyces cerevisiae* is, wat die beste presteer het. Tans word die geisoleerde gis spesies blootgestel aan die gaskromotografie–massaspektrometrie (GCMS) en geanaliseer om te identifiseer wat die vlugtige stowwe is wat verantwoordelik is vir die aanloklikheid. Verskeie vrug biotoetse is ook gedoen met Break-THRU® S240 en melasse om die larwe mortaliteit verder te verhoog en semi-veld proewe met *P. kudriavzevii* en *S. cerevisiae* word tans ondersoek. Bykomende larwe beweging, eierlegging voorkeure en aanloklikheid toetse word tans nog uitgevoer.

3.2.18 **PROGRESS REPORT: Improving baculovirus virulence against the false codling moth by repeated passage and virus combinations**.

Project 1199 (2019 – 2020/21) by D Taylor, P Iita, M Jukes, C Knox, S Moore and M Hill (RU/CRI)

Summary

Cryptophlebia leucotreta granulovirus (CrleGV-SA) has been used to control *T. leucotreta* in citrus orchards for over a decade as part of an integrated pest management (IPM) strategy. Although this is the only baculovirus species registered for use against *T. leucotreta* in citrus orchards, two others also infect and kill *T. leucotreta*: Cydia pomonella granulovirus (CpGV) and the recently described Cryptophlebia peltastica nucleopolyhedrovirus (CrpeNPV). Not only are these alternatives to CrleGV, but they provide the opportunity to test mixtures of baculoviruses to determine whether any two of them can work synergistically to be more effective against *T. leucotreta.* Should any combination of viruses lead to a change in lethal concentration or speed of kill, a more effective baculovirus-based biopesticide for *T. leucotreta* control could be developed. Mixtures of viruses with improved efficacy could prove to be an effective management tool, should host resistance to CrleGV arise in the field. The first part of this study extends previous studies to investigate the virulence and speed of kill of different mixtures of CrleGV-SA, CrpeNPV and CpGV against *T. leucotreta* in laboratory bioassays. The ultimate goal is to determine whether baculovirus mixtures, as opposed to individual virus, should be considered as an active ingredient in future biopesticides to provide improved control of *T. leucotreta* in citrus orchards. The second part of this study is to determine whether the virulence of CrpeNPV can potentially be improved against *T. leucotreta* by repeated passaging through this heterologous host, the homologous host being the litchi moth, *C. peltastica*.

Opsomming

Cryptophlebia leucotreta granulovirus (CrleGV-SA) word al langer as 'n dekade gebruik om *T. leucotreta* in sitrusboorde te beheer as deel van 'n geïntegreerde plaagbestuurprogram (IPM). Alhoewel dit die enigste bakulovirus spesie is wat geregistreer is vir gebruik teen *T. leucotreta* in sitrusboorde, is daar nog twee wat *T. leucotreta* kan besmet en doodmaak: Cydia pomonella granulovirus (CpGV) en die onlangs beskryfte Cryptophlebia peltastica nukulêrepolihedrovirus (CrpeNPV). Hierdie is nie net alternatiewe opsies vir CrleGV nie, maar bied ook die geleentheid om mengsels van bakulovirusse te toets om te bepaal of enige twee van hulle sinergisties kan werk om meer effektief teen *T. leucotreta* te wees. As enige kombinasie van virusse tot 'n verandering in die dodelike konsentrasie of die spoed van die doodmaak lei, kan 'n meer effektiewe bakulovirus-gebaseerde biologiese plaagdoder vir die beheer van *T. leucotreta* ontwikkel word. Mengsels van virusse met 'n verbeterde effektiwiteit kan ook ŉ oplossing bied as weerstand teen CrleGV in die veld ontwikkel. Die eerste deel van hierdie studie brei uit op ŉ vorige studie om die virulensie en spoed van doodmaak van verskillende mengsels van CrleGV-SA, CrpeNPV en CpGV teen *T. leucotreta* in laboratorium-biotoetse te ondersoek. Die uiteindelike doel is om te bepaal of bakulovirusmengsels, in teenstelling met individuele virusse, as 'n aktiewe bestanddeel in toekomstige biologiese plaagdoders beskou moet word om sodoende

beter beheer van *T. leucotreta* in sitrusboorde te bied. Die tweede deel van hierdie studie is om te bepaal of die virulensie van CrpeNPV moontlik teen *T. leucotreta* verbeter kan word deur herhaalde deurgang deur larwes van hierdie heteroloë gasheer, waarvan die homoloë gasheer die lietjie-mot, *C. peltastica* Is.

3.3 PROGRAMME: FRUIT FLY

Programme coordinator: Aruna Manrakhan (CRI)

3.3.1 Programme summary

Fruit flies are pests of phytosanitary concern to all export markets of citrus from southern Africa. Either a systems approach (integration of pre and post-harvest management) or stand-alone post-harvest cold disinfestation treatment should be used, depending on markets, for mitigation of risk of fruit flies in citrus consignments originating from southern Africa. Projects carried out under the fruit fly programme addressed aspects of both pre-harvest and post-harvest management of fruit fly pests. These are summarised below, following the order in which they are presented in the fruit fly programme annual report.

A new fruit fly pest of citrus present in the north and north eastern parts of South Africa - *Bactrocera dorsalis* (Oriental fruit fly) raised concern for citrus exports to Japan in 2017. The Japanese Ministry of Agriculture, Forestry and Fisheries (MAFF) queried the efficacy of the existing *Ceratitis capitata* (Mediterranean fruit fly or Medfly) cold treatment schedule for the oriental fruit fly. The cold sensitivity of Medfly and oriental fruit fly was compared in artificial medium following a request from MAFF (3.3.2). Medfly was found to be more cold tolerant than the oriental fruit fly for all life stages tested. This implied that the current cold treatment schedule for Medfly in citrus fruit exports from South Africa to Japan would also be efficacious against the oriental fruit fly. These results were accepted by MAFF and led to uninterrupted citrus exports to Japan.

Another new fruit fly species: *Ceratitis quilicii* (Cape fly) present in South Africa was described in 2016. The Cape fly was split from *Ceratitis rosa* (Natal fly), a recognized pest of citrus in South Africa. The distribution, abundance and host range of Cape fly and Natal fly were quantified (3.3.3). The Cape fly was found in all citrus production regions in Mpumalanga and Limpopo and was more abundant than Natal fly in most sites. No infestation of citrus by either Cape fly or Natal fly was recorded in this study.

Pre and post-harvest treatments for fruit flies are tested to a large extent using laboratory reared flies. Colonies of five fruit fly species continued to be maintained under the long term Project 407 (3.3.4). These colonies are refreshed every year by addition of wild flies reared mostly from fruit.

Under a fruit fly systems approach for citrus other than lemons and limes destined for the European markets, cold shipping conditions with resulting pulp temperatures above 3°C are being used. The efficacy of a treatment at 3.5°C (pulp temperature) on fruit fly mortality is being quantified (3.3.5). Medfly was found to be more cold tolerant than oriental fruit fly and Natal fly at 3.5°C. Trials on the treatment at 3.5°C will now continue only on Medfly.

Effects of interruptions in cold treatment are being investigated (3.3.6). Results obtained so far have demonstrated that interruptions resulting in pulp temperatures reaching 2°C or 4°C above the upper threshold of a cold treatment did not change the efficacy of the cold treatment.

Production and export of mandarins are increasing in South Africa. Fruit fly bait sprays can be phytotoxic on fruit of some mandarin cultivars at the green and colour break stages. The efficacy of different densities of registered bait stations for fruit fly suppression in mandarin orchards is being investigated (3.3.7). The registered bait stations – M3 and Magnet Med were both equally effective in suppressing fruit fly adult populations in mandarin orchards. The use of M3 bait stations at the lower end of the recommended density (300 units per ha) led to infestation of mandarin fruit at harvest in one trial site.

A fruit fly species, which is not considered a fruit fly pest in South Africa, *Ceratitis cosyra* (marula fly), raised concern for citrus export from eSwatini to the USA market. Similar concerns might be expected for future export of citrus from the northern regions of South Africa to USA. This fly was also identified as one of the intercepted fruit fly species in citrus exports to Europe in 2006 and in 2018. The cold tolerance of marula fly and Medfly are being compared and the efficacy of a Medfly cold treatment for disinfestation of marula fly in citrus will be quantified (3.3.8). In the cold tolerance tests, marula fly was found to be more cold sensitive than Medfly. Further ecological studies are being conducted on the Cape fly, this time throughout South Africa (3.3.9). Its distribution and abundance across South Africa are being investigated. Its host range and fruit dependent demographic parameters will also be determined.

The use of electronic traps and decision support systems based on climatic models for optimal detection of the oriental fruit fly in space and time will be investigated (3.3.10). Efficacy of a new bait station – AVIMA bait station – which was found to be promising in laboratory assays on Medfly and oriental fruit fly is now being field tested (3.3.11).

Fruit fly prevalence is usually high in the Western Cape Province, due to cultivation of mixed fruit types in close proximity (deciduous and citrus). An early start of fruit fly control in citrus production regions in that Province is recommended. Trials comparing the efficacy of early baiting (October/November) versus late baiting (January/February) are being carried out (3.3.12).

Fruit fly prevalence in an area is gauged using attractant-baited traps. The role of temperature, relative humidity and fly physiology on the efficiency of fruit fly attractants are being investigated (3.3.13).

Information obtained from the fruit fly projects presented in this report will be used for better management of fruit fly pests along the citrus production chain.

Programopsomming

Vrugtevlieë is plae van fitosanitêre belang vir alle uitvoermarkte van sitrus vanaf suider-Afrika. Óf ŉ sisteembenadering (integrasie van voor- en na-oes bestuur) óf alleenstaande na-oes koue-ontsmettingsbehandeling moet gebruik word, afhangende van markte, vir die versagting van die risiko van vrugtevlieë in sitrusbesendings met oorsprong in suider-Afrika. Projekte wat onder die vrugtevliegprogram uitgevoer is, het aspekte van beide voor- en na-oes bestuur van vrugtevliegplae aangespreek. Dit word hieronder opgesom in die volgorde wat hulle in die vrugtevliegprogram se jaarlikse verslag aangebied word.

ŉ Nuwe vrugtevliegplaag van sitrus wat in die noordelike en noord-oostelike dele van Suid-Afrika teenwoordig is, *Bactrocera dorsalis* (Oosterse vrugtevlieg), het kommer laat ontstaan vir sitrus-uitvoere na Japan in 2017. Die Japannese Ministerie van Landbou, Bosbou en Visserye (*MAFF*) het die effektiwiteit van die bestaande *Ceratitis capitata* (Mediterreense vrugtevlieg of Medvlieg) koue-behandelingskedule vir die oosterse vrugtevlieg bevraagteken. Die koue-sensitiwiteit van Medvlieg en oosterse vrugtevlieg is, op versoek van *MAFF*, in kunsmatige medium vergelyk (3.3.2). Medvlieg was vir alle lewensstadia wat getoets is, meer koue-tolerant as die oosterse vrugtevlieg. Dit het geïmpliseer dat die huidige koue-behandelingskedule vir Medvlieg in sitrusvrug-uitvoere vanaf Suid-Afrika na Japan, ook effektief teen die oosterse vrugtevlieg sal wees. Hierdie resultate is deur *MAFF* aanvaar, en het tot ononderbroke sitrus-uitvoere na Japan gelei.

ŉ Ander nuwe vrugtevliegspesie, *Ceratitis quilicii* (Kaapse vlieg), teenwoordig in Suid-Afrika, is in 2016 beskryf. Die Kaapse vlieg is vanaf *Ceratitis rosa* (Natalse vlieg) geskei, ŉ erkende plaag van sitrus in Suid-Afrika. Die verspreiding, voorkoms en gasheerreeks van Kaapse vlieg en Natalse vlieg is gekwantifiseer (3.3.3). Die Kaapse vlieg is in alle sitrusproduksiestreke in Mpumalanga en Limpopo gevind en was in die meeste persele meer volop as die Natalse vlieg. Geen besmetting van sitrus deur óf die Kaapse vlieg, óf die Natalse vlieg, is in hierdie studie aangeteken nie.

Voor- en na-oes behandelings vir vrugtevlieë word tot ŉ groot mate getoets deur gebruik te maak van laboratorium-geteelde vlieë. Kolonies van vyf vrugtevliegspesies word voortdurend in stand gehou onder die lang-termyn Projek 407 (3.3.4). Hierdie kolonies word elke jaar hernu deur die toevoeg van wilde vlieë wat meestal vanaf vrugte geteel is.

Onder ŉ vrugtevlieg sisteembenadering vir sitrus, behalwe vir suurlemoene en lemmetjies, wat vir die Europese markte bestem is, word koue-verskepingstoestande, met pulptemperature bó 3°C gebruik. Die effektiwiteit van ŉ behandeling teen 3.5°C (pulptemperatuur) op vrugtevlieg mortaliteit, word gekwantifiseer (3.3.5). Daar is gevind dat Medvlieg meer koue-tolerant as die oosterse vrugtevlieg en Natalse vlieg teen 3.5°C was. Proewe met die behandeling teen 3.5°C sal nou slegs op Medvlieg voortgaan.

Gevolge van onderbrekings in koue-behandeling word ondersoek (3.3.6). Resultate sover het aangedui dat onderbrekings wat tot pulptemperature lei wat 2°C of 4°C bó die boonste drempel van ŉ koue-behandeling bereik, nie die effektiwiteit van die koue-behandeling verander het nie.

Produksie en uitvoer van mandaryne neem toe in Suid-Afrika. Vrugtevlieg lokaasspuite kan fitotoksies vir vrugte van sommige mandarynkultivars wees, teen die groen en kleurbreek stadiums. Die effektiwiteit van verskillende digthede van geregistreerde lokstasies vir vrugtevlieg onderdrukking in mandarynboorde is ondersoek (3.3.7). Die geregistreerde lokstasies, M3 en Magnet Med, was ewe effektief in die onderdrukking van volwasse vrugtevliegpopulasies in mandarynboorde. Die gebruik van M3 lokstasies aan die laer kant van die aanbevole digtheid (300 eenhede per ha) het tot besmetting van mandarynvrugte teen oestyd, by een van die proefpersele, gelei.

ŉ Vrugtevliegspesie, wat nie as ŉ vrugtevliegplaag in Suid-Afrika beskou word nie, *Ceratitis cosyra* (maroelavlieg), het kommer gewek vir sitrus-uitvoer vanaf eSwatini na die VSA mark. Soortgelyke kommer mag ontstaan vir toekomstige uitvoer van sitrus vanaf die noordelike streke van Suid-Afrika na die VSA Hierdie vlieg is ook geïdentifiseer as een van die onderskepte vrugtevliegspesies in sitrus-uitvoere na Europa in 2006 en in 2018. Die koue-toleransie van maroelavlieg en Medvlieg word vergelyk en die effektiwiteit van ŉ Medvlieg koue-behandeling vir ontsmetting van maroelavlieg in sitrus sal gekwantifiseer word (3.3.8). In die koue-toleransie toetse is dit gevind dat maroelavlieg meer koue-sensitief as Medvlieg was.

Verdere ekologiese studies word op die Kaapse vlieg uitgevoer, hierdie keer regdeur Suid-Afrika (3.3.9). Sy verspreiding en voorkoms regdeur Suid-Afrika word ondersoek. Sy gasheerreeks en vrug-afhanklike demografiese parameters sal ook bepaal word.

Die gebruik van elektroniese lokvalle en besluitneming ondersteuningsisteme, gebaseer op klimaatsmodelle, vir optimale opsporing van die oosterse vrugtevlieg sal ruimtelik en tydelik ondersoek word (3.3.10). Effektiwiteit van ŉ nuwe lokstasie, AVIMA lokstasie, wat belowend in laboratoriumtoetse op Medvlieg en oosterse vrugtevlieg was, word nou in die veld getoets (3.3.11).

3.3.2 FINAL REPORT: Cold tolerance of immature stages of *Ceratitis capitata* (Wiedemann) and *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae)

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

Summary

The cold sensitivity of Medfly and the oriental fruit fly was determined following a request by the Japanese Ministry of Agriculture, Forestry and Fisheries. The cold tolerances of the two species were compared at four immature stages on a carrot-based medium at -0.59°C ± 0.00°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, mature larvae of Medfly were found to be more cold tolerant than younger larvae. Since this result was different to an outcome 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 at 1°C between different life stages of Medfly reared *in vivo* (in citrus) and *in vitro* (in the carrot based medium). There were survivors of young and mature Medfly larvae reared *in vitro* for up to 9 days of cold treatment. There were no survivors of any of the immature stages of Medfly reared *in vivo* after 7 days of cold treatment. In fruit, mortality of mature larvae of Medfly was lower than mortality of the other life stages on the 7th day of cold treatment.

Opsomming

Die koue-sensitiwiteit van Medvlieg en die oosterse vrugtevlieg is, ná ŉ versoek van die Japannese Ministerie van Landbou, Bosbou en Visserye, bepaal. Die koue-toleransies van die twee spesies is by vier onvolwasse stadiums op wortel-gebaseerde medium teen -0.59°C ± 0.00°C by ses blootstellingsperiodes oor 11 opeenvolgende dae vergelyk. Daar is gevind dat Medvlieg meer koue-tolerant as die oosterse vrugtevlieg was vir alle lewensstadia wat getoets is. Die resultate het dus gedemonstreer dat die huidige koue-behandelingskedule vir sitrusvrug-uitvoere vanaf Suid-Afrika na Japan, effektief teen beide Medvlieg en die oosterse vrugtevlieg kan wees. In die koue-toleransie studie op kunsmatige medium, is dit gevind dat ouer larwes van Medvlieg meer koue-tolerant as jonger larwes is. Aangesien hierdie resultaat verskil het van ŉ uitkoms van vorige navorsing wat by CRI uitgevoer is, waar dit gevind is dat jong Medvlieg larwes die mees koue-tolerant in sitrus was, is ŉ opvolgstudie begin ten einde die koue-toleransie teen 1°C tussen verskillende lewensstadia van Medvlieg wat *in vivo* (in sitrus) en *in vitro* (in die wortel-gebaseerde medium) geteel is, te vergelyk. Daar was lewendige jong en ou Medvlieg larwes wat *in vitro* geteel is vir tot op 9 dae van koue-behandeling. Daar was geen lewendiges van enige van die onvolwasse stadia van Medvlieg wat *in vivo* geteel is, ná 7 dae van koue-behandeling nie. Mortaliteit van ou larwes van Medvlieg in vrugte was laer as mortaliteit van die ander lewensstadia op die 7de dag van koue-behandeling.

Introduction

The Japanese Ministry of Agriculture, Forestry and Fisheries (MAFF), in a letter to the South African Department of Agriculture, Forestry and Fisheries (DAFF) dated 01 November 2017, requested that the cold sensitivity of *Ceratitis capitata* (Wiedemann), Mediterranean fruit fly (Medly), and *Bactrocera dorsalis* (Hendel), oriental fruit fly, be compared. The Project 1213 was thus initiated at Citrus Research International (CRI) in January 2018 with the main objective being to determine the cold sensitivity of *B. dorsalis* in comparison to *C. capitata* on artificial larval medium following an experimental design suggested by MAFF. Results of the study on cold tolerance of *B. dorsalis* were written up in an official report and submitted to DAFF for submission to MAFF. In the latter study, exposure periods for 99% mortality of *C. capitata* were estimated to be higher for older larvae (3rd instar larvae) than younger larvae (2nd and 1st instar larvae). Moreover, estimated exposure periods for 50%-99% mortality of the egg stages were higher than those estimated for the young *C. capitata* larvae at the same mortality levels. These results might put in question the large scale validation tests of the disinfestation treatment of <1.4°C in which young *C. capitata* larvae were used. In order to ascertain that younger larvae of *C. capitata* in citrus are more cold tolerant than other stages, a second study was initiated to concurrently compare the tolerances of immature stages of *C. capitata* reared in vivo (in Benny Valencia oranges) and in vitro (in artificial larval medium) at the same cold treatment temperature.

Stated objectives

1. To compare the cold sensitivity of *Ceratitis capitata* (Wiedemann) and *Bactrocera dorsalis* (Hendel) in vitro (in artificial larval medium)
2. To compare the tolerances of immature stages of *C. capitata* reared in vivo (in Benny Valencia oranges) and in vitro at the same cold treatment temperature

Materials and methods

1. Compare cold sensitivity of Ceratitis capitata *(Wiedemann) and* Bactrocera dorsalis *(Hendel) in vitro (in artificial larval medium)*

<u>Test insects</u>

Two fruit fly species were tested: *C. capitata* and *B. dorsalis*. The two fruit fly species were reared at the Citrus Research Centre (CRC) of Citrus Research International (CRI), Nelspruit, Mpumalanga, South Africa. The *C. capitata* colony originated from deciduous fruit collected from the Western Cape Province, South Africa in 1998 (Grout et al., 2011). The *C. capitata* colony was periodically refreshed with wild males reared from *Coffee* *canephora* Pierre ex Froehner cv. Robusta from Burgershall Agricultural Research Council (ARC) experimental station (25°06'43.5"S, 31°05'16.0"E), Hazyview, Mpumalanga Province, South Africa. The *C. capitata* colony was refreshed in September 2016 and again in September 2017 by crossing wild males reared from coffee sampled at Burgershall ARC experimental station with females from the CRI laboratory colony. In 2016, 711 wild males were crossed with 623 females. In 2017, 305 wild males were crossed with 386 females.

The *B. dorsalis* colony originated from live wild females and males collected in December 2014 from insecticide free 3-component Biolure (Chempac (Pty) Ltd, Suider Paarl, South Africa) baited Chempac bucket traps (Chempac (Pty) Ltd, Suider Paarl, South Africa) and methyl eugenol (Invader-Lure, River Bioscience (Pty) Ltd, Port Elizabeth, South Africa) baited Chempac bucket traps placed on mango trees situated near Louw's Creek, Mpumalanga. The *B. dorsalis* colony was also periodically refreshed with wild males recovered from insecticide-free methyl eugenol traps placed near Louw's Creek (25°41'09.3"S, 31°11'05.0"E). The last colony refreshment before this study was on 17 February 2016 where 300 wild males were crossed with a similar number of laboratory reared females.

The *C. capitata* colony was maintained on a wheat bran diet consisting mainly of brewer's yeast (Organic World, Johannesburg, South Africa) and granulated white sugar (Selati, RCL Foods, South Africa). The *B. dorsalis* colony was maintained on a carrot based diet consisting mainly of brewer's yeast and carrot powder (Amidor (Pty) Ltd., Johannesburg, South Africa).

Diets with immature stages of both species were held in a room at 26.01°C ± 0.04°C, 69.50% ± 0.29% RH and exposed to natural light supplemented with artificial lights between 08:00 and 16:00 on weekdays. Pupae formed for each species were packed in brown paper bags and left to emerge in the bags. Once emerged, adults were transferred to adult cages which were kept in a separate room. Adults of both species were kept in nylon mesh cages of 42 cm in diameter and 150 cm in height supported at both ends with plastic bowls of 42 cm in diameter (Fig. 3.3.2.1). The adult cages of the two species were kept in the same room maintained at 25.71°C ± 0.05°C and exposed to natural light. Adults were provided *ad libitum* with water and a mixture of granulated sugar and yeast extract (Amberex 1003, Juneau, USA) at a ratio of 3:1.

Figure 3.3.2.1. Adult cages of *C. capitata* and *B. dorsalis* colonies maintained at CRC, CRI, Nelspruit, South Africa.

In the *C. capitata* colony, adult females laid their eggs through the nylon mesh. Eggs of *C. capitata* were collected from a water container placed under the adult cages. For collection of *B. dorsalis* eggs, one apple *Malus domestica* Borkh. cv Granny Smith was placed in each cage containing adults of this species. Before placement in the cage, the apple was pricked on each of two sides with a row of pins. Eggs were laid inside the holes created by the pins. The peel areas where eggs were deposited were cut out from the fruit and split open in order to expose the egg clusters which were then rinsed and collected in water.

Artificial larval diet

The larval diet used for the two species in the cold tolerance study was a carrot based diet with carrot powder (Amidor (Pty) Ltd., Johannesburg, South Africa) and brewer's yeast (Organic World, Randburg, South Africa) in the ratio of 2:1. The carrot powder and brewer's yeast mixture constituted 29.3% of the diet. The remaining ingredients were water (70.4%) and the preservatives, methyl 4-hydroxybenzoate and sorbic acid (both from Sigma-Aldrich Pty. Ltd., Kempton Park, South Africa) at 0.2% and 0.1% of the diet mixture respectively.

Preparation of immature stages

Four immature stages of *C. capitata* and *B. dorsalis* were prepared: eggs (≤ 24 hours old), first instar larvae, second instar larvae and third instar larvae. In order to obtain the different immature stages, eggs of the two species were collected from the colonies as described above over a 20 to 21 hour period. Most of the eggs collected were between 14 and 21 hours old since water containers or apples were placed at 11:00 the previous day and removed at 07:00 or 08:00 on the following day (with no oviposition occurring during darkness between 18:00 and 06:00 for both species). An egg-water mixture was prepared for each species by placing one ml of eggs of *C. capitata* and *B. dorsalis* in 25 and 20 ml of deionized filtered water respectively in order to get ~20 eggs of each species per 0.025 ml aliquot of the egg-water mixture. Aliquots (0.025 ml each) of egg-water mixture were placed on moist blue blotting squares (~1 cm x ~1 cm) (Fig. 3.3.2.2 A). Eggs on moist blotting squares (Fig. 3.3.2.2B) were counted until a total count of 100 was reached. Once a count of 100 eggs was reached, the moist blotting squares were transferred onto the surface of 100 g of the larval diet (Fig. 3.3.2.2C) contained in a plastic dish (12.5 cm diameter and 2.5 cm height). Two holes (4 mm in diameter) were pierced on opposite sides of the plastic dish to allow aeration and to avoid water condensation inside the dish. The diet dish containing 100 eggs was covered with a plastic lid.

Figure 3.3.2.2. Inoculation of eggs onto carrot-based larval medium: (A) Placement of aliquots (0.025ml) of egg:water mixture onto moist blotting paper squares, (B) Counting of eggs on moist blotting paper squares and (C) Placement of moist blotting paper squares with eggs on the surface of the carrot-based larval medium.

For development of immature stages of each species, diet containers with eggs were placed in an environmental chamber (Fig. 3.3.2.3) (modified Conviron CMP3023, Controlled Environments, Manitoba, Canada). The temperature setting in the chamber was adjusted to achieve a target temperature of 26°C. For each species, development in the diet was allowed for 0.13 (3 hours), three, four and six days after egg inoculation in order to obtain the egg stage (≤ 24 hours old), first instar, second instar and third instar larvae respectively. The selected incubation times for the different life stages were based on a previous study on the immature development of both *C. capitata* and *B. dorsalis* on the same artificial diet at a similar temperature (Manrakhan et al. 2018). In the previous study, first egg hatch (eggs of ≤ 21hours old) of both species occurred after one day of incubation. First instar, second instar and third instar larvae of both species were mostly recorded on the third, fourth and sixth day of incubation.

Figure 3.3.2.3. Incubation of immature stages of *C. capitata* and *B. dorsalis* in carrot-based medium contained in aerated plastic dishes and placed in an environmental chamber set at a target temperature of 26°C.

For each life stage, species and replicate, there were five diet containers prepared for each of six cold treatment exposure periods and an untreated control. There were therefore 35 diet containers in total for each life stage, species and replicate. The diet containers were labelled according to the species, life stage and replicate number. Additionally, for each life stage, species and replicate, there were two diet containers with eggs (200 eggs in total) which were prepared to verify the composition of the immature stages on the day of the exposure to the cold treatment.

Air temperature inside the environmental chamber and temperatures inside five diet containers, each with 100 g of larval diet and no eggs, were recorded every hour using a logger (Grant Squirrel 1000 series Type 1025, Monitoring and control Laboratories, Johannesburg, South Africa) with temperature probes of the type-T thermocouple system. Temperature probes were moved to fresh diet containers with no eggs at the start of each incubation for each replicate. The probes were distributed in representative sections of the stacks of diet dishes in the chamber.

Lights were switched off in the chamber throughout incubation. A humidifier was placed in the environmental chamber in order to achieve a relative humidity of 60%. A relative humidity logger (Hygrochron Hi-Resolution iButton, Johannesburg, South Africa) was placed inside the chamber to measure hourly relative humidity. The mean (± SE) air and media temperatures during incubation before the cold treatment and for the untreated control inside the chamber were 25.76°C ± 0.03°C and 25.95°C ± 0.02°C respectively. The mean (± SE) relative humidity inside the chamber was 65.28% ± 0.28% during incubation before the treatment and for the untreated control.

Cold treatment

A built-in cold room (4.40 m length x 3.45 m width x 2.14 m height) located at CRC, CRI, Nelspruit, Mpumalanga, South Africa was used for the tests. Refrigeration was supplied by an air cooled condensing unit (FRIGA Systems single pack, Model no – FSQR 15/4.2MD) with R22 as refrigerant, a compressor (Sanyo compressor type C-SC863H8H) with a cooling capacity of 199.1 cm^3/h and an induced draft evaporator RECAM RE918A8, fitted with three 500 mm five bladed fans which circulated the air at an average flow rate of 5860 m^3/h. Defrost cycles were set at four hourly intervals.

The target temperature tested was -0.6°C ± 0.6° C for a total period of 11 days. The susceptibility of the four immature life stages of *C. capitata* and *B. dorsalis* to the cold treatment was compared concurrently at six exposure periods: one, three, five, seven, nine and 11 days after the start of the cold treatment.

There were four replicates in this trial. The four replicates of these tests were carried out between 16 January and 23 March 2018 (experiment schedule provided in Annexure A). Each replicate of the cold treatment was conducted separately such that the treatment from precooling through to the final exposure period was also separately repeated.

Just before starting the cold treatment, each diet container of each life stage of each species was transferred to a similarly labelled transparent flip-top container (12.5 cm x 12.5 cm x 6 cm) with about 50 holes (< 2 mm in diameter) pierced on the top (Fig. 3.3.2.4). This transfer was carried out in order to enable jumping larvae to leave diet dishes during storage following a cold treatment.

Figure 3.3.2.4. Transfer of diet dishes with immatures of *C. capitata* and *B. dorsalis* into flip-top containers in preparation for cold treatment.

For each replicate, the flip-top containers with the different life stages of the two species were packed in 12 aerated plastic crates (50 cm x 32 cm x 29 cm) with two crates packed for each exposure period. The two crates for each exposure period had a total of 40 flip-top containers for all species and life stages. The life stages and two species were equally distributed among the two crates. Each crate also contained an extra diet container with no eggs for temperature monitoring during storage after the cold treatment. The 12 crates containing diet containers were placed in two rows in the cold room over a platform of empty crates which were in turn placed on top of a wooden pallet (Fig. 3.3.2.5A). The crates were randomized per row for each replicate (Annexure B). At the end of each exposure period, crates which were removed were replaced with empty crates each containing 20 empty flip-top containers. These replacements were done in order to maintain the layout of the treated crates and thus similar airflow within the arena. At the start of the cold treatment, all crates were covered with a layer of blanket (Fig. 3.3.2.5B) in order to stabilize temperature in the treated arena.

Figure 3.3.2.5. Layout of diet containers with immature stages of *C. capitata* and *B. dorsalis* in cold room: (A) Crates containing diet containers were set out in two rows with each row containing a crate to be removed at

the end of each exposure period and (B) Crates covered with a layer of blanket at the start of the cold treatment to maintain temperature stability in treated arena.

Inside the cold room, air temperatures at two points: one at air delivery and one at the air return and temperatures inside five diet containers, each with 100 g of larval diet and no eggs, were recorded every 5 minutes using a logger (Grant Squirrel 2020 2F8, Monitoring and control Laboratories, Johannesburg, South Africa) with temperature probes of the type-T thermocouple system. Diet containers with no eggs used for temperature measurements were prepared on the day of preparation of the egg stages for each replicate. The diet containers for temperature measurements in the cold room were also each placed in a flip-top container. Temperature probes in diet containers were distributed in the two rows of crates inside the cold chamber (Fig. 3.3.2.6). As a precaution against the malfunctioning of one of the temperature probes, a spare probe was included in the arena. The readings from the spare probe were however not considered in calculations of temperature averages.

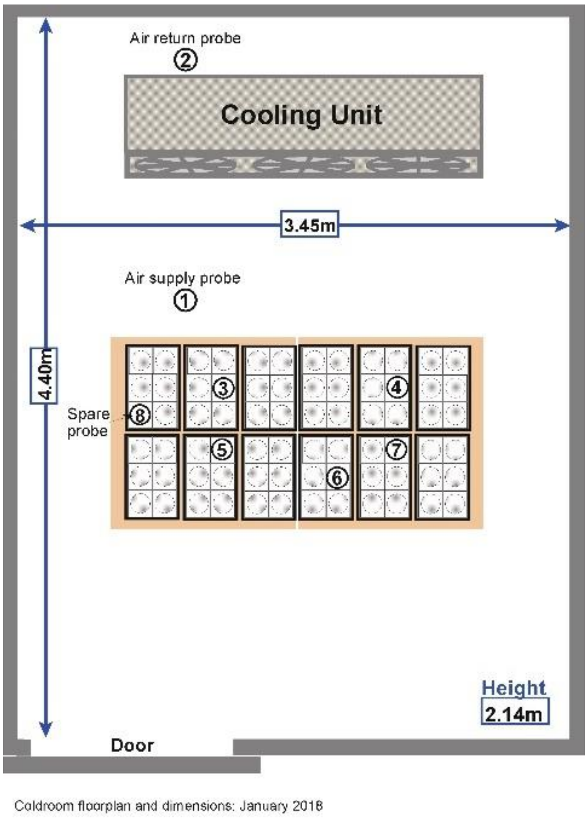

Figure 3.3.2.6. Distribution of probes in the cold room for measurement of air temperatures (Probes 1 and 2) and for measurement of diet temperatures (Probes 3-8 with 8 being a spare probe which was discounted in calculations of averages).

Prior to the start of temperature measurement in the cold chamber for each replicate, temperature probes were calibrated by immersion in ice-water as follows. Ice made from purified water was first crushed into particles of less than 20 mm diameter and then placed in a 2.2 L insulated flask with about 400 ml purified water. The ice/water mixture was left for at least ten minutes for the temperature to stabilise and then a glass mercury thermometer (Brannan, England) was inserted to confirm a stable reading of exactly 0°C (Fig. 3.3.2.7). When temperature readings of the probes were stabilised, calibration runs were started. At least three calibration runs with logging intervals of three minutes were conducted. After at least 10 readings, each run was stopped, the ice mixture was agitated and then the next run was started. The readings of each probe were first averaged for each run and then averaged over the three runs. A calibration factor was derived for each probe: Calibration factor = True temperature of ice/water (0°C) +/- average probe reading (Annexure C). Calibration factors were all within ± 0.3°C from 0°C.

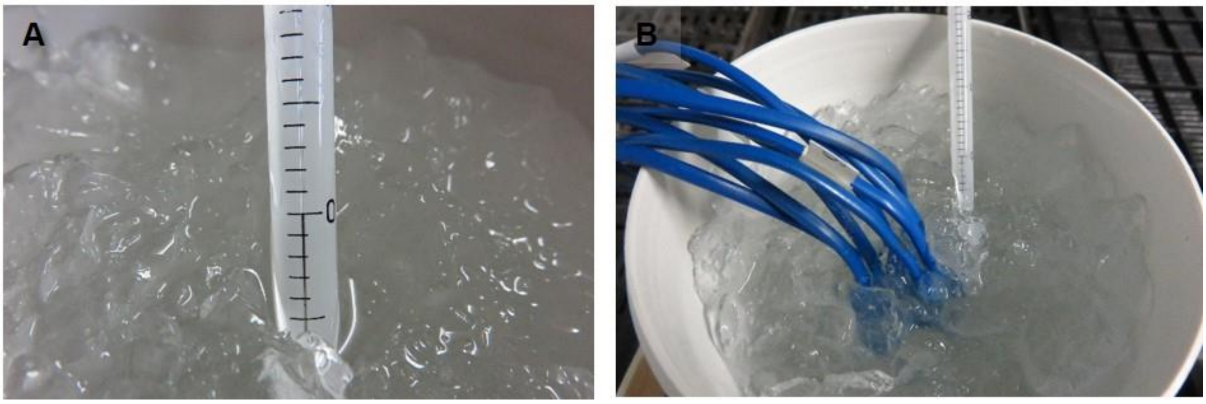

Figure 3.3.2.7. Calibration of temperature probes used in the cold room: (A) Confirmation of 0°C of ice/water mixture using a glass mercury thermometer for calibration of probes and (B) Placement of temperature probes in ice/water mixture for calibration.

The start point of the cold treatment was considered when three of the probes reached -0.6°C or below (excluding the spare probe). At the end of each exposure period, the cold treated diet containers were moved to a second environmental chamber (modified Conviron CMP3023, Controlled Environments, Manitoba, Canada) set at a target temperature of 26°C. Air temperature inside the second chamber was recorded every hour using temperature probes of the type-T thermocouple system connected to the same logger as the one used for the incubation of immature stages before the cold treatment. Temperatures inside the two cold treated diet containers with no eggs were recorded for a period of six days after removal from the cold room. Relative humidity inside the second chamber was measured using a logger (Hygrochron Hi-Resolution iButton, Johannesburg, South Africa). The containers for the egg, first larval, second larval and third larval stages were held for six, three, two and two days respectively before being dissected to determine mortality. The mean (± SE) air and media temperatures inside the second chamber during storage of the diet containers after the cold treatment were 25.83°C ± 0.02°C and 25.80°C ± 0.02°C respectively. The mean (± SE) relative humidity inside the second chamber during storage of the diet containers after the cold treatment was 62.56% ± 0.16%. The untreated control for the egg, first larval, second larval and third larval stages were kept in the first environmental chamber set at a target temperature of 26°C for similar periods as the treated dishes before being dissected to determine the untreated control mortality.

Verification of immature stage composition

For each life stage, species and replicate, two dishes were examined separately. In each dish, unhatched and hatched eggs were first determined. Live and dead larvae were counted. All live larvae found were removed and killed in water just off the boil following the methods of White & Elson-Harris (1994). The killed larvae were then preserved in 100% ethanol for larval instar determination based on the characteristics of their mouthparts (White and Elson-Harris, 1994).

First instars of both *C. capitata* and *B. dorsalis* were characterised by the presence of preapical teeth and non-sclerotized mandibles (Annexure D). Second instars of both fruit fly species were characterised by the presence of preapical teeth and sclerotized mandibles (Annexure D). Third instars of both species were characterised by the absence of preapical teeth and presence of sclerotized mandibles (Annexure D). Additionally, the body length (from mandible to end of last body segment) of each larva from a sample of 10 larvae from each dish was measured using a Vernier calliper.

Mortality assessment

In the determination of mortality, live and dead larvae in each dish were examined and counted under a stereomicroscope (Fig. 3.3.2.8). Live and dead larvae were first checked and counted on the surface of the diet in a dish. The diet was then washed in water onto a fine stainless steel sieve of 180 µm aperture (Madison test sieve, Johannesburg, South Africa) in order to loosen the diet for easier retrieval and identification of larvae. A larva was considered dead if there was no visible movement even when prodded. Fully formed pupae found were considered alive.

Figure 3.3.2.8. Assessment of larval mortality in diet medium.

Data analysis

Data were summarized as average percentage mortality for each life stage of each species at each cold exposure period. For each life stage of each species and each exposure period Abbot's formula (Abbott, 1925) was used to correct for untreated control mortality. Effects of species, stages and exposure periods on mortality were analysed using logistic regression (XLSTAT, Addinsoft). Estimates of cold exposure periods to achieve 50%, 90%, 95% and 99% mortality levels for each life stage and species were derived using the Probit model (XLSTAT, Addinsoft).

2. Compare the tolerances of immature stages of C. capitata *reared in vivo (in Benny Valencia oranges) and in vitro (in artificial larval diet) at 1°C*

Test insects

Immatures of *C capitata* used in the tests were sourced from a colony maintained at CRC described in the first part of the study.

Larval environment

Immatures were tested in two larval environments: in *Citrus sinensis* (L.) Osbeck (cv. C100 Valencia) oranges (*in vivo*) and in artificial larval diet (*in vitro*).

Fruit

Valencia oranges sourced directly from a commercial citrus farm (Crocodile Valley) in Ehlanzeni District, Mpumalanga Province, South Africa were used for the tests. Fruit collected were dipped in a Sporekill and 2,4-D Sodium salt mix for 1 minute (1 ml of Sporekill and 0.01 ml of 2,4-D in 1 L of water) in a 100 L plastic tub. Sporekill and 2,4-D mix dips were refreshed for every 500 fruit. After the sporekill and 2,4-D dips, fruit were then dipped in a fungicide mixture of Imazalil as sulphate and Guazatine (0.67 g of Imazalil and 4.8 ml of Guazatine in 1 L of water) for 1 minute in a plastic tub. The temperature of the fungicide mixture was at approximately 35°C. The pH of the mixture was approximately 5. Fruit were stored for up to four months in a cold room set at 4°C prior to the start of inoculations. Before each test (replicate), the external and internal characteristics of 12 randomly selected fruit were measured. The colour of each fruit was first graded according to the rind colour using Set Number 34 of the Colour Prints for Blemish and Appearance Standards (2015) published by Citrus Research International and endorsed by the Department of Agriculture, Land Reform and Rural Development, South Africa. Each fruit was weighed and measured with a Vernier caliper at the equator. The firmness of each fruit was tested using a fruit pressure tester (Model FT 327, Facchini, Italy). The tester was pressed against each side of the fruit at the equator and the pressure at which the oil glands burst was then recorded (appearance of liquid on paper placed in between tester and fruit surface). The mean pressure was calculated for each fruit. Each fruit was then cut in half and the thickness of the peel at the thicker and thinner side of the fruit was measured using a Vernier Caliper. The mean thickness of the peel was calculated for each fruit. The thickness included the flavedo and albedo. The sample of 12 fruit was then juiced at the Citriculture laboratory at CRC. Fruit juice was passed over a muslin cloth to trap pulp fibre. The pH of the juice was recorded using a pH meter. Fruit juice sugar (total soluble solids) was recorded with a hand held refractometer. Acidity (percentage of citric acid) was measured by titration with 0.1N NaOH and using phenolpthalein ($C_{20}H_{14}O_4$) as an indicator.

Fruit were prepared a day before the tests by first removing calyces and secondly dipping in a mixture of Imazalil as sulphate and Guazatine (0.67 g of Imazalil and 4.8 ml of Guazatine in 1 L of water) for 1 minute in a plastic tub. The temperature of the water was between 35°C and 40°C and pH was at 5.

Artificial larval diet

The larval diet used in the study for the two species was a carrot based diet as described in the first part of the study.

<u>Preparation of developmental stages for testing</u>

Three immature stages: eggs, young larvae and mature larvae of *C. capitata* were tested concurrently in the two larval media described above. *Ceratitis capitata* eggs were collected from a water container placed under a cage with fine mesh containing *C. capitata* adults of different ages. Eggs used were collected over 20-21 hours (placement of water container at 11:00 the previous day and collection at 07:00/08.00 the next day). An egg-water mixture was prepared using deionized filtered water in order to obtain approximately 45 eggs per 0.025 ml aliquot of the mixture. In order to obtain the mean number of eggs per aliquot, 10 0.025 ml aliquots were placed onto a black cloth inside one Petri dish. The number of eggs in each aliquot were counted and averaged. This was repeated two times (30 aliquots in total) for each life stage and each larval environment. In order to determine egg hatch rate, each Petri dish containing aliquots of egg:water mixture was held for 4 days (96 hours) before a count of the number of hatched eggs was carried out for each aliquot in each Petri dish. The numbers of eggs that hatched were then averaged per aliquot.

Fruit inoculation

In each fruit, a 6 mm-diameter hole was bored at about 30 mm deep in the fruit beneath the calyx using a cork borer (of 5 mm-diameter). A brewer’s yeast:water mixture (1:2) was placed into the hole (between 0.2 and 0.5 ml). One aliquot 0.025 ml of egg:water mixture was inserted in the hole of the fruit using an automatic pipette. The hole was then plugged with cotton wool before being sealed with molten wax. Each inoculated fruit was placed in a brown paper bag.

Larval diet inoculation

A 100 g lot of larval diet was placed in a plastic dish (12.5 cm diameter and 2.5 cm height). Aliquots (0.025ml) of the egg-water mixture were placed on 3-4 moist blue blotting paper squares (1 cm x 1 cm) until a total of 100 eggs was reached. The blotting squares containing 100 eggs were then placed on the surface of the diet in each plastic dish. Each diet dish was placed inside an aerated flip-top container.

Incubation

For larval development, fruit and diet dishes with eggs were placed in a temperature controlled room. The temperature setting in the room was adjusted to achieve a target temperature of 26°C. In both fruit and diet, development was allowed for one and four days after egg inoculation in order to obtain eggs at 50% and above of the egg stage duration and young larvae respectively. For mature larvae, development in fruit was allowed for nine days after egg inoculation whilst in diet, development was allowed for six days after egg inoculation. For each life stage in fruit, 30 fruit with eggs were prepared for use in each of six cold treatment exposures and an untreated control. There were therefore 210 fruit in total for each life stage. For each life stage in diet, five diet containers with eggs (500 eggs in total) were prepared for use in each of six cold treatment exposures and an untreated control. There were therefore 35 diet containers in total for each life stage. The numbers of fruit and diet containers prepared were to ensure that there were more than 200 individuals tested for each treatment and control. Additionally, for each life stage, there were 12 fruit and two diet containers with eggs (200 eggs in total) which were prepared to verify the composition of the immature stages on the day of the exposure to the cold treatment in both larval environments.

Air temperature inside the temperature controlled room and temperatures inside three fruit and three diet containers with no eggs were recorded every hour using a logger (Grant Squirrel 1000 series Type 1025, Monitoring and control Laboratories, Johannesburg, South Africa). The logger used temperature probes of the type-T thermocouple system. The probes were distributed in representative sections of the stacks of fruit and diet dishes. Prior to the start of temperature measurement in each replicate, temperature probes were calibrated by immersion in ice-water as previously described. Lights were switched off in the temperature controlled room throughout the study. A humidifier was placed in the environmental chamber in order to achieve a relative humidity of 60%. A relative humidity logger was placed inside the chamber to measure hourly relative humidity.

<u>Cold treatment tested</u>

The cold room described in part 1 of the study was used for the tests. The target temperature was 1.0°C for a total period of 11 days. The susceptibility of the three life stages of *C. capitata* in the two types of larval environments to the cold treatment were compared concurrently at six exposure periods: one, three, five, seven, nine and eleven days after the start of the cold treatment. Fruit and diet containers with the three life stages of *C. capitata* were placed simultaneously in the cold room. Fruit and diet containers were divided in the cold room by exposure day so that they could be removed at the end of each exposure period. For each exposure day, fruit and diet containers were distributed randomly in two crates (Fig. 3.3.2.9). There was a total of 12 crates laid out in two rows in the cold room.

Figure 3.3.2.9. Layout in cold room with crates containing both fruit and diet containers.

Inside the cold room, air temperatures at two points: one at air delivery and one at the air return and temperatures inside five fruit and five diet containers (each with 100 g of larval diet), with no eggs, were recorded every 5 minutes using a logger (Grant Squirrel 2020 2F8, Monitoring and control Laboratories, Johannesburg, South Africa) with temperature probes of the type-T thermocouple system. Fruit and diet containers with no eggs for temperature measurements were prepared on the last egg inoculation before cold treatment. Temperature probes were distributed in representative sections of the chamber. Prior to the start of temperature measurement in each replicate, temperature probes were calibrated as described in the previous section.

The start point of the treatment in fruit was when three of the probes in fruit reached target temperature. Similarly, the start point of the treatment in diet was when three of the probes in diet reached target temperature.

At the end of each exposure period, 30 fruit and five diet containers of each life stage were removed from the cold room. Treated fruit and diet containers were placed in the same temperature controlled room as the one used for incubation which was set at a target temperature of 26°C. After the cold exposure and placement in the warm temperature room (less than 24 hours in warm room), treated fruit were dipped in a mixture of Imazalil as sulphate and Guazatine (0.67 g of Imazalil and 4.8 ml of Guazatine in 1 L of water) for one minute in a plastic tub. The temperature of the water was between 35°C and 40°C and pH was at 5. The fruit were packed in fresh bags. The fungicide and repacking was to reduce the risk of fungal growth in the fruit batch.

In order to measure temperatures inside fruit and diet containers after cold treatment, two fruit and two diet containers were also removed from the cold room. Temperatures inside two cold treated fruit and two cold treated diet containers with no eggs were recorded using another logger (Grant Squirrel 2040 4F16, Monitoring and control Laboratories, Johannesburg, South Africa). The logger used temperature probes of the type-T thermocouple system. The warming up of the cold treated fruit or diet containers was recorded for a period of 6 days after removal from the cold room. Fruit and diet containers with egg stages were held for eight and five days respectively before being dissected to determine mortality. Fruit and diet containers with young larval stages were held for five and two days respectively before being dissected to determine mortality. Fruit and diet containers with mature larvae were held for two days before being dissected to determine mortality.
The untreated fruit and diet for the egg, young larvae and mature larvae were kept in the first environmental chamber set at a target temperature of 26°C for similar periods as the treated dishes before being dissected to determine mortality.

In the determination of mortality, dead and live larvae in each dish and fruit were counted. A larva was considered dead if there was no visible movement even when prodded. There were four replicates of these tests. Each replicate was conducted at a time such that the treatment from precooling through to the final exposure period was separately repeated.

Data analysis

Data were summarized as average percentage mortality for each life stage at each cold exposure period in each rearing medium. In each rearing medium, Abbot's formula (Abbott, 1925) was used to correct for untreated control mortality in each life stage for each cold exposure period. Uncorrected and corrected mortality percentages were arcsine square root transformed to reduce variances in the datasets before being analysed using ANOVA (XLSTAT, Addinsoft). Fixed effects in the ANOVA were rearing medium, lifestage, exposure period, and replicate was considered a random effect. Estimates of cold exposure periods to achieve 50%, 95% and 99% mortality levels for each life stage and medium were derived using the Probit model (GENSTAT). In the Probit model, data from the second replicate of the trial were excluded due to high mortality rates in the control for the mature larvae.

Results and discussion

1. Compare cold sensitivity of Ceratitis capitata *(Wiedemann) and* Bactrocera dorsalis *(Hendel) in vitro (in artificial larval medium)*

Composition of immature stages on the day of start of cold exposure

On the first day of cold exposure, only eggs were found in dishes treated as eggs for both *C. capitata* and *B. dorsalis*. The mean percentages egg hatch (± SE) in the dishes and fruit treated as eggs were both at 0.00% ± 0.00%. In the diet dishes, the mean egg hatch (±SE) for cohorts treated as young larvae and those treated as mature larvae were 89.46% ± 1.19% and 74.71% ± 2.01% respectively.

In all four replicates for the two species, the majority or all of the larvae in dishes treated as third instars were at the third instar larval stages (Table 3.3.2.1). Larvae in diet dishes treated as first and second instars for both *C. capitata* and *B. dorsalis* were less consistently separated according to the target stages (Table 3.3.2.1).

Table 3.3.2.1. Larval characteristics of diet dishes of *C. capitata* and *B. dorsalis* which were treated as first instars, second instars and third instars in the four replicates of the cold tolerance study. For each replicate, there were two diet dishes (inoculated with 100 eggs each) for each life stage and species.

Replicate	Species	Larval stage targeted	Mean larval length ± SE (mm)	Total number of larvae found	% Instar composition		
					First	Second	Third
1	*C. capitata*	First	1.45 ± 0.03	60	100	0	0
		Second	3.52 ± 0.09	151	5	87	9
		Third	7.70 ± 0.10	169	0	0	100
	B. dorsalis	First	2.14 ± 0.06	60	73	27	0
		Second	4.55 ± 0.21	113	0	79	21
		Third	9.45 ± 0.07	104	0	2	98
2	*C. capitata*	First	1.52 ± 0.04	93	100	0	0
		Second	2.15 ± 0.12	82	72	28	0
		Third	8.24 ± 0.06	137	0	0	100
	B. dorsalis	First	1.87 ± 0.03	46	100	0	0
		Second	3.69 ± 0.10	51	16	84	0
		Third	10.55 ± 0.09	73	0	0	100
3	*C. capitata*	First	1.92 ± 0.09	94	27	73	0
		Second	3.44 ± 0.05	87	0	100	0
		Third	8.24 ± 0.05	139	0	0	100
	B. dorsalis	First	2.25 ± 0.09	68	78	22	0
		Second	4.62 ± 0.20	85	0	87	13
		Third	10.24 ± 0.07	104	0	0	100
4	*C. capitata*	First	1.73 ± 0.16	135	81	19	0
		Second	3.15 ± 0.08	98	0	100	0
		Third	8.65 ± 0.07	155	0	0	100
	B. dorsalis	First	2.13 ± 0.06	125	41	59	0
		Second	4.02 ± 0.08	108	0	100	0
		Third	10.63 ± 0.08	100	0	0	100

Temperature records in cold room

In this study, the time taken from start of cooling until at least three probes in the diet containers were at ≤ 0.6°C was 3.83 hours, 3.42 hours, 3.58 hours and 4.25 hours in the first, second, third and fourth replicate respectively. After the start of the cold treatment, the mean (± SE) diet temperature for 11 consecutive days in the cold room was -0.59°C ± 0.00°C, -0.59°C ± 0.00°C, -0.60°C ± 0.00°C and -0.59°C ± 0.00°C for the first, second, third and fourth replicate respectively (Fig 3.3.2.10). The mean minimum (± SE) diet temperature during the cold treatment was -0.64°C ± 0.00°C, -0.65°C ± 0.00°C, -0.67°C ± 0.00°C and -0.66°C ± 0.00°C for the first, second, third and fourth replicate respectively. The mean maximum (± SE) diet temperature during the cold treatment was -0.52°C ± 0.00°C, -0.53°C ± 0.00°C, -0.50°C ± 0.00°C and -0.50°C ± 0.00°C for the first, second, third and fourth replicate respectively.

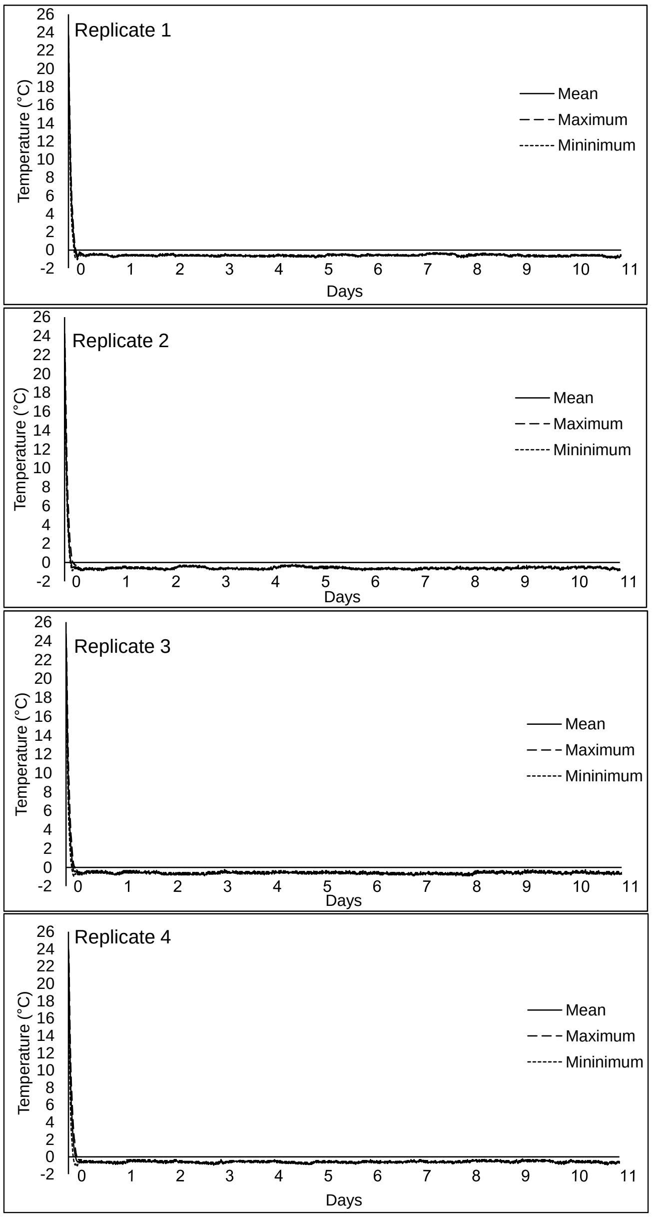

Figure 3.3.2.10. Mean, maximum and minimum temperature profiles of diet containers in four replicates of the cold tolerance test over 11 consecutive days. Temperatures in diet containers were measured by five temperature probes at 5 minute intervals in each replicate. The treatment was considered to start (day 0) when three of the probes were ≤ -0.6°C.

<u>Mortality of immatures of *C. capitata* and *B. dorsalis*</u>

Mortality rates of all immature stages of *B. dorsalis* were consistently higher than those of *C. capitata* at all exposure periods of the cold treatment (Table 3.3.2.2). There were no survivors of any life stages of the two species beyond 7 days of cold treatment. For *C. capitata*, there were no survivors of eggs and first instar larvae beyond 5 days of cold treatment. There were no survivors of the second instar and third instar larvae of *C. capitata* beyond 7 days of cold treatment. There were no survivors of eggs and first instar larvae of *B. dorsalis* beyond 3 days of cold treatment. For second and third instar larvae of *B. dorsalis*, there were no survivors beyond 5 days of cold treatment. There were significant effects of species, life stage and exposure periods on mortality (Species: χ^2=4970.08, df=1, P<0.0001; Stage: χ^2=201.59, df=3, P<0.0001; Exposure period: χ^2=14923.83, df=5, P<0.0001).

Results from the Probit analysis to estimate the number of days of cold exposure to achieve 50%, 90%, 95% and 99% mortality are presented in Table 3.3.2.3. Estimated cold exposure periods to achieve 50%, 90%, 95% and 99% mortality levels were higher for *C. capitata* than *B. dorsalis* for all immature stages tested indicating that *C. capitata* is more cold tolerant than *B. dorsalis*.

The data obtained here support previous findings by Armstrong et al. (1995) that *C. capitata* is more cold tolerant than *B. dorsalis*. In Armstrong et al. (1995), a Probit 9 quarantine security was estimated at 9 days of cold treatment at 1.1°C ± 0.6°C for *C. capitata* in papaya fruit while the same quarantine security level was estimated at 7 days of cold treatment at 1.1°C ± 0.6°C for *B. dorsalis* in the same fruit. Studies carried out by Hallman et al. (2013; 2011) at 0.94°C ± 0.65°C and 1.0°C ± 0.1°C respectively also showed that *B. dorsalis* was no more cold tolerant than *C. capitata* in both fruit and in artificial diet.

Table 3.3.2.2. Mean (± SE) mortality of eggs, first instar larvae, second instar larvae and third instar larvae of *C. capitata* and *B. dorsalis* treated in a carrot-based diet at -0.59°C ± 0.00°C (overall mean across all four replicates) at different exposure periods over 11 consecutive days.

Life stage	Exposure period (days)	Total number of inoculated eggs	Total number of live larvae		Mean (± SE) Uncorrected mortality (%)		Mean (± SE) Corrected mortality (%)	
			C. capitata	*B. dorsalis*	*C. capitata*	*B. dorsalis*	*C. capitata*	*B. dorsalis*
Eggs	0	2000	1316	1119	34.20 ± 2.75	44.05 ± 5.69		
	1	2000	1406	253	29.70 ± 1.75	87.35 ± 9.02	0.41 ± 0.41	79.72 ± 13.15
	3	2000	945	10	52.75 ± 3.92	99.50 ± 0.44	27.44 ± 8.22	99.24 ± 0.65
	5	2000	229	0	88.55 ± 1.66	100.00 ± 0.00	82.77 ± 1.90	100.00 ± 0.00
	7	2000	0	0	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
	9	2000	0	0	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
	11	2000	0	0	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
First instar larva	0	2000	1555	1223	22.25 ± 2.07	38.85 ± 2.66		
	1	2000	1226	931	38.70 ± 6.16	53.45 ± 9.29	21.37 ± 7.17	25.85 ± 11.50
	3	2000	336	142	83.20 ± 5.61	92.90 ± 5.64	78.18 ± 7.55	88.19 ± 9.36
	5	2000	1	0	99.95 ± 0.05	100.00 ± 0.00	99.94 ± 0.06	100.00 ± 0.00
	7	2000	0	0	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
	9	2000	0	0	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
	11	2000	0	0	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
Second instar larva	0	2000	1493	1184	25.35 ± 4.27	40.80 ± 3.01		
	1	2000	1305	863	34.75 ± 3.76	56.85 ± 7.61	12.36 ± 3.85	28.24 ± 9.66
	3	2000	466	121	76.70 ± 4.52	93.95 ± 1.70	68.77 ± 5.97	89.96 ± 2.53
	5	2000	29	3	98.55 ± 0.72	99.85 ± 0.15	97.94 ± 1.07	99.76 ± 0.24
	7	2000	1	0	99.95 ± 0.05	100.00 ± 0.00	99.94 ± 0.06	100.00 ± 0.00
	9	2000	0	0	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
	11	2000	0	0	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
Third instar larva	0	2000	1478	1023	26.10 ± 4.87	48.85 ± 3.53		
	1	2000	1356	583	32.20 ± 7.47	70.85 ± 2.59	11.43 ± 6.40	42.57 ± 5.05
	3	2000	782	135	60.90 ± 3.71	93.25 ± 3.63	45.89 ± 7.61	86.31 ± 7.42
	5	2000	86	5	95.70 ± 2.43	99.75 ± 0.15	93.44 ± 4.05	99.53 ± 0.29
	7	2000	2	0	99.90 ± 0.10	100.00 ± 0.00	99.88 ± 0.12	100.00 ± 0.00
	9	2000	0	0	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
	11	2000	0	0	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00

Table 3.3.2.3. Estimated exposure periods at -0.6°C required for 50%, 90%, 95% and 99% mortality of eggs, first instars second instars and third instars of *C. capitata* and *B. dorsalis* in a carrot-based medium.

Life stage	Species	Estimated exposure days (95% CI) for four mortality levels			
		50%	90%	95%	99%
Eggs	*C. capitata*	3.59 (3.54-3.64)	5.37 (5.38-5.47)	6.03 (5.90-6.16)	7.47(7.27-7.70)
	B. dorsalis	0.58 (0.53-0.62)	1.35 (1.29-1.41)	1.71 (1.62-1.82)	2.69 (2.46-3.00)
First Instar	*C. capitata*	1.66 (1.62–1.70)	3.43 (3.33-3.53)	4.21 (4.08-4.36)	6.21 (5.94-6.50)
	B. dorsalis	1.45 (1.41-1.49)	2.90 (2.81-2.99)	3.53 (3.41-3.66)	5.11 (4.87-5.36)
Second instar	*C. capitata*	2.01 (1.97-2.06)	4.00 (3.90-4.11)	4.86 (4.72-5.01)	7.00 (6.73-7.28)
	B. dorsalis	1.39 (1.35-1.43)	2.80 (2.72-2.92)	3.42 (3.30-3.56)	4.97 (4.74-5.23)
Third instar	*C. capitata*	2.44 (2.39-2.49)	5.01 (4.89-5.14)	6.15 (5.98-6.33)	9.01 (8.69-9.37)
	B. dorsalis	1.18 (1.14-1.22)	2.88 (2.78-2.98)	3.70(3.56-3.86)	5.93(5.62-6.28)

2. Compare the tolerances of immature stages of C. capitata reared in vivo and in vitro at 1°C

<u>Composition of immature stages on the day of start of cold exposure</u>

On the first day of cold exposure, only eggs were found in diet dishes and fruit treated as eggs. The mean egg hatch rates (± SE) in diet treated as young and mature larvae were 92.95% ± 0.43% and 91.98% ± 0.46% respectively. The mean egg hatch rates (± SE) in fruit treated as young and mature larvae were 92.93% ± 0.42% and 93.08% ± 0.37% respectively. In all four replicates, the majority or all of the larvae in diet dishes and fruit treated as mature larvae were at the third instar stages (Table 3.3.2.4). For diet dishes and fruit treated as young larvae, second instars were predominantly found (Table 3.3.2.4).

Table 3.3.2.4. Larval characteristics of *C. capitata* in diet and fruit which were treated as young and mature larvae in the four replicates of the cold tolerance study. In each replicate, the number live larvae examined were from two diet dishes (inoculated with 100 eggs each) and 15 fruit (inoculated with approximately 50 eggs) for each life stage and species.

Replicate	Medium	Larval stage targeted	Mean larval length ± SE (mm)	Total number of larvae found	% Instar composition		
					First	Second	Third
1	Diet	Young	3.80 ± 0.09	135	3.70	96.30	0.00
		Mature	8.14 ± 0.07	164	0.00	0.00	100.00
	Fruit	Young	2.82 ± 0.10	258	12.79	87.21	0.00
		Mature	7.45 ± 0.11	195	0.00	6.15	93.85
2	Diet	Young	3.48 ± 0.06	124	7.26	92.74	0.00
		Mature	8.59 ± 0.09	177	0.00	0.00	100.00
	Fruit	Young	2.59 ± 0.06	209	9.57	90.43	0.00
		Mature	7.29 ± 0.16	97	0.00	0.00	100.00
3	Diet	Young	3.09 ± 0.13	40	0.00	100.00	0.00
		Mature	7.15 ± 0.07	175	0.00	0.00	100.00
	Fruit	Young	4.41 ± 0.33	238	2.52	91.18	6.30
		Mature	7.56 ± 0.17	166	0.00	0.00	100.00
4	Diet	Young	4.67 ± 0.04	134	0.00	100.00	0.00
		Mature	7.45 ± 0.03	133	0.00	0.75	99.25
	Fruit	Young	4.00 ± 0.18	287	13.24	86.41	0.35
		Mature	8.44 ± 0.05	260	0.00	1.15	98.85

<u>Temperature records in cold room</u>

The time taken from start of cooling until at least three probes in the diet containers were at ≤ 1°C was 4.33 hours, 4.92 hours, 4.92 hours and 4.92 hours in the first, second, third and fourth replicate respectively. In fruit, the time taken from start of cooling until at least three probes in fruit were at ≤ 1°C was longer: 6.92 hours, 6.83 hours, 7.50 hours and 6.50 hours in the first, second, third and fourth replicate respectively. After the start of the cold treatment, the mean (± SE) diet temperature for 11 consecutive days in the cold room was 1.03°C ± 0.00°C, 1.04°C ± 0.00°C, 1.04°C ± 0.00°C and 1.00°C ± 0.00°C for the first, second, third and fourth replicate respectively (Fig 3.3.2.11). After the start of the cold treatment, the mean (± SE) temperature in fruit for 11 consecutive days in the cold room was 1.03°C ± 0.00°C, 1.04°C ± 0.00°C, 1.01°C ± 0.00°C and 1.03°C ± 0.00°C for the first, second, third and fourth replicate respectively (Fig 3.3.2.12) The mean minimum (± SE) diet temperature during the cold treatment was 0.95°C ± 0.00°C, 0.97°C ± 0.00°C, 0.93°C ± 0.00°C and 0.95°C ± 0.00°C for the first, second, third and fourth replicate respectively. The mean minimum (± SE) temperature in fruit during the cold treatment was 0.95°C ± 0.00°C, 0.96°C ± 0.00°C, 0.94°C ± 0.00°C and 0.97°C ± 0.00°C for the first, second, third and fourth replicate respectively. The mean maximum (± SE) diet temperature during the cold treatment was 1.11°C ± 0.00°C, 1.11°C ± 0.00°C, 1.13°C ± 0.00°C and 1.04°C ± 0.00°C for the first, second, third and fourth replicate respectively. The mean maximum (± SE) temperature in fruit during the cold treatment was 1.11°C ± 0.00°C, 1.14°C ± 0.00°C, 1.08°C ± 0.00°C and 1.10°C ± 0.00°C for the first, second, third and fourth replicate respectively.

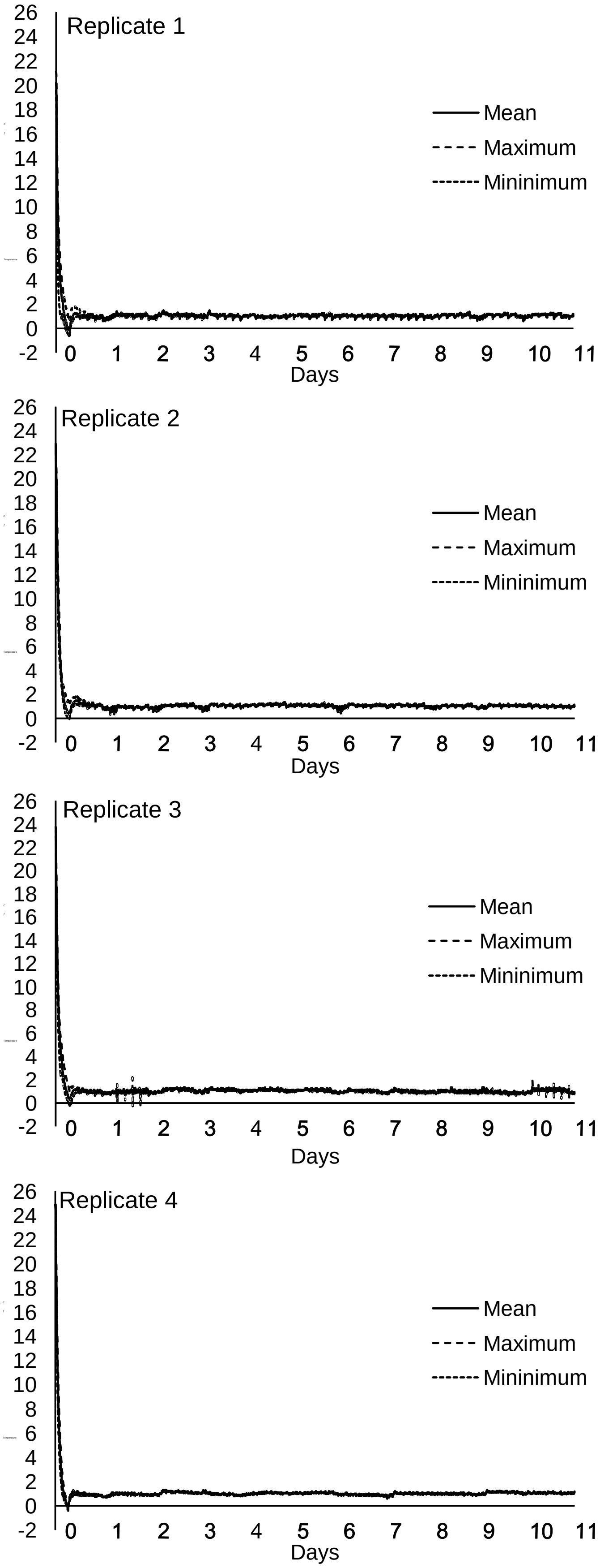

Figure 3.3.2.11. Mean, maximum and minimum temperature profiles of diet containers in four replicates of the cold tolerance test over 11 consecutive days. Temperatures in diet containers were measured by five temperature probes at 5 minute intervals in each replicate. The treatment was considered to start (day 0) when three of the probes were ≤ 1.0°C.

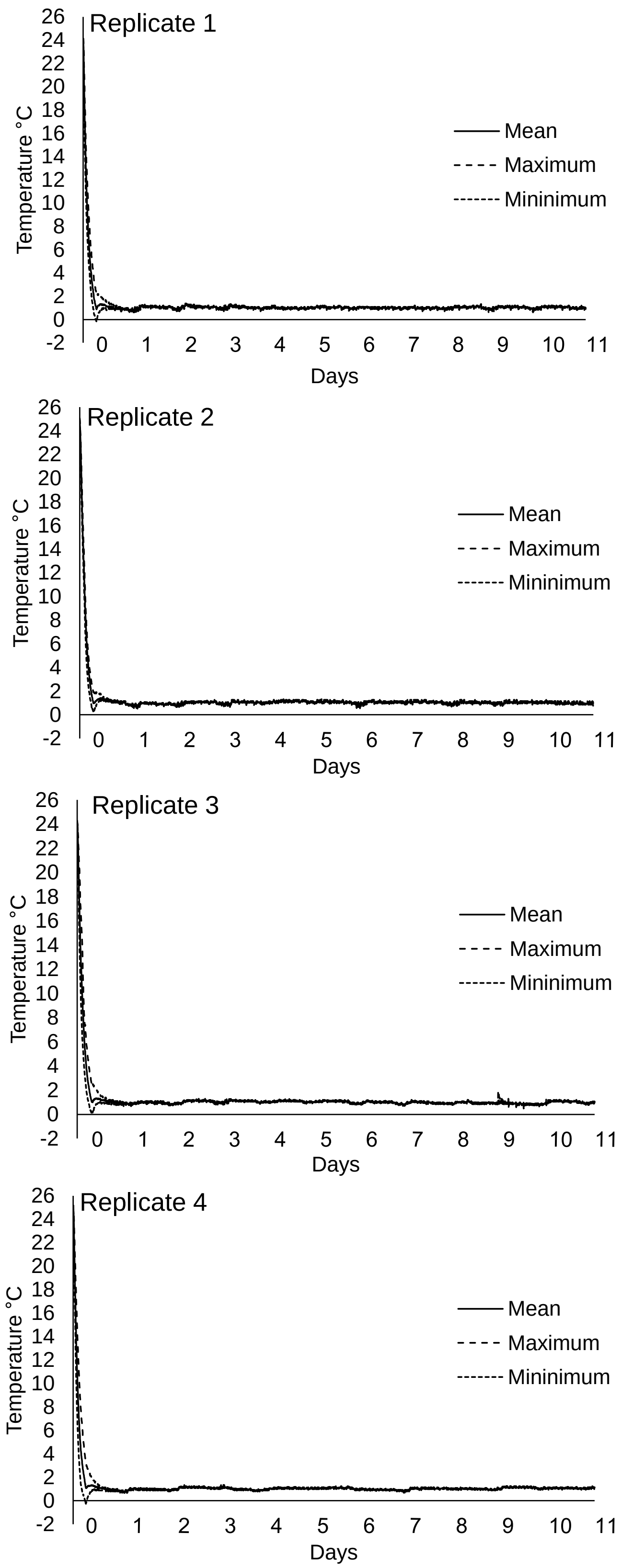

Figure 3.3.2.12. Mean, maximum and minimum temperature profiles in oranges cv Valencia C100 in four replicates of the cold tolerance test over 11 consecutive days. Temperatures in fruit were measured by five temperature probes at 5 minute intervals in each replicate. The treatment was considered to start (day 0) when three of the probes were ≤ 1.0°C.

Fruit quality

Fruit used in the four replicates of this study generally had similar external and internal characteristics (Table 3.3.2.5).

Table 3.3.2.5. External and internal fruit characteristics used in the four replicates of the cold tolerance tests.

Replicate	Colour grade mode	Average weight/ g	Average diameter/ mm	Average firmness/ kg	Average peel thickness/ mm	pH	Acidity (%)	Sugar content (° Brix)
1	1	230.3 ± 8.2	78.1 ± 1.0	4.0 ± 0.2	5.0 ± 0.1	3.5	1	11.1
2	2	216.4 ± 9.0	76.2 ± 1.3	4.1 ± 0.2	4.9 ± 0.1	3.7	1	11.0
3	1	199.5 ± 8.8	74.0 ± 1.1	3.6 ± 0.2	5.0 ± 0.0	3.4	1.4	11.3
4	1	185.8 ± 6.9	72.0 ± 1.0	4.4 ± 0.2	4.7 ± 0.1	3.5	1.1	11.7

Mortality of immatures of *C. capitata* reared *in vitro* and *in vivo*

At the target temperature of 1°C, uncorrected mortality rates of all immature stages were higher when reared *in vivo* (in fruit) compared to when reared *in vitro* (in diet) (Table 3.3.2.6 & 3.3.2.7). When mortality rates were corrected (using control), rearing medium was no longer an influential factor in mortality (Table 3.3.2.7). Exposure period and life stage, on the other hand, significantly influenced mortality, even when corrected for control (Table 3.3.2.7). There was a significant interaction between rearing medium (diet and fruit) and life stage on corrected mortality rates (Table 3.3.2.7).

In fruit, there were generally lower corrected mortality rates for mature larvae and eggs compared to young larvae as the cold exposure period increased beyond day one but differences in cold tolerance between life stages were not significant (Table 3.3.2.6 and 3.3.2.7). Estimates of the number of cold exposure days for 99% mortality of immatures of *C. capitata* at 95% CI were higher for mature larvae than eggs and young larvae in fruit although there were large overlaps in these estimates between the life stages (Table 3.3.2.8).

In diet, corrected mortality rates were significantly lower for eggs and young larvae than mature larvae and estimates for the number of cold exposure days required for 99% mortality (at 95% CI) were higher for eggs than for young and mature larvae (Tables 3.3.2.6, 3.3.2.7 & 3.3.2.8). Similar to what was observed in fruit, there were large overlaps in probit estimates for the different life stages in diet (Table 3.3.2.8). In diet, there were however no survivors of the egg stages beyond seven days of cold exposure whilst young and mature larvae survived until day nine of the cold treatment. The higher residual survivorship of young and mature larvae compared to eggs during exposure to cold in diet was similar to what was observed in the first part of this study.

Probit estimates for the number of days required to kill 99.9% of *C. capitata* immatures were generally higher in fruit compared to medium. An upper estimate of 12 days at 1°C to kill 99% of *C. capitata* at 95% CI falls within an internationally approved cold treatment protocol for this species (1.11°C for 14 days).

The relative tolerances of immature stages of *C. capitata* to cold treatments have previously been studied using different fruit commodities, treatment temperatures and experimental methods. Different conclusions on the relative cold tolerances of *C. capitata* larval stages were reached in the previous studies with no specific trends associated with larval environment (fruit type: citrus versus non citrus), inoculation methods, incubation times, treatment temperature, pre-cooling times and methods of mortality assessment. Eggs of *C. capitata* were found to be the most susceptible stage to cold (Back and Pemberton, 1915; Hill et al., 1988) except in a more recent study when mortality of different life stages of this species and another fruit fly species – *Zeugodacus cucurbitae* (Coquillett) were compared in navel oranges at 1.5°C and 2.0°C (Follett et al., 2018). In the latter study (Follett et al., 2018), immature stages were reared in artificial diet first before being placed inside navel oranges and this method might have contributed to their contrasting results. Some studies have reported differences in cold tolerances between younger and older larvae (Back and Pemberton, 1915; De

Lima et al., 2011; De Lima, 1992; De Lima et al., 2007; Jessup et al., 1993; Willink et al., 2006) while others have reported no significant differences in cold tolerances between young and old larvae (Hill et al., 1988; Santabella et al., 2009).

While differences in cold tolerances of different life stages in fruit flies and other Dipterans have been demonstrated, the actual mechanisms behind age related variation in thermal tolerance have still not been elucidated although a number of hypotheses such as morphological reorganisation during development and carry over of temperature tolerance have been postulated (Bowler and Terblanche, 2008). Based on the results of this study, the use of young larvae in cold treatment trials on *C. capitata* in citrus can still be justified. The use of mature larvae in cold treatment trials on *C. capitata* in citrus can also be considered.

Table 3.3.2.6. Mean (± SE) mortality of eggs, young larvae and mature larvae of *C. capitata* reared in a carrot-based diet (*in vitro*) and in sweet oranges (*in vivo*) both at 1.03°C ± 0.00°C (overall mean across all four replicates) at different exposure periods over 11 consecutive days.

Life stage	Exposure period	Total number of eggs inoculated		Total number of live larvae		Mean (± SE) uncorrected mortality (%)		Mean (± SE) corrected mortality (%)	
		In diet	In fruit	In diet	In fruit	In diet	In fruit	In diet	In fruit
Eggs	0	2000	5490	1678	2567	16.10 ± 1.29	54.45 ± 6.37		
	1	2000	5490	1458	1823	27.10 ± 9.82	67.55 ± 4.03	15.00 ± 10.60	27.84 ± 4.25
	3	2000	5490	995	1292	50.25 ± 13.05	77.14 ± 6.39	44.29 ± 13.04	49.72 ± 11.96
	5	2000	5490	472	847	76.40 ± 8.58	84.60 ± 1.98	72.19 ± 9.89	64.44 ± 5.34
	7	2000	5490	97	79	95.15 ± 3.53	98.62 ± 0.52	94.31 ± 4.11	98.06 ± 1.17
	9	2000	5490	0	0	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
	11	2000	5490	0	0	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
Young larvae	0	2000	5940	1721	1591	31.85 ± 13.90	61.47 ± 2.68		
	1	2000	5940	1101	673	36.05 ± 16.44	75.72 ± 3.10	13.83 ± 10.58	36.11 ± 9.78
	3	2000	5940	534	782	62.05 ± 10.34	84.54 ± 0.99	46.16 ± 10.29	58.96 ± 4.62
	5	2000	5940	59	315	91.20 ± 4.69	94.97 ± 0.45	89.06 ± 5.33	86.63 ± 1.86
	7	2000	5940	0	51	99.20 ± 0.39	99.71 ± 0.07	98.95 ± 0.55	99.22 ± 0.22
	9	2000	5940	1	0	99.90 ± 0.10	100.00 ± 0.00	99.86 ± 0.14	100.00 ± 0.00
	11	2000	5940	0	0	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
Mature larvae	0	2000	5580	1363	2154	13.95 ± 1.01	73.51 ± 7.08		
	1	2000	5580	1279	1377	44.95 ± 8.70	88.63 ± 2.09	37.12 ± 9.09	48.63 ± 16.65
	3	2000	5580	759	854	73.30 ± 8.37	86.75 ± 1.49	69.72 ± 9.24	45.87 ± 15.78
	5	2000	5580	176	284	97.05 ± 1.59	94.64 ± 1.08	96.69 ± 1.78	67.46 ± 18.04
	7	2000	5580	16	17	100.00 ± 0.00	99.16 ± 0.11	100.00 ± 0.00	95.93 ± 1.27
	9	2000	5580	2	0	99.95 ± 0.05	100.00 ± 0.00	99.94 ± 0.06	100.00 ± 0.00
	11	2000	5580	0	0	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00

Table 3.3.2.7. ANOVA results showing effects of rearing medium, life stage and cold exposure period on uncorrected and corrected mortality of *C. capitata*. Results shown are the degrees of freedom (DF), sum of squares (SS), mean squares (MS), F-value (F) and p value (Pr>F) on a significance level of 5%. Significant parameters are highlighted in bold.

Mortality measurement	Source	DF	Sum of squares	Mean squares	F	Pr > F
Uncorrected mortality	Replicate	3	0.147	0.049	2.463	0.065
	Medium	**1**	**1.188**	**1.188**	**59.616**	**<0.0001**
	Stage	**2**	**0.448**	**0.224**	**11.250**	**<0.0001**
	Exposure	**6**	**18.270**	**3.045**	**152.807**	**<0.0001**
	Medium*Stage	2	0.010	0.005	0.252	0.777
	Medium*Stage*Exposure	**12**	**1.334**	**0.111**	**5.577**	**<0.0001**
	Error	141	2.810	0.020		
Corrected mortality	**Replicate**	**3**	**0.724**	**0.241**	**7.053**	**0.000**
	Medium	1	0.002	0.002	0.050	0.824
	Stage	**2**	**0.260**	**0.130**	**3.797**	**0.025**
	Exposure	**5**	**23.256**	**4.651**	**135.926**	**<0.0001**
	Medium*Stage	**2**	**0.372**	**0.186**	**5.439**	**0.005**
	Medium*Stage*Exposure	10	0.458	0.046	1.337	0.219
	Error	120	4.106	0.034		

Table 3.3.2.8. Estimated exposure periods at 1°C required for 50%, 95% and 99% mortality of eggs, young larvae and mature larvae of *C. capitata* in a carrot-based medium and fruit.

Medium	Life stage	Estimated exposure days (95% CI) for three mortality levels		
		50%	95%	99%
Diet	Eggs	2.88 (2.24-3.43)	6.38 (5.55-7.73)	7.83 (6.73-9.70)
	Young larvae	2.81 (2.27-3.31)	5.72 (4.98-6.93)	6.92 (5.96-8.58)
	Mature larvae	1.50 (0.63–2.08)	4.66 (3.87-6.18)	5.97 (4.88-8.21)
Fruit	Eggs	2.81 (2.14-3.37)	7.37 (6.53-8.62)	9.26 (8.11-11.03)
	Young larvae	2.13 (1.48-2.65)	6.06 (5.30-7.23)	7.68 (6.64-9.37)
	Mature larvae	0.66 (-0.88-1.61)	6.88 (5.87-8.53)	9.46 (7.96-12.10)

Conclusions

1. Current cold treatment schedule for citrus fruit exports from South Africa to Japan would be efficacious against both Medfly and oriental fruit fly.
2. Use of young larvae in cold treatment trials on Medfly in citrus can still be justified. The use of mature larvae in cold treatment trials on Medfly can also be considered.

Future research

Mortality data obtained during this project and previous projects at CRI can be mined to develop predictive models of time-temperature treatments.

Technology transfer

Results on the comparative cold tolerance of Medfly and Oriental fruit fly were presented at the 10th CRI Research Symposium, August 2018.

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Annexure A.

Schedule of inoculations, cold exposure periods and dissections for the four replicates of the cold tolerance study.

	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	Monday	Tuesday
	16/01/2018	17/01/2018	18/01/2018	19/01/2018	20/01/2018	21/01/2018	22/01/2018	23/01/2018	24/01/2018	25/01/2018	26/01/2018	27/01/2018	28/01/2018	29/01/2018	30/01/2018
Rep 1	Inoculation 3rd		Inoculation 2nd	Inoculation 1st			Inoculation eggs								
							In cold; Instar check; reached temperature at 16:45	D1		D3		D5		D7	
							Control 3rd		Control 2nd	Control 1st			Control eggs		
											Treated D1 3rd&2nd	Treated D1 1st	Treated D3 3rd&2nd	Treated D3 1st	Treated D1 Eggs; Treated D5 3rd&2nd
Rep 2															Inoculation 3rd

Egg inoculations

Cold treatment exposure

Control dissections

Dissections of treatments

	Wednesday	Thursday	Friday	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	Monday	Tuesday	Wednesday
	31/01/2018	**01/02/2018**	**02/02/2018**	**03/02/2018**	**04/02/2018**	**05/02/2018**	**06/02/2018**	**07/02/2018**	**08/02/2018**	**09/02/2018**	**10/02/2018**	**11/02/2018**	**12/02/2018**	**13/02/2018**	**14/02/2018**
Rep 1															
	D9		D11												
	Treated D5 1st	Treated D3 eggs; Treated D7 3rd&2nd	Treated D7 1st	Treated D5 eggs; Treated D9 3rd&2nd	Treated D9 1st	Treated D7 eggs; Treated D11 3rd& 2nd	Treated D11 1st	Treated D9 eggs		Treated D11 eggs					
Rep 2		Inoculation 2nd	Inoculation 1st			Inoculation eggs									
						In cold; Instar check	D1		D3		D5		D7		D9
						Control 3rd		Control 2nd	Control 1st			Control eggs			
										Treated D1 3rd&2nd	Treated D1 1st	Treated D3 3rd&2nd	Treated D3 1st	Treated D1 Eggs; Treated D5 3rd&2nd	Treated D5 1st
Rep 3														Inoculation 3rd	

Egg inoculations

Cold treatment exposure

Control dissections

Dissections of treatments

	Thursday	Friday	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday
	15/02/2018	16/02/2018	17/02/2018	18/02/2018	19/02/2018	20/02/2018	21/02/2018	22/02/2018	23/02/2018	24/02/2018	25/02/2018	26/02/2018	27/02/2018	28/02/2018	01/03/2018
Rep 2															
		D11													
	Treated D3 eggs; Treated D7 3rd&2nd	Treated D7 1st	Treated D5 eggs; Treated D9 3rd&2nd	Treated D9 1st	Treated D7 eggs; Treated D11 3rd& 2nd	Treated D11 1st	Treated D9 eggs		Treated D11 eggs						
Rep 3	Inoculation 2nd	Inoculation 1st			Inoculation eggs										
					In cold; Instar check	D1		D3		D5		D7		D9	
					Control 3rd		Control 2nd	Control 1st			Control eggs				
									Treated D1 3rd&2nd	Treated D1 1st	Treated D3 3rd&2nd	Treated D3 1st	Treated D1 Eggs; Treated D5 3rd&2nd	Treated D5 1st	Treated D3 eggs; Treated D7 3rd&2nd
Rep 4													Inoculation 3rd		Inoculation 2nd

Egg inoculations

Cold treatment exposure

Control dissections

Dissections of treatments

	Friday	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
	02/03/2018	03/03/2018	04/03/2018	05/03/2018	06/03/2018	07/03/2018	08/03/2018	09/03/2018	10/03/2018	11/03/2018	12/03/2018	13/03/2018	14/03/2018	15/03/2018	16/03/2018
Rep 3															
	D11														
	Treated D7 1st	Treated D5 eggs; Treated D9 3rd&2nd	Treated D9 1st	Treated D7 eggs; Treated D11 3rd& 2nd	Treated D11 1st	Treated D9 eggs		Treated D11 eggs							
Rep 4	Inoculation 1st			Inoculation eggs											
				In cold; Instar check	D1		D3		D5		D7		D9		D11
				Control 3rd		Control 2nd	Control 1st			Control eggs					
								Treated D1 3rd&2nd	Treated D1 1st	Treated D3 3rd&2nd	Treated D3 1st	Treated D1 Eggs; Treated D5 3rd&2nd	Treated D5 1st	Treated D3 eggs; Treated D7 3rd&2nd	Treated D7 1st
	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
	17/03/2018	18/03/2018	19/03/2018	20/03/2018	21/03/2018	22/03/2018	23/03/2018	24/03/2018	25/03/2018	26/03/2018	27/03/2018	28/03/2018	29/03/2018	30/03/2018	31/03/2018
Rep 4															
	Treated D5 eggs; Treated D9 3rd&2nd	Treated D9 1st	Treated D7 eggs; Treated D11 3rd& 2nd	Treated D11 1st	Treated D9 eggs		Treated D11 eggs								

Egg inoculations

Cold treatment exposure

Control dissections

Dissections of treatments

Annexure B

Randomisation of crates containing diet containers with immature stages of *C. capitata* and *B. dorsalis* for the six cold exposure periods in cold room for each replicate.

Rep 1

Fan

D11	D1	D3	D7	D9	D5
D7	D9	D5	D3	D1	D11

Door

Rep 2

Fan

D7	D3	D9	D1	D5	D11
D1	D3	D9	D7	D11	D5

Door

Rep 3

Fan

D9	D5	D3	D7	D11	D1
D1	D7	D9	D3	D11	D5

Door

Rep 4

Fan

D5	D3	D1	D11	D7	D9
D7	D1	D5	D9	D3	D11

Door

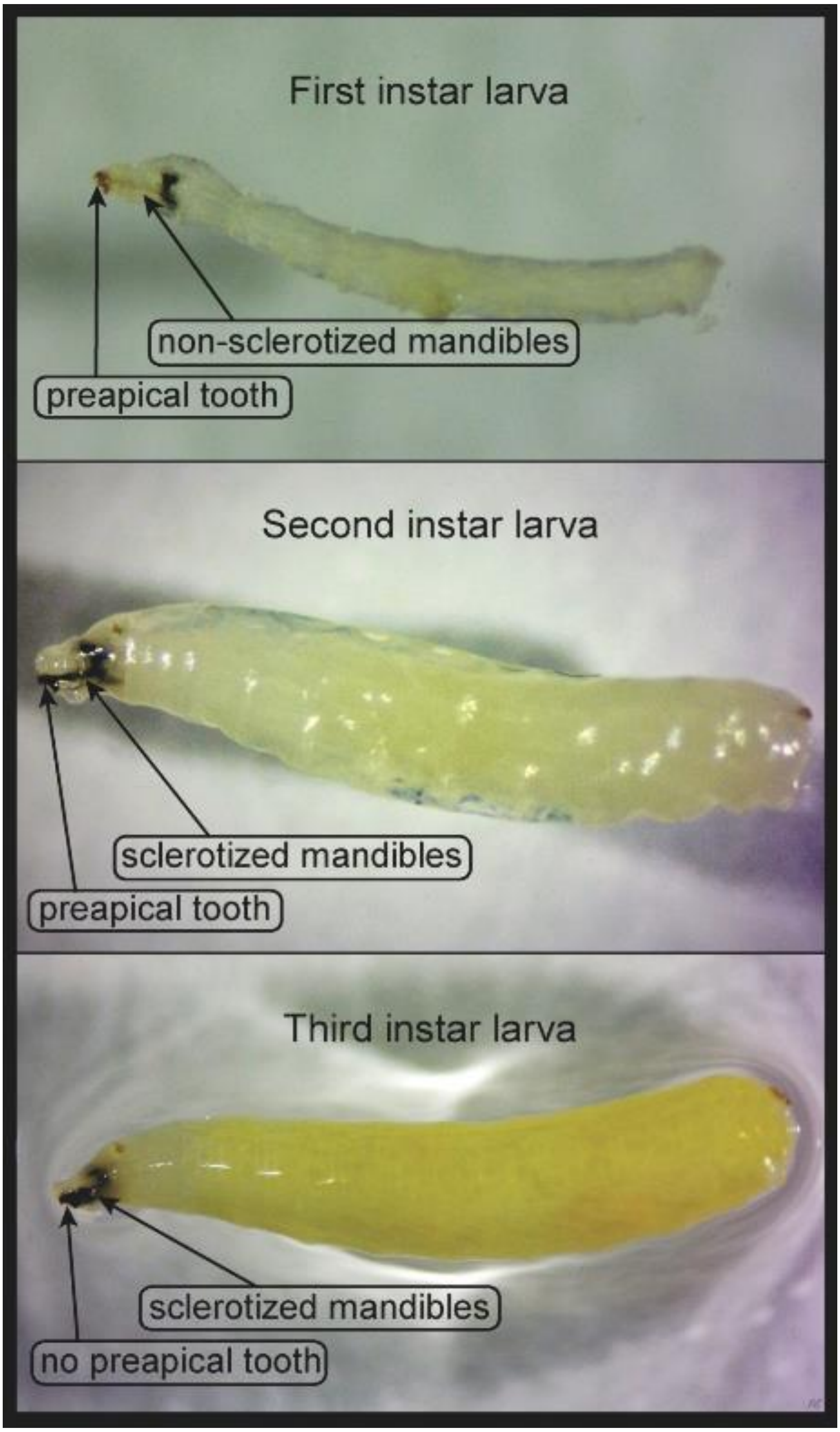
Annexure C

Calibration of sensors by ice water immersion

Replicate (date of calibration)	Sensor number	Readings			Calibration factor
		Run 1	Run 2	Run 3	
1 (21/01/2018)	1	0.07	0.09	0.05	-0.07
	2	0.07	0.09	0.04	-0.07
	3	0.05	0.07	0.00	-0.04
	4	0.06	0.09	0.02	-0.06
	5	0.18	0.19	0.12	-0.16
	6	0.10	0.09	0.09	-0.09
	7	0.15	0.16	0.12	-0.14
	8	0.18	0.18	0.14	-0.17
2 (04/02/2018)	1	-0.02	0.01	0.01	0.00
	2	-0.01	-0.01	0.00	0.01
	3	-0.02	0.00	-0.02	0.01
	4	-0.02	0.00	-0.02	0.01
	5	0.17	0.18	0.16	-0.17
	6	-0.09	-0.02	-0.05	0.05
	7	0.12	0.11	0.11	-0.11
	8	0.16	0.14	0.18	-0.16
3 (18/02/2018)	1	0.10	0.10	0.07	-0.09
	2	0.01	0.06	0.04	-0.04
	3	0.07	0.09	0.07	-0.08
	4	0.05	0.08	0.05	-0.06
	5	0.15	0.20	0.14	-0.16
	6	0.02	0.08	0.06	-0.05
	7	0.24	0.20	0.15	-0.20
	8	0.22	0.23	0.19	-0.21
4 (04/03/2018)	1	0.00	0.01	0.03	-0.01
	2	-0.02	-0.05	0.00	0.02
	3	0.08	0.05	0.08	-0.07
	4	0.02	-0.01	0.03	-0.01
	5	0.13	0.12	0.17	-0.14
	6	0.02	0.02	0.06	-0.03
	7	0.09	0.10	0.11	-0.10
	8	0.19	0.16	0.18	-0.18

Annexure D

Morphology of first, second and third instar larvae of *C. capitata* and *B. dorsalis*



3.3.3. **FINAL REPORT: Biology and ecology of *Ceratitis rosa* and *Ceratitis quilicii* (Diptera: Tepritidae) in citrus**

Project 1170 (2017 - 2019) by John-Henry Daneel (CRI), Vaughan Hattingh (CRI), Johnnie van den Berg (NWU) and Aruna Manrakhan (CRI)

Summary

Ceratitis rosa Karsch s.l. (Diptera: Tephritidae), an indigenous pest of commercial fruit including citrus in South Africa, belongs to a complex of cryptic species (*Ceratitis* FAR). *Ceratitis rosa* s.l. was recently split into *C. rosa* and *Ceratitis quilicii* De Meyer, Mwatawala & Virgilio and this impacts the pre- and postharvest management of these species. In this project, (1) the effectiveness of attractant-based traps for monitoring of *C. rosa* and *C. quilicii* in citrus orchards, (2) the relevant abundance of these species in citrus in the northern parts of South Africa, and (3) the rate of larval development of the two species in fruit of different citrus types, were quantified. Traps baited with three types of attractants (EGO Pherolure, Capilure, and three-component Biolure) were set out in orchards on nine farms, for one year, in the northern parts of South Africa. Larval development of *C. rosa* and *C. quilicii* were compared in *Citrus limon*, *C. paradisi*, *C. reticulata* and *C. sinensis*. Eggs were artificially inoculated into fruit of each citrus type and larval development assessed daily over 15 days, by dissecting sub-samples of the infested fruit. EGO Pherolure and Biolure were effective in trapping both fly species. *Ceratitis quilicii* was more abundant than *C. rosa* through almost all of the study areas. *Ceratitis rosa* was negatively affected by low temperatures. The development of these two fruit fly species did not differ in each of the citrus types. There were, however, differences in larval and pupal survival rates between the species, depending on citrus type. For both species, larval development was optimal in *C. sinensis* and poor in *C. reticulata*. *Ceratitis rosa* had higher larval survival rates than *C. quilicii* in *C. limon* and *C. sinensis*. Survival was also highest in *C. sinensis* and it is the most suitable fruit to conduct further cold sterilization trials with.

Opsomming

Ceratitis rosa Karsch s.l. (Diptera: Tephritidae), ŉ inheemse pes op kommersiële vrugte, wat in Suid-Afrika sitrus insluit, behoort tot ŉ kompleks van kriptiese spesies (*Ceratitis* FAR). *Ceratitis rosa* was onlangs onderverdeel in twee spesies: *C. rosa* en *Ceratitis quilicii* De Meyer, Mwatawala & Virgilio wat die voor en na-oes bestuur van hierdie spesies beinvloed. Hierdie ondersoek was geloods om te bepaal: (1) hoe effektief lokaas gebaseerde lokvalle vir hierdie spesies is in sitrusboorde, (2) die relatiewe rykdom van die twee spesies in sitrusboorde in die noordelike gedeeltes van Suid-Afrika en (3) die ontwikkelingstempo van die larwes in verskillende sitrusvrugsoorte. Lokvalle gelaai met drie soorte lokmiddels (EGO Pherolure, Capilure en drie-komponent Biolure) was in boorde op nege plase, oor ŉ tydperk van een jaar, in die noordelike gedeeltes van Suid-Afrika uitgesit. Die ontwikkeling van die larwes van *C. rosa* en *C. quilicii* was in *Citrus limon*, *C. paradisi*, *C. reticulata* en *C. sinensis* vergelyk. Die eiers was kunsmatig in die vrugte van elke sitrus soort geïnokkuleer en die larwe ontwikkeling was daagliks oor 15 dae bepaal, deur gedeeltelike monsters van die geïnvesteerde vrugte te dissekteer. EGO Pherolure en Biolure was effektief om beide vliegspesies te lok. *Ceratitis quilicii* was meer volop as *C. rosa* deur feitlik al die studie areas. *Ceratitis rosa* was negatief geaffekteer deur lae temperature. Die ontwikkeling van die vrugtevlieg spesies het nie verskil in die sitrussoorte nie. Daar was egter verskille in die oorlewing van die larwes en papies tussen die spesies afhangende van die sitrussoort. Vir beide spesies was die larwe ontwikkeling optimaal in *C. sinensis* en treurig in *C. reticulata. Ceratitis rosa* het beter larwe ontwikkelingstempos as *C. quilicii* in *C. limon* en *C. sinensis* gehandhaaf. Oorlewing was ook die beste in *C. sinensis* en dit is dus die beste vrug om verdere koue sterilisasie proewe mee te doen.

Introduction

Ceratitis rosa s.l. Karsch (Natal fruit fly) belongs to the subtribe Ceratitidina (tribe Dacini), an Afrotropical group of fruit flies with populations also occurring in Mauritius and Reunion (De Meyer 2001). *Ceratitis rosa* s.l. attacks a wide variety of indigenous and commercial fruit (White & Elson-Harris 1992; De Meyer 2001) and is considered a pest of quarantine (phytosanitary) importance (Badii *et al*. 2015). In South Africa, *C. rosa* s.l. is distributed in the northern and eastern parts and along most of the coastal areas (including the south western areas) of the country, while it is largely absent in the drier inland areas and arid regions (De Villiers *et al*. 2013). *Ceratitis rosa* s.l. prefers higher elevations and a wetter climate (Normand *et al*. 2000). In commercial citrus orchards in South Africa, *C. rosa* s.l. is managed by means of aerial and ground insecticidal bait applications, bait stations, male annihilation techniques (MAT), and orchard sanitation (Manrakhan 2019). For some export markets, additional phytosanitary risk mitigation assurances must be provided, with mandatory postharvest cold treatment of the fruit being required by official bilateral trade protocols (Grout *et al*. 2011b).

The taxonomy of *C. rosa* s.l. was recently reviewed and new insights established. *Ceratitis rosa* s.l. is one of the species in the *Ceratitis* FAR-complex (acronym for *fasciventris*, *anonae* and *rosa*) which consists of three species: *Ceratitis fasciventris* (Bezzi), *C. anonae* Graham and *C. rosa* s.l. (Hendrichs *et al.* 2015). Through the use of microsatellites, it was established that the FAR-complex consisted of five genotypic clusters, with *C. anonae* having a single cluster, *C. fasciventris* consisting of two clusters (F1 and F2) with allo- and parapatric distributions, and *C. rosa* with two clusters (R1 and R2), with an allo- and sympatric distribution (Virgilio *et al*. 2013). The sympatric clusters of *C. rosa* were both reported to occur in South Africa (Virgilio *et al.* 2013). Laboratory studies showed differences in thermal biology of the two *C. rosa* types (Tanga *et al*. 2015). Trapping studies in Tanzania also confirmed different climatic requirements of the two *C. rosa* types with R1 being more abundant in hot areas and R2 being more abundant in cooler areas (Mwatawala *et al.* 2015). The R1 and R2 clusters were further differentiated morphologically using feathering on the midtibia of the males (De Meyer *et al.* 2015). After it was suggested that the *C. rosa* R2 should be considered a new species (De Meyer *et al*. 2015), De Meyer *et al*. (2016) subsequently described morphotype R2 as *Ceratitis* (*Pterandrus*) *quilicii* De Meyer, Mwatawala & Virgilio and R1 was referred to as the true type of *C. rosa*.

There is currently a gap of knowledge regarding whether *C. rosa* or C. *quilicii* might be problematic to the citrus industry in South Africa, if not both. Moreover, previously collected data on pre- and postharvest treatments for *C. rosa* s.l. might now be questionable for use on either species. The research described below addresses the biology and ecology of *C. rosa* and *C. quilicii* and identifies similarities and differences between the two species.

Stated objectives

This study aimed to quantify similarities and differences in the biology and ecology of *C. rosa* and *C. quilicii* in citrus.

The specific objectives were:

A. To compare the responses of *C. rosa* and *C. quilicii* to two male attractants and one food-based attractant in field cages.
B. To establish the relative abundance of *C. rosa* and *C. quilicii* in citrus orchards using attractant-based traps and fruit sampling.
C. To compare the developmental rates of all life stages of *C. rosa* and *C. quilicii* in citrus fruit and artificial medium under constant temperature rearing conditions.
D. To determine the relative tolerance of immature stages of *C. rosa* and *C. quilicii* to post-harvest cold treatment temperatures.

Some of the objectives set out in the original proposal were either modified or not achieved due to low fruit fly numbers in the *C. quilicii* colonies. Objective A was modified. The efficacy of attractants was not determined in field cages with known numbers of the two species as originally proposed. This would have allowed comparison of attractants within and between species. Efficacy of attractants for monitoring of the two species was instead quantified in orchards with natural populations of the pests. This enabled comparison of attractants within a species. Objective D could not be achieved.

Materials and methods

A & B. Fruit fly attractants and abundance

Study sites

Since the Limpopo and Mpumalanga Provinces collectively make up 49% of the citrus production area of South Africa (38 073 ha) (CGA, 2018), this study was conducted in these areas. Nine citrus farms were selected as study sites. At each farm, traps were placed in a *Citrus sinensis* (L.) Osbeck orchard. Sites were chosen based on their altitude (m a.s.l.). The study sites were grouped into three categories: high altitude (900 – 1100 m a.s.l.), medium altitude (400 – 600 m a.s.l.), and low altitude (150 – 300 m a.s.l.). Geographical data were taken with a Garmin GPSMAP 60 Cx. Registered pest and fruit fly control practices were applied on each farm, based on each farm's historic fruit fly experience and current method of spray application preference.

According to the Köppen-Geiger climate classification, these farms are all situated in a temperate climate, known to have dry winters and hot summers (Peel *et al*. 2007). In South Africa, citrus cultivation is categorised in six climatic zones: Hot-Humid (less than 200 km from the sea and below 300 m a.s.l.), Hot-Dry (less than 600 km from the sea and below 600 m a.s.l.), Intermediate (between the hot low areas and cool high areas, 600 to 900 m a.s.l.), Cold/Semi-Coastal (semi-coastal areas of the eastern, southern and western Cape), Cool-Inland (above 900 m a.s.l.), and Semi-Desert (hot summers and cold winters in the Northern Cape) (Barry 1996). The nine farms were distributed in four of these climatic regions with the Cold/Semi-Coastal and Semi-Desert not represented in this study.

The weather data used in this study were supplied by Agroclimatology, Agricultural Research Council – Institute for Soil, Climate and Water (ARC - ISCW), Stellenbosch, South Africa. Weather stations were located between 5.2 and 24.4 km from each of the study sites. Daily weather data were collected from May 2016 to May 2017. The weather data were averaged per month and monthly data were subsequently averaged for each site. The total rainfall for each farm was also recorded. Due to the close proximity of two of the sites (Whisky and Vergenoeg), the same weather station was used to supply data for these farms.

Trapping survey

A trapping survey was carried out at each site from May 2016 to May 2017 to characterise the adult populations of *C. rosa* and *C. quilicii*.

Three attractants, known for their efficacy in attracting both *C. rosa* and *C. quilicii* (Manrakhan *et al*. 2017a) were used in this study. These were: Biolure (Chempac (Pty) Ltd., Suider Paarl, South Africa), consisting of three-components in a single sachet (single 9.5 cm square with a 3 cm opening on one side to assist with vapour release); Capilure (Green Trading (Pty) Ltd. Brits, South Africa) consisting of mainly trimedlure (tert-butyl 4 (or 5)–chloro-2-methylcyclohexane carboxylate) and proprietary extenders (Nakagawa *et al*. 1981); and Egolure (Insect Science (Pty) Ltd, Tzaneen, South Africa) consisting of enriched ginger root oil (EGO) which contains alpha-copaene (Mwatawala *et al*. 2013; Shelly & Pahio 2002). Biolure is a food-based attractant (FAO/IAEA 2018) while Capilure and Egolure are male attractants (FAO/IAEA 2018; Mwatawala *et al*. 2013).

Biolure was placed in a Chempac Bucket trap which consisted of two parts: a plastic opaque lid that screwed onto a yellow plastic base which had three entrance holes (2.0 cm diameter holes, with window-tubes fitted) in the top part of the base section. A fourth entrance hole (2.5 cm diameter) was situated at the tip of an inverted cone moulded into the base of the trap. The trap with both sections fitted together was 16 cm in height, with a diameter of 13.5 cm at its widest point. Capilure was dispensed from a plastic capsule which contained 3 ml of attractant absorbed onto a cotton cloth. Egolure (2 ml) was contained in a small polyethylene bulb. Each of these two attractant types were placed individually and separately in a Sensus trap. The Sensus trap also consisted of two parts: a blue screw top lid, slightly elevated into a dome, which fitted onto a hyaline plastic bottom, with a total height of 13 cm and a diameter of 11.5 cm. The lid was 1 cm wider than the bottom section to prevent any rain entering through the 12 entrance holes (0.6 x 0.7 cm), made around the top circumference of the bottom section.

There were a total of nine traps per farm, three for each lure. Traps were placed at least 30 metres apart. Traps were set at a height of 1.4 m to 1.9 m inside the trees, on the south-eastern side. A thin, easily bendable wire was used for hanging each trap. Sticky ant barriers or petroleum jelly were added on each wire to protect the contents of the traps against potential crawling predators. A 1 cm^3 strip of 2,2-dichlorovinyl dimethyl phosphate (DDVP)-block (Dichlorvos, 195 g/kg active ingredient) (River Bioscience, Port Elizabeth, South Africa) was placed in the bottom of each trap as a killing agent. Biolure and Capilure were replaced monthly, but Egolure was replaced only once after six months. DDVP blocks were replaced once a month in all traps. Every month, adult flies were collected from the traps, placed in numbered vials and transported to the laboratory at Citrus Research International (CRI), Nelspruit, South Africa for identification.

Specimen identification

Specimens collected were identified to species level and sexed using published keys which are based on morphological features (De Meyer 1996, 1998; De Meyer & Copeland 2005; De Meyer *et al*. 2015; De Meyer & Freidberg 2006; White 2006). A Zeiss Stemi 2000 – C dissecting microscope (Carl Zeiss (Pty) Ltd, Randburg, South Africa) with a magnification that ranged from 6.5 to 50x was used during identification. The male flies of *C. rosa* and *C. quilicii* were differentiated based on the pattern of black colouration on the midtibia (De Meyer *et al*. 2016). In the midtibia of a *C. rosa* male, is a solid black area which extends to the dorsal and ventral margins whereas for a *C. quilicii* male, the black area on the midtibia does not extend to the dorsal and ventral margins (De Meyer *et al*. 2016).

All female flies of *C. rosa* s.l. and male flies with no midtibias were placed in 99% alcohol and shipped to the Royal Museum of Central Africa, Tervuren, Belgium (RMCA) for identification by means of molecular diagnostics, described in Virgilio *et al*. (2019). A reduced panel of six microsatellite markers (FAR4, FAR6, FAR7, FAR11, FAR16) was used to identify female specimens (Virgilio *et al*. 2019). In this study identification of the two species was confirmed using a threshold of 95% for the coefficient of the model used for clustering the species (Virgilio *et al*. 2019).

Fruit collection

Mature citrus fruit were collected from the ground or trees in citrus orchards. Fruit were placed in brown paper bags (42.5 cm x 30 cm) and brought back to the laboratory at CRI, Nelspruit. All fruit were dipped in a 0.1% Sporekill mixture (Didecyldimethylammoniun chloride, 120 g/L active ingredient) (ICA, Stellenbosch, South Africa). Fruit were counted, weighed and placed in rectangular plastic emergence containers with aerated lids, with volumes ranging from 13 L (35.5 x 24.5 x 18 cm) to 2.2 L (17.5 x 12.5 x 12 cm), and filled with a 2 cm layer of fine sand. The containers were kept in a room at ambient temperature for not less than 5 weeks and were inspected twice a week for adult emergence. Adult fruit flies emerging after eclosion were aspirated and placed in Petri dishes (using separate dishes for separate samples) allowing the colour of the flies to fully develop in order to facilitate identification of the flies as described above.

Statistical analysis

Trapping data were summarised as flies per trap per day (FTD) which is defined as the number of flies of a species collected in each trap and divided by the number of days between the service intervals. Trapping data were averaged for each attractant type, altitude, climatic region and time of the year. Relative abundance indexes (RAI) were calculated according to the methods used by Segura *et al*. (2006) in order to directly compare abundances of *C. rosa* and *C. quilicii*. RAI values were calculated from FTD values of each species. The RAI value was calculated as follows: RAI = FTD *C. quilicii/* (FTD *C. quilicii* + FTD *C. rosa*) (Segura *et al.* 2006). A value of 1 indicates the exclusive presence of *C. quilicii*, and zero, the exclusive presence of *C. rosa*. RAI values were averaged for each location, attractant type, altitude, climatic region and time of the year. Effects of location, attractant type, altitude, climatic region and time of the year on RAI values were analysed using a hierarchical linear model (HLM) in SPSS Statistics Version 25. The dependence of data from the same site were taken into account when comparing means of the different variables. The correlations of weather data and trap catches were analysed by using PROC SURVEYREG in SAS, where the dependence of data from the same farm were taken into account.

Fruit infestation data were summarized for each fruit type sampled and were expressed as the total number of fruit collected in kilograms and the total number of adult flies reared from each citrus species.

C. Comparing the developmental rates of *C. rosa* and *C. quilicii* in citrus fruit

Environment

The study was conducted at the Citrus Research International (CRI) premises in Nelspruit, Mpumalanga, South Africa, during July to October 2016. A temperature controlled room, with a photoperiod L12: D12 was used for incubation of fruit used in the study. Temperature in the room was monitored on an hourly basis using an ibutton, (Maxim ibutton, Fairbridge Technologies, (PTY) Ltd., Sandton, South Africa). Average air temperatures recorded during trials on different citrus types are provided in Table 3.3.3.1. An oscillating desk fan was used to create airflow over the fruit and to keep any unwanted secondary pests such as vinegar flies (Diptera: Drosophilidae) away.

Table 3.3.3.1. Mean temperatures recorded in the incubation room where fruit were kept during the trial for the control fruit and the dissection fruit.

Mean temperature (°C ± SE) in the incubation room for the control fruit			
Eureka	**Star Ruby**	**Nadorcott**	**Late Valencia**
26.35 ± 0.05	26.50 ± 0.04	26.39 ± 0.05	26.18 ± 0.04
Mean temperature (°C ± SE) in the incubation room for the fruit kept to be dissected			
Eureka	**Star Ruby**	**Nadorcott**	**Late Valencia**
26.31 ± 0.07	26.36 ± 0.06	26.76 ± 0.06	26.06 ± 0.06

Fruit

Four citrus export grade fruit types were sourced from growers, depending on seasonal availability. These were lemon (*Citrus limon* (L.) Burman f. cv. Eureka) (hereafter referred to as Eureka), grapefruit (*Citrus paradisi* cv. Star Ruby) (hereafter referred to as Star Ruby), mandarin (*Citrus reticulata* cv. Nadorcott) (hereafter referred to as Nadorcott), and Valencia (*Citrus sinensis* cv. Late Valencia) (hereafter referred to as Late Valencia). The first available fruit, Eureka, was received on 17 June 2016 from Vutsela Iglobhu Investments (Pty) Ltd, (Ryton Estates), Star Ruby was received on 27 June 2016 from Golden Frontiers (Vergenoeg), Nadorcott, on 27 July 2016 from Indigo Fruit (Pty) Ltd, trading as Larten, and Late Valencia, received on 16 August 2016 from Crocodile Valley.

The fruit used in these experiments were neither waxed nor exposed to any other packhouse treatments. Upon arrival at the CRI facilities in Nelspruit, all fruit were dipped into water baths that contained mixtures of fungicides and sanitizers: Citricure (210 g/ l guazatine, ICA International Chemicals Pty. Ltd.), Imazacure (imidazole 750 g/ kg, ICA International Chemicals Pty. Ltd., Stellenbosch, South Africa), Sporekill (120 g/ l didecyldimethylammonium chloride, ICA International Chemicals Pty. Ltd., Stellenbosch, South Africa), TBZ (thiabendazole 500 g/ l, ICA International Chemicals Pty. Ltd., Stellenbosch, South Africa) and Protector (pyrimethanil 400 g/ l, ICA International Chemicals Pty. Ltd., Stellenbosch, South Africa). Different combinations were used for different citrus types. The combinations used were Citricure (4.8ml/ l) + Sporekill (1ml/ l), Citricure (4.8ml/ l) + Sporekill (1ml/ l) + Imazacure (0.67g/ l), Citricure (4.8ml/ l) + Protector (2.5 ml/ l) + TBZ (2ml/ l), Citricure (9.6ml/ l) + Sporekill (2ml/ l) + Imazacure (1.34g/ l), and Citricure (4.8ml/ l) + Sporekill (1ml/ l) + Protector (2.5 ml/ l). All treatments were done in 70 L plastic containers, big enough to facilitate the dipping of a lug box containing fruit. These treatments were done to protect fruit from fungal decay during storage and the experimental phase. All fruit were inspected to ensure that there were no signs of any previous infestations by dipterans, or any physical damage that would allow secondary infestations by pests such as vinegar flies. The dipped fruit were placed into the incubation room the day before the inoculation in order to allow the fruit to warm up to the target incubation temperature of 26°C ± 1 °C, to minimize the potential effect of colder or warmer fruit when the fruit were inoculated.

To determine the external and internal quality of the fruit used during the trial, twelve fruit were randomly selected for each citrus type and for every replicate (in total 36 fruit per citrus type). These fruit were not inoculated with fruit fly eggs. The fruit were graded according to their colour using Set Numbers 37 (Eureka), 35C (Star Ruby), 36 (Nadorcott), 34 (Late Valencia) of the Colour Prints for Blemish and Appearance Standards (1997), published by CRI, endorsed by the National Department of Agriculture, Directorate: Food safety and Quality Assurance described in CRI (1995). Each fruit was weighed on an analytical balance (Shimadzu ELB3000, Roodepoort, South Africa) and their equatorial diameter determined using a fruit size caliper. The fruit were then peeled and the thickness of the skin determined using a Vernier caliper. The fruit were then sent to the Citriculture Laboratory, Citrus Research International, Nelspruit, South Africa, for an internal quality test to establish the percentage extractable juice (%), Brix (°) and acidity (%) and to determine the pH content of the fruit (DAFF 2018).

Insect rearing

Laboratory-reared *C. rosa* and *C. quilicii* were used in this study. Both species were reared in gauze cages at ambient temperatures, inside rooms with the walls consisting of either fine meshed screens or windows, so that the fruit fly cultures had exposure to natural light conditions, to allow for the adult flies' requirement for crepuscular conditions (natural dusk conditions) (Grout & Stoltz 2007). The *C. rosa* culture was renewed in September 2015 by adding flies reared from *Eriobotrya japonica* (Thunb.) Lindl. (Family: Rosaceae), collected in Nelspruit, South Africa. The *C. quilicii* culture was first renewed by flies reared from *Eriobotrya japonica* collected in Pretoria during September 2015, and again from *Acca sellowiana* (O. Berg) Burret (Family: Myrtaceae), collected during February 2016 from Pretoria, South Africa. The adult flies were transferred to the insectaries where they were reared on a carrot-based diet.

Fruit fly eggs for use in bioassays were obtained by placing apples (*Malus domestica* Borkh cv. Granny Smith) inside the mesh cages the day before the start of bioassays. The apples were removed from cages on the day of the inoculation, to ensure that the eggs were never older than 24 hours.

Each apple was punctured four times around its equator with a small pinning device (consisting of eight 6 mm x 0.8 mm diameter pins placed in a row, 2.5 mm apart), to create wounds for females to lay their eggs in and to facilitate easy recovery of eggs according to methods described by Grout & Stoltz (2007). Four males and four females from each of the two fly species were collected from the adult culture (the same culture from where the eggs were collected), and placed in 1.5 ml Boilproof tubes filled with absolute alcohol, to serve as voucher specimens of the test insects as recommended by PMRG (2019). Voucher specimens are kept at CRI.

Inoculation procedure

During inoculation, 20 aliquot samples (0.025 ml per aliquot) of eggs of each fruit fly species were placed on a moistened black cloth inside two separate 90 mm Petri dishes and each aliquot counted to determine how many eggs were placed inside each fruit. Similar aliquots were placed on five Petri dishes per species, to allow the eggs to hatch and to confirm the viability of the eggs after 72 hours.

The inoculation always commenced with *C. quilicii.* On the day of an inoculation a hole of 6 mm in diameter and 20 mm deep was made at the calyx end of each fruit, using a cork borer. After inoculation with eggs of *C. quilicii* was completed, fruit were stored and all equipment washed, before inoculation with *C. rosa* commenced to avoid contamination between the two species. It was estimated that 1 ml of eggs of each species contained approximately 15000 eggs. In order to inoculate 35 eggs into each fruit, 1 ml of eggs was placed into 10.71 ml of water. The water added was adjusted according to the available volume of eggs on the inoculation day. A 0.025 ml aliquot of this egg-water mixture was pipetted into the hole in each fruit. The mixture was always well agitated before an aliquot was drawn to ensure a homogenous mixture. Prior to placement of eggs, Torula yeast (Organic World, Randburg, South Africa), mixed with freshly boiled distilled water in a 1:2 ratio, was added (0.2 to 0.5 ml) into the hole to serve as a food source for the developing larvae (Grout *et al*. 2011b). The hole containing the eggs was filled with a piece of cotton wool and sealed with microwax (Sasolwax 7835, Sasol South Africa (PTY) Ltd., Sasolburg, South Africa) to prevent infestation by other arthropods or fungal infections.

For each fruit type and each fly species, 130 individual fruit were used. Of these, 105 were dissected and 25 served as a control treatment to determine adulthood development. Each fruit for dissection was enclosed individually in a brown paper bag to avoid any fungal growth from spreading to other fruit. Inoculated fruit of each citrus type with the different fly species were randomly selected and placed in crates according to day of dissection. The crates were then covered with shade cloth to further protect them from any secondary infections by other dipterans. The 25 fruit used as control were incubated in bulk on sterilised sand in 9 L aerated plastic containers to facilitate pupation of the jumping larvae. The control fruit were used to determine development period and success rate from eggs to adulthood. All inoculated fruit were kept in the incubation room. The above 130 fruit per citrus type was considered a replicate. For each citrus type, there were three replicates conducted with three separate batches of eggs.

Assessment of development and survival

Seven fruit inoculated with each species were dissected daily for 15 days, commencing one day after inoculation. During dissection, live and dead larvae were counted and recorded. All live larvae from each fruit were collected and kept separate for determination of larval instar and measurement of length. In order to preserve larvae in a good state for subsequent analyses, larvae were killed in boiling hot water and placed in 70% ethanol. Larvae were kept in small microcentrifuge tubes (1.5 ml) with caps. Larvae collected each day, were placed on slides and inspected under a stereomicroscope (10 – 25x magnification) (Leica EZ 4 D, Leica Microsystems Ltd, Heerbrugg, Switzerland) to photograph each individual larva. Mouth part characteristics (presence or absence of pre-apical tooth), presence of a shed mouth hook, and body length were used to determine the larval stage (White & Elson-Harris 1992; Carroll 1998; Steck & Ekesi 2015).

For fruit incubated as control, sand was sieved each day from five days after inoculation onwards, until no more pupae were recovered. All pupae were weighed individually using a Mettler Toledo New Classic MS scale (Microsep (PTY) Ltd, Sandton, South Africa) after which they were placed on sand in Petri dishes. One half of the bottom of the Petri dish was covered with a thin layer of sand and the other half was covered by a piece of filter paper, cut in a semi-circle. The contents of each Petri dish was moistened daily with distilled water to avoid desiccation of the pupae. Emerging adults were allowed to age and to complete full colouring before sex was recorded.

Statistical analysis

The number of larvae collected per day were averaged for each species, fruit type and replicate. For each day after inoculation, the numbers of different instars were recorded to calculate development rate. For each fruit fly species in each citrus type, total gross larval survival rates were calculated by dividing the total number of larvae recovered from a fruit over the 15 days by the total number of eggs inoculated into the fruit. Total net larval survival rates were calculated by dividing the total number of larvae by total number of viable eggs (hatched eggs). When determining the larval survival rate of the two species, the 3rd replicate of the Late Valencia trial was not included in the analysis due to no larvae recorded for *C. quilicii* from day 10 onwards, and for *C. rosa* from day 8 onwards. The absence of larvae could have been due to high fungal growth. The percentage of the different instars per fruit per day was determined. For each instar and citrus type, mean body length was calculated for each fly species. For fruit that were set as control, the larval development was determined by recording the number of jumping larvae per day after inoculation until 27 days after inoculation. Pupal mass was also recorded separately for each citrus type and the mean pupal mass was analysed as per species per cultivar.

The data were analysed using SPSS Statistics Version 25. The effect of citrus type on larval length was analysed by applying a two-way analysis of variance (ANOVA).

A three-way ANOVA was used to determine the effects of fruit fly species, citrus type and time on survival of all the larval stages, from day 2 until day 15. A three-way ANOVA was used to determine the effects of fruit fly species, citrus type and time on survival of only mature larvae recorded over the last 6-day period. Mean pupal mass was compared between the citrus types and fruit fly species by means of a two-way ANOVA.

Results and discussion

A. Fruit fly attractants

During the trial period, male flies were captured by all three lures (Table 3.3.3.2). Higher catches of *C. quilicii* and *C. rosa* were recorded in Egolure baited traps. Females were largely collected from Biolure traps with the exception of two flies caught in Egolure traps. The combined trap catches from the nine locations were 1052 for *C. quilicii* and 89 for *C. rosa* males, and 494 and 17 females respectively of *C. quilicii* and *C. rosa.* The RAI average value for Biolure was similar to that of Egolure and both were significantly different from that of Capilure (Table 3.3.3.2) (P = 0.04).

Table 3.3.3.2. Total number of male and female flies caught in traps at nine localities over a 12-month period for *C. rosa* and *C. quilicii*.

Species	Biolure	Capilure	Egolure	Total
Average RAI value	0.89 a	0.76 b	0.88 a	0.86
***C. quilicii* males**	378	108	566	1052
***C. rosa* males**	21	19	49	89
***C. quilicii* females**	492	0	2	494
***C. rosa* females**	17	0	0	17
Total	921	127	617	1652

*Means within rows followed by the same letter do not differ significantly at P = 0.05

Male and female catches of each species were compared in the different sites for each species. Although *C. quilicii* males were caught at all nine sites, females were only caught at eight sites (Figure 3.3.3.1). A ratio of 1.1:1 *C. quilicii* males in Egolure traps to *C. quilicii* females in Biolure traps was obtained when all nine sites were pooled together. For *C. rosa* males, the ratio of males in Egolure traps to females in Biolure traps was 1.3:1 when catches from all sites, excluding Vergenoeg, were pooled. In Vergenoeg, catches of *C. rosa* males in Egolure traps were 8.5 times higher than catches of females in Biolure traps.

The female ratio of the two species in the Biolure traps and the male ratio of the two species in the Egolure traps were also compared at each site (Figure 3.3.3.1). In most sites, except the low altitude and Hot-Humid climate zone where *C. rosa* dominated, the ratio between the males and females of the two species were similar, irrespective of whether a male or a female trap was employed. In the low altitude and hot humid climate zone (Vergenoeg and Whisky) ratios of *C. rosa* and *C. quilicii* males in Egolure traps were not similar to the ratios of females of the two species in Biolure traps (Figure 3.3.3.1).

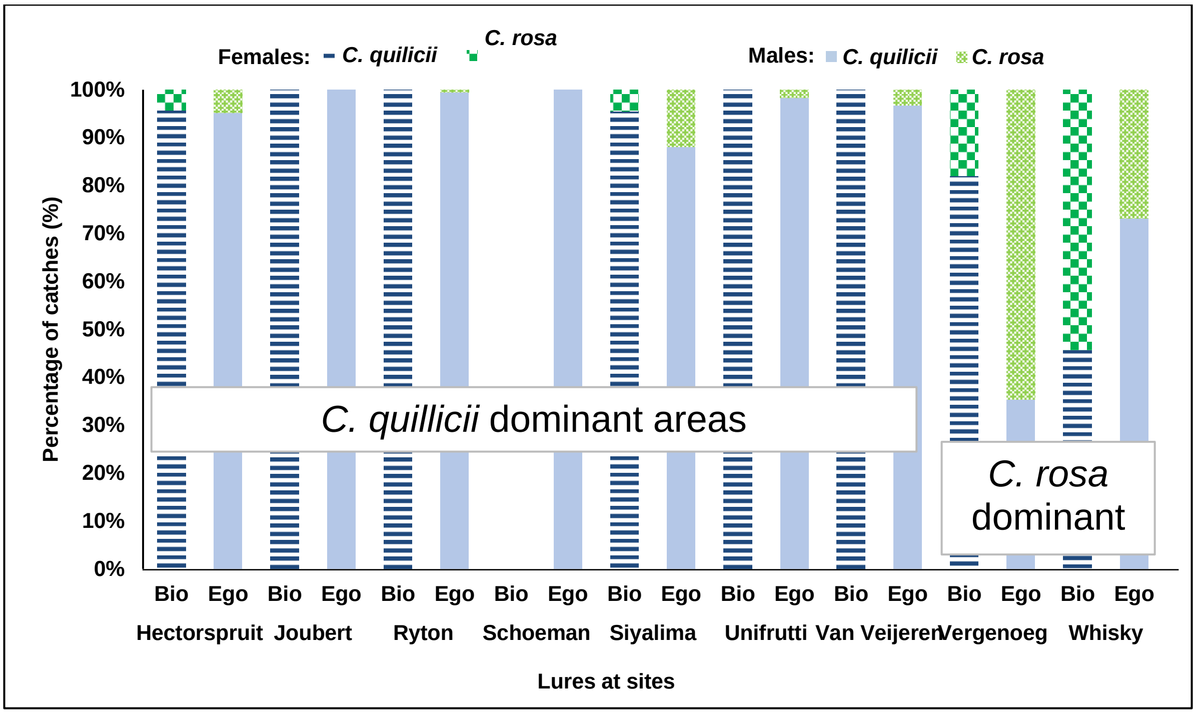


Figure 3.3.3.1. A comparison between the ratio of *C. quilicii* and *C. rosa*, female and male catches at nine sites.

B. Abundance

Ceratitis quilicii was the dominant species and was caught at all nine sites (Figure 3.3.3.2). *Ceratitis rosa* was only collected from seven of the nine sites, and it never represented more than 5% of the total number of trapped flies except at two of the trial sites. The two sites where an abundance of *C. rosa* was equal or higher than *C. quilicii* were situated in the low altitude Hot-Humid climatic zone (Figure 3.3.3.2).

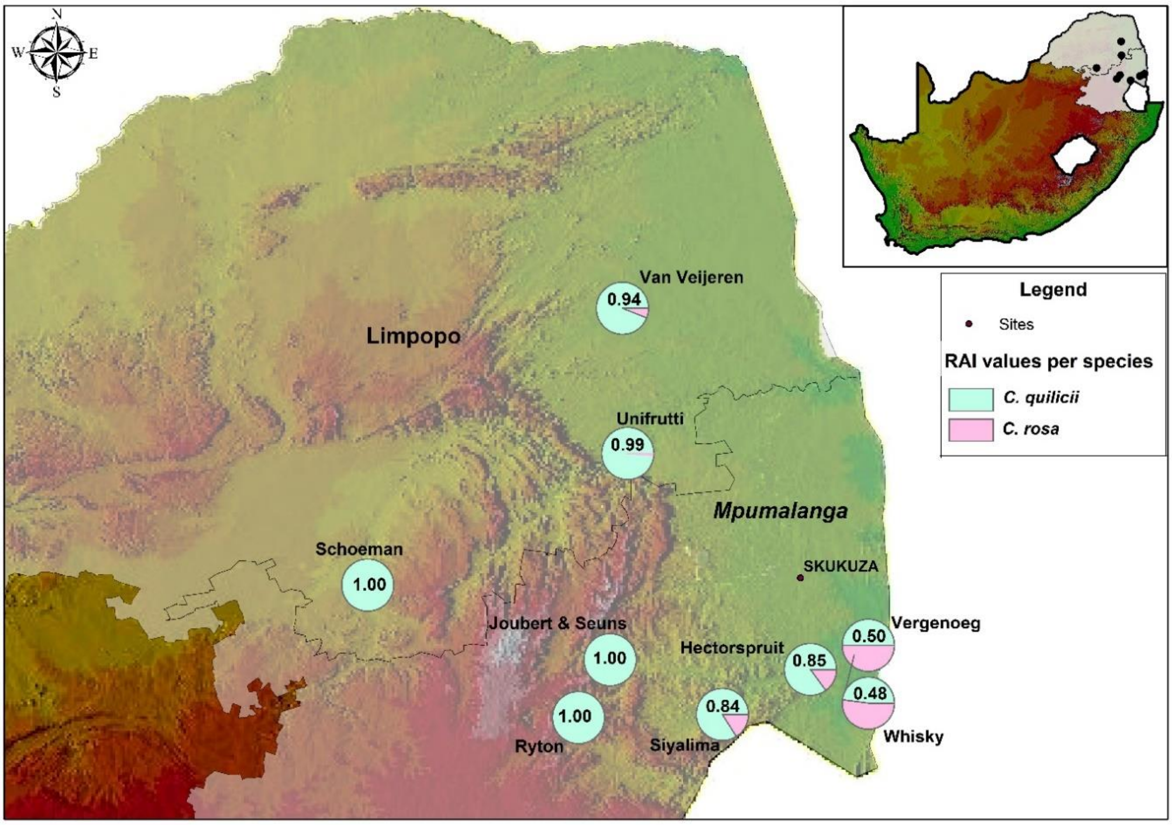


Figure 3.3.3.2. Relative Abundance Index (RAI) values of *C. quilicii* and *C. rosa* at the nine sampling sites in the Mpumalanga and Limpopo provinces. Total trap catches (males and females) of all sampling months and of all lures were combined.

The higher abundance of *C. quilicii* was reflected in all the lures used in the study: Biolure, Capilure and Egolure confirmed (Table 3.3.3.3).

Table 3.3.3.3. The relative abundance index (RAI) values of C*. quilicii* and *C. rosa* in the nine sampling sites using three different lures. A RAI value of 1 indicates total dominance of *C. quilicii* and a RAI value of 0, total dominance of *C. rosa*.

	Number of sites in each RAI category for each lure					
Lure	**Zero Catches**	**RAI = 0**	**0 - 0.33**	**0.34 - 0.66**	**0.67 - 1**	**RAI = 1**
Biolure	1	0	0	1	4	3
Capilure	1	1	1	1	1	4
Egolure	0	0	0	1	5	3

The effect of altitude and the four climatic zones on abundance of C. quilicii and C. rosa

There was a significant effect of altitude on the relative abundance of *C. rosa* and *C. quilicii* (P=0.04) irrespective of which lure was used (Figure 3.3.3.3). At the lower altitudes, the RAI values were lower, indicating an increased presence of *C. rosa* (Figure 3.3.3.3). There was no interaction between altitude and lure (*P* = 0.154).

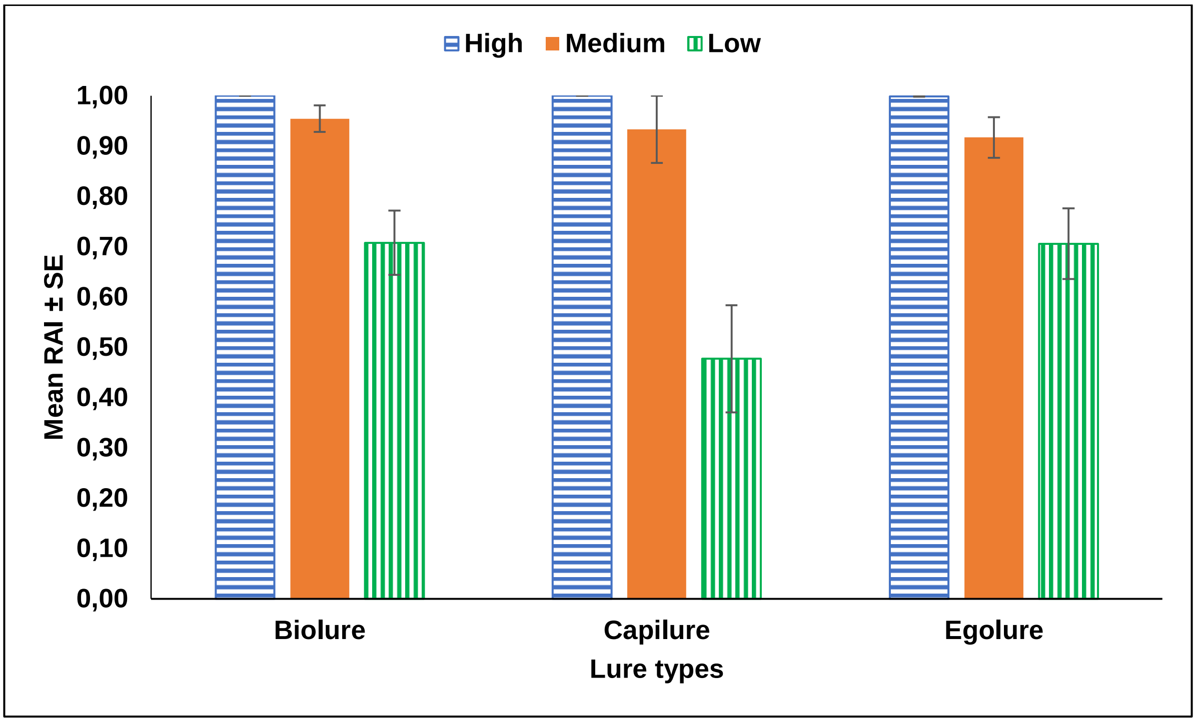


Figure 3.3.3.3. Average relative abundance index (RAI) values of fly captures by means of three lures at different altitudes. Bars indicate standard errors. The RAI value was calculated as follows: RAI = FTD *C. quilicii/* (FTD *C. quilicii* + FTD *C. rosa*).

Temperatures increased with a decrease in altitude, which resulted in a lower RAI value for *C. rosa*. The mean maximum temperature at the high altitude sites was 3.6 °C lower than at low-altitude sites. The mean minimum temperature was 4.7 °C lower at the high altitude sites than low altitude sites. With increases in maximum temperatures, the abundance of *C. rosa* also increased (lower RAI value). The average humidity recorded at the three different altitudes was similar while rainfall was highest in the high altitude region, followed by the low altitude region (Table 3.3.3.4).

There were no significant differences (P = 0.10) in RAI values between the climatic zones when data where pooled for the three lure types. However, there was a statistically significant interaction between climatic region and lure (P = 0.05). In Capilure baited traps, catches in the Intermediate zone consisted of only *C. rosa* males (Figure 3.3.3.4) whilst this was not the case with the other lures. In the Hot-Humid zone, *C. rosa* numbers dominated in Capilure baited traps (RAI = 0.48) but not in Biolure or Egolure traps (Figure 3.3.3.4).

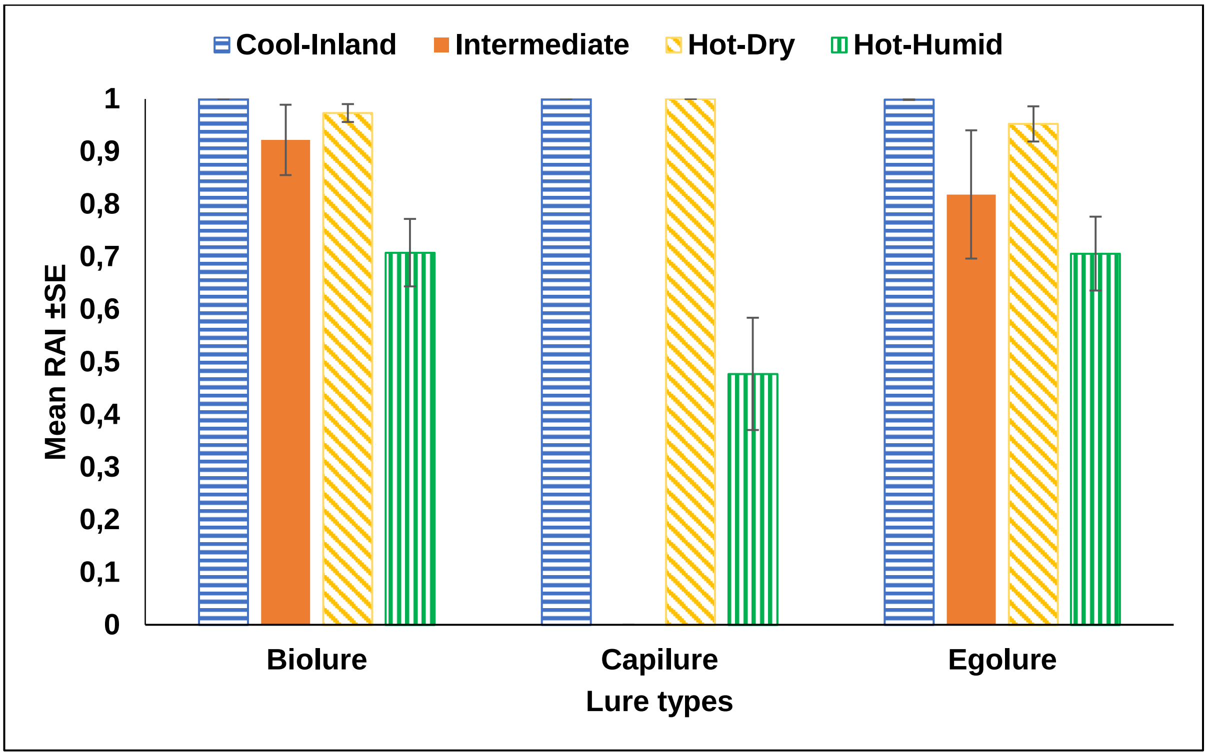


Figure 3.3.3.4. Mean relative abundance index (RAI) values distributed over four climatic zones according to numbers of flies caught by the different attractants. Bars represent standard errors.

Ceratitis quilicii was dominant in all four climatic zones (pooled data for different lures). The average RAI value was at its highest in the Cool-Inland climatic zone. Lower numbers of *C. rosa* were captured in the zone with lower temperatures. Temperatures were the highest in the Hot-Humid and the Hot-Dry zones. The RAI was again lowest in the Hot-Humid zone (RAI =0.65) but high in the Hot-Dry area (RAI = 0.97). The average humidity was the highest in the Hot-Humid zone and lowest in the Hot-Dry zone (Table 3.3.3.4). The total rainfall recorded was the highest in the Cool-Inland zone followed by Hot-Humid zone, Intermediate and the Hot-Dry zone (Table 3.3.3.4).

Table 3.3.3.4. The mean daily relative abundance index (RAI) values, maximum and minimum average humidity and total rainfall for each altitude region and climatic zone.

		Mean RAI	Mean daily humidity (rH)	Mean total rainfall (mm)
Altitude	**High**	1.00	61.21	795.50
	Med	0.94	59.99	732.37
	Low	0.65	64.14	832.79
Climatic zone	**Cool-Inland**	1.00	62.92	916.30
	Intermediate	0.85	60.47	789.27
	Hot-Dry	0.97	58.42	586.24
	Hot-Humid	0.65	64.14	832.79

Ceratitis rosa catches were positively correlated with minimum temperature (Table 3.3.3.5). *Ceratitis rosa* catches were also negatively correlated with altitude (Table 3.3.3.5). This indicates a higher abundance of *C. rosa* with increasing minimum temperatures and a lower abundance of *C. rosa* at higher altitudes.

Table 3.3.3.5. Correlation between mean fruit fly numbers caught per day (all traps) and abiotic factors: altitude, maximum temperature, minimum temperature, maximum relative humidity and total relative humidity.

		Altitude (m a.s.l.)	Max Average temp. (°C)	Min Average temp. (°C)	Max Average rH (%)	Min Average rH. (%)	Total rainfall (mm)
C. quilicii	**Correlation Coefficient**	0.017	-0.402	-0.033	-0.017	-0.218	0.402
	***P*-value**	0.966	0.284	0.932	0.966	0.574	0.284
C. rosa	**Correlation Coefficient**	-0.869**	0.633	0.751*	0.380	0.278	0.270
	***P*-value**	0.002	0.067	0.020	0.313	0.468	0.482

** Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed)

Seasonal effect

In the high and medium altitude sites, *C. quilicii* was more abundant than *C. rosa* across the year (Figure 3.3.3.9). In May, *C. rosa* numbers increased in the medium altitude sites but *C. quilicii* still remained dominant in that month (Figure 3.3.3.5). In the low altitude sites, C. *quilicii* was only dominant during winter (July and August) and summer (January and February) months (Figure 3.3.3.5).

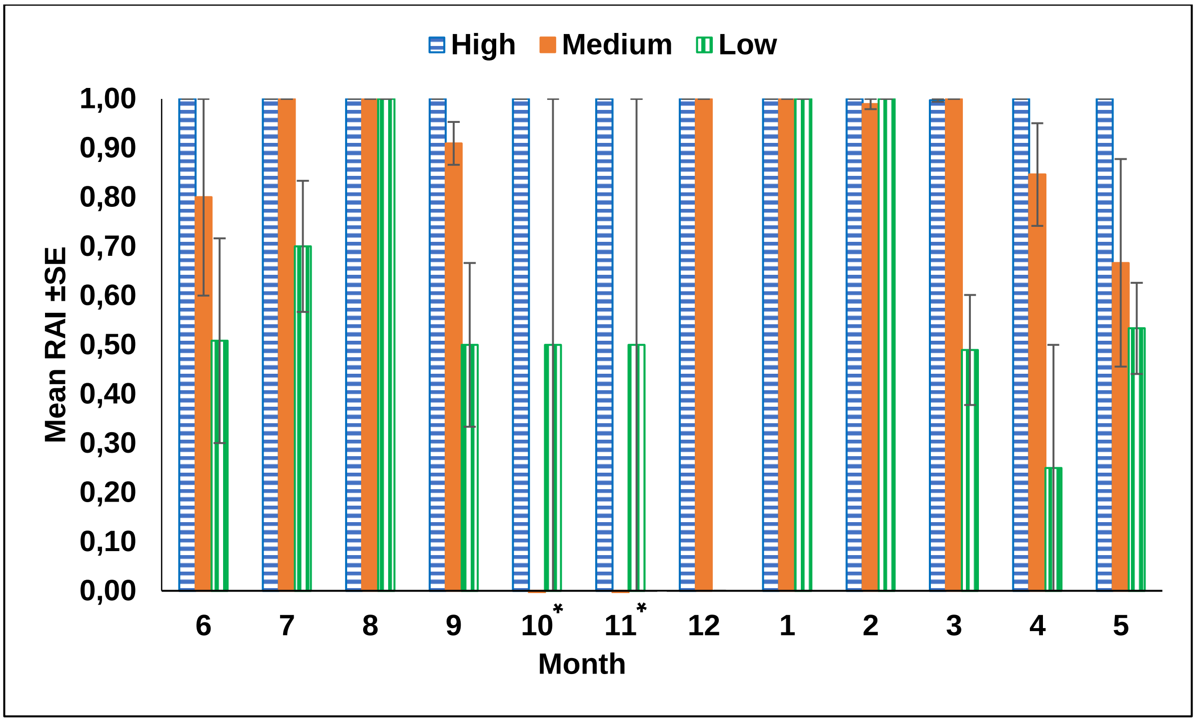


Figure 3.3.3.5. Mean relative abundance index (RAI) values for all lures combined for both males and females over a twelve-month period (where month 1 equals the first month of the year i.e. January, with the rest of the months represented consecutively) at three different altitudes. The two months (10 and 11) denoted by an asterisk indicate no catches at medium altitude.

In all climate zones except Hot-Humid, *C. quilicii* was more abundant than *C. rosa* for most parts of the year (Figure 3.3.3.6). In the Intermediate zone, *C. rosa* was more dominant during April and May (Figure 3.3.3.6). In the Hot-Humid zone, *C. rosa* was more abundant than *C. quilicii* across the year except in the winter (July and August) and summer (January and February) months (Figure 3.3.3.6).

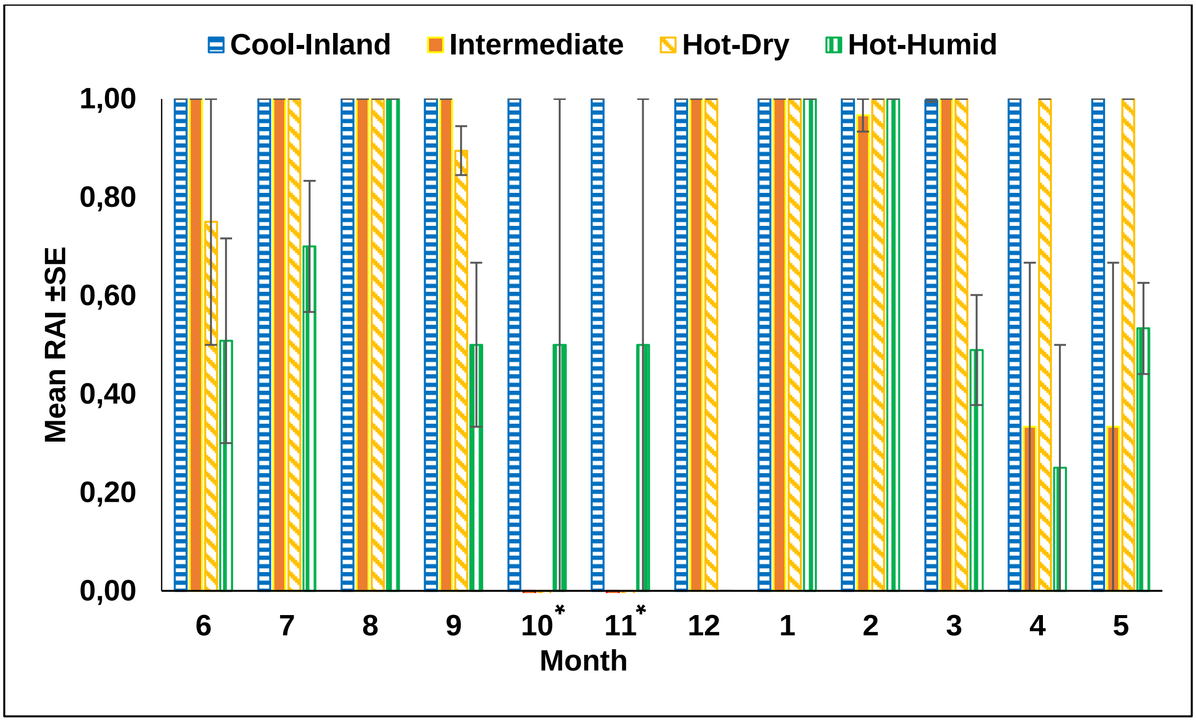


Figure 3.3.3.6. Mean relative abundance index (RAI) values, all lures combined for both males and females over a twelve-month period (where month 1 equals the first month of the year i.e. January, with the rest of the

months represented consecutively), in four different climatic zones. The two months (10 and 11) denoted by an asterisk indicate no catches at medium altitude.

Ceratitis quilicii male and female catches showed similar peaks in September (spring) and February (late summer) (Figure 3.3.3.7). Catches of *Ceratitis rosa* males and females peaked between March and May (autumn) (Figure 3.3.3.8). High catches of *C. rosa* males also occurred in June (winter) and September (spring), (Figure 3.3.3.8).

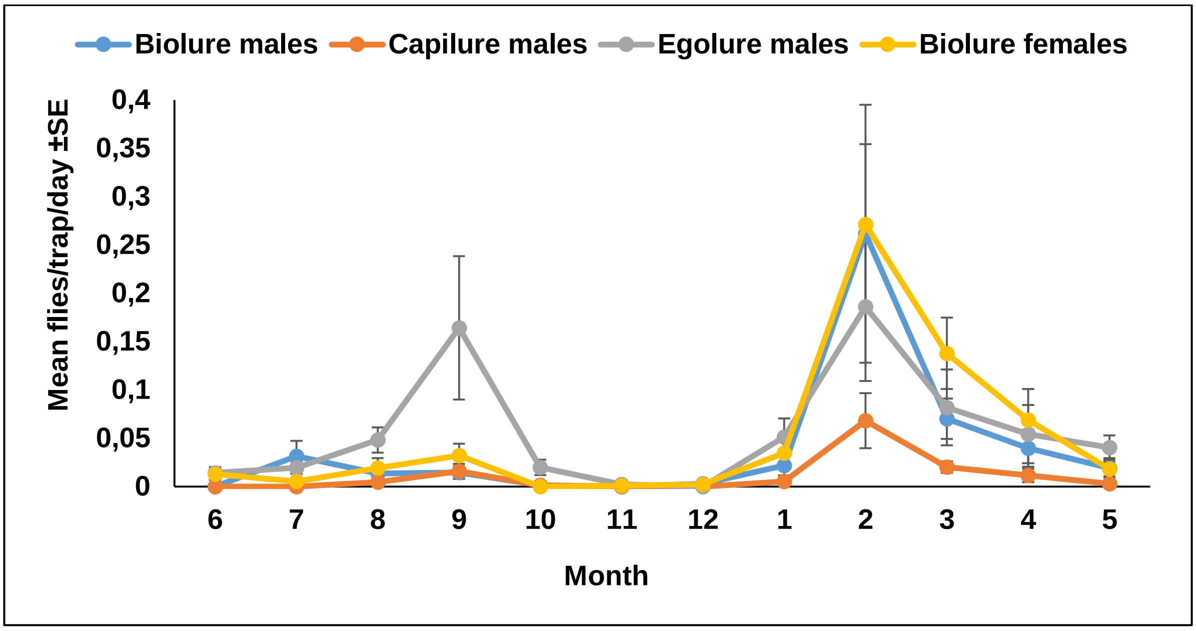


Figure 3.3.3.7. Mean numbers of male and female *C. quilicii* flies caught with different attractants over a twelve-month period (where month 1 equals the first month of the year i.e. January, with the rest of the months represented consecutively). Bars indicate standard error.

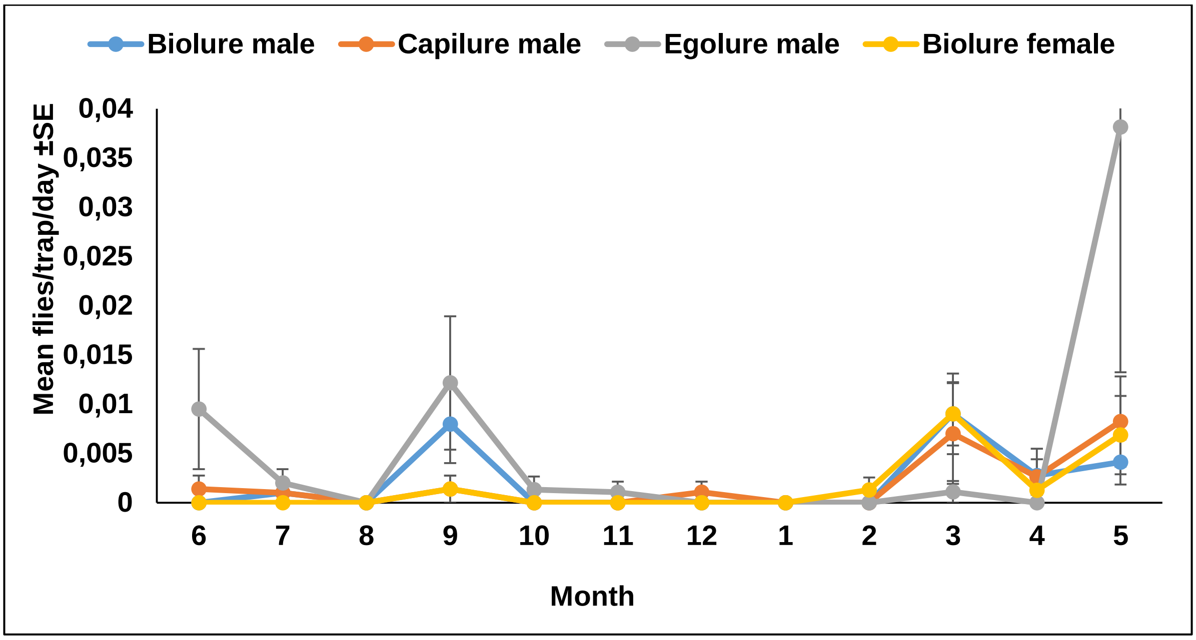


Figure 3.3.3.8. Mean numbers of male flies of *C. rosa* caught with different attractants over a twelve-month period (where month 1 equals the first month of the year i.e. January, with the rest of the months represented consecutively). Bars indicate standard error.

Host fruit

During the trial period, 188 kg of citrus, Clementine, grapefruit, lemon and oranges, were collected from the ground but neither *C. rosa* nor any *C. quilicii* adults were reared from the citrus collected from the commercial orchards.

A & B. Discussion

Ceratitis quilicii was highly abundant and more widespread than *C. rosa* in the northern provinces of South Africa. Karsten *et al*. (2016) reported similar results, however, they did not collect any *C. rosa* from the sites where they sampled, even though some sites (Komatipoort and Nelspruit) were very close to the sites used in this study. The difference in the results between this study and the study by Karsten *et al.* (2016) could have been as a result of sampling period. Karsten *et al.* (2016) did not sample the populations of *C. rosa* and *C. quilicii* continuously throughout the year. The authors described a year as their sampling period and the samples analysed could have been from a once off sampling. Karsten *et al.* (2016) discussed the importance to sample at the right time of the year when the fly numbers are at their highest due to the availability of ripening fruit, especially with species such as *C. rosa* that might occur in lower numbers, which was confirmed in this study. This study sampled over a twelve-month period improving the chances of collecting *C. rosa* adults.

Ceratitis quilicii dominated at the two higher-altitude regions (426 – 1086 m a.s.l.). This is in agreement with Mwatawala *et al*. (2015) who reported that at low altitudes (< 550 m), *C. rosa* was more dominant, and at high altitudes (> 1170 m), *C. quilicii* was the dominant species. In this study, *C. rosa* was not recorded in two of the higher altitude sites. There was however a record of it in one of the high altitude sites. Mwatawala *et al*. (2015), on the other hand, found that the two species occurred together at all altitudes between 540 – 1650 m a.s.l. in Tanzania. In a study on the temperature related developmental times of *C. rosa* and *C. quilicii*, Tanga *et al*. (2015) estimated a lower developmental threshold for *C. quilicii* than for *C. rosa* (lower threshold of Kenyan *C. rosa* population was 11.27 °C, and that of the *C. rosa* South African population was 8.99 °C). In the Cool-Inland zone in this study, average minimum air temperature was 11.05 °C which possibly limited the growth of *C. rosa* populations in these regions. According to Begon *et al*. (2006) there is a temperature drop of 1 °C for every 100 m gain in altitude in dry air, and 0.6 °C in moist air, with a corresponding decrease in species richness. In this study, an increase in *C. rosa* was observed with an increase in minimum air temperature and a decrease in altitude.

No significant relationships were observed in this study between species distribution and either humidity or total rainfall, leaving temperature as the main abiotic factor that could partition the distribution of the two species. Duyck *et al*. (2006) reported that *C. rosa* s.l. was more sensitive to low humidity than *C. capitata,* but that it was more tolerant to low temperatures than *C. capitata.* The optimum temperature for *C. rosa* s.l. is between 22 - 23 °C, with an annual rainfall of 3000 – 3500 mm (Duyck *et al.* 2006). De Villiers *et al*. (2013) confirmed that *C. rosa* s.l. was absent from the drier regions of South Africa and found a positive correlation between its distribution and precipitation. In Réunion, *C. rosa* s.l. occurs on the wetter side of the island (Normand *et al*. 2000). It is possible then that both *C. quilicii* and *C. rosa* will be affected with changes in humidity and rainfall.

A comparison between the two species did not show any change in ratio over twelve months at high altitude or the Cool-Inland zone, with total dominance of *C. quilicii* in the trap catches. This result was also similar for the medium altitude and Hot-Dry zone, except for May when more male C*. rosa* flies, trapped by Biolure and Capilure, were caught at medium-altitude sites. In the low-altitude and Hot-Humid zone, the dominance of the two species changed over time, with RAI-values of ≤ 0.5 in seven of the twelve months. A study conducted by Kounatidis *et al*. (2008) reported movement of *Bactrocera oleae* (Rossi) between higher and lower altitudes as seasons changed. During warm months there were more *B. oleae* flies in orchards at high and medium altitudes, while during winter, flies were more abundant in low-lying regions. Geurts *et al*. (2012) found that *C. rosa* s.l. was more abundant in high-altitude areas in Tanzania, during the rainy season (February and March), similar to the observations on *C. quilicii* in this study

The effectiveness of trimedlure as a lure for *C. rosa* was demonstrated by Georgala (1964). Trimedlure has been reported to be more effective in attracting *C. capitata* than *C. rosa* s.l. (Grout *et al*. 2011a) and is also considered an effective lure for monitoring the FAR complex (Virgilio *et al*. 2008). Grout *et al*. (2011a) found that immature *C. rosa* males were less attracted to trimedlure. Egolure, which was recently introduced into the South African citrus industry, contains α-copaene (Shelly & Pahio 2002) and, like trimedlure, gives a mating advantage to *C. rosa* (Quilici *et al.* 2013; Manrakhan *et al*. 2017b). One of the advantages of Egolure is that it attracts a wide range of *Ceratitis* species (Manrakhan *et al*. 2017b; Mwatawala *et al*. 2013, 2015). A study conducted by Manrakhan *et al*. (2017a) found that *C. rosa* and *C. quilicii* were attracted in similar numbers by Egolure and trimedlure. In this study most of the males of both species were caught using Egolure.

In this study, catches of *C. quilicii* males with Biolure, an attractant which is not species-specific and female biased, were lower compared to catches of conspecific females. Grové & De Beer (2019), trapped fruit flies in the Mbombela Local Municipality, Mpumalanga, using Biolure and reported higher catches of *C. rosa* s.l. females than males with this attractant. Results on catches of *C. rosa* with Biolure in this study were, however, different with catches of males being very similar to the female catches.

A practical problem for managing fruit flies in orchards is that only the males of *C. quilicii* and *C. rosa* can be differentiated morphologically, and this needs to be done by means of dissecting microscopes which are not available to all farmers (De Meyer *et al*. 2016; Virgilio *et al*. 2019). This causes some practical difficulties in fruit fly monitoring particularly where species have to be differentiated in attractant-baited traps. In citrus, there are specific thresholds for *C. rosa* in capilure and trimedlure baited traps since *C. rosa* is a recognized pest of citrus. There is however no threshold for *C. quilicii* given that the latter is not yet considered a pest of citrus in South Africa. The results of this study can be used to estimate *C. rosa* catches in capilure or trimedlure baited traps, particularly for citrus production regions of Limpopo and Mpumalanga. About 5% of the *C. rosa* s.l. captured in capilure baited traps in citrus production regions at the high and intermediate altitudes can be assumed to be *C. rosa* while 50% of the *C. rosa* s.l. males captured in Capilure baited traps in citrus production regions at the low altitudes can be assumed to be *C. rosa*.

There was no evidence in this study that citrus was utilized as a host for either *C. rosa* or *C. quilicii* although this result could have been affected by pesticide applications. However, since various citrus species are listed as hosts for *C. rosa*, the host status of citrus to these two species should be further investigated using semi field set ups as recommended by Aluja & Mangan (2008).

C. Compare the developmental rates of *C. rosa* and *C. quilicii* in citrus fruit

Fruit condition and internal quality

Different fruit conditions, internal quality of the fruit, or fruit that is too green or too ripe, may enhance or delay development and survival of the larvae in the fruit. Cold sterilization trials were conducted on export quality fruit and therefore all fruit used in this trial were selected with this as the standard. The internal quality of the fruit was evaluated against the Minimum Standards, Standards and Requirements for Citrus, No. 634 (DAFF 2018) and found to be satisfactory.

Results on the external and internal characteristics of the four citrus types are provided in Table 3.3.3.6. Fruit of Star Ruby were the largest. Nadorcott fruit had the thinnest rind. Star Ruby, Nadorcott and Valencia fruit, had higher juice content and Brix-values. Acidity value for Eureka was much higher than that of other cultivars.

Table 3.3.3.6. External and internal characteristics of the four citrus types (Eureka, Star Ruby, Nadorcott, and Late Valencia) used in the trial. Fruit that were ready to be harvested, internally and externally mature, and of export quality were selected for determining these parameters. Colour grades were determined according to the scale of Grade 1 = fully coloured fruit to Grade 8 = totally green fruit.

Citrus types	Date tests were conducted	Site	Mean fruit size (mm)	Juice (%)	Brix (°)	Acid (%)	Ratio (Brix to Acid)	pH	Mean rind thickness (mm)	Mean weight (g)	External colour grade
Eureka	2016/07/06	Ryton	74.3	45.82	8.8	9.49	1.0	2.32	5.2	227.7	1
Star Ruby	2016/07/08	Vergenoeg	87.4	51.73	12.8	1.36	9.4	3.13	5.3	274.0	2
Nadorcott	2016/08/02	Larten	74.8	55.46	13.8	1.16	11.9	3.34	3.8	141.4	1
Late Valencia	2016/08/31	Croc Valley	78.2	55.56	11.6	1.27	9.2	3.61	5.0	212.7	1

Egg viability

Egg viability was tested outside the fruit by placing aliquots of an egg and water mixture on a black cloth in Petri dishes on the day of the inoculation. Mean egg hatch rates were lower for *C. quilicii* compared to *C. rosa* except for when trials were conducted with the Late Valencia fruit when *C. quilicii* was higher than *C. rosa* (Table 3.3.3.7).

Table 3.3.3.7. Inoculation dates, average number of eggs and average percentage egg hatch in the aliquots placed on the Petri dishes during each inoculation. All eggs used during the inoculations were less than 23 hours old.

		Ceratitis quilicii		***Ceratitis rosa***	
Citrus types	**Date of inoculation**	**Mean number of eggs**	**Mean % hatched**	**Mean number of eggs**	**Mean % hatched**
Eureka	2016/07/04	34.17 ± 2.32	54.64 ± 3.27	35.93 ± 1.94	87.47 ± 1.06
	2016/07/06				
	2016/07/09				
Star Ruby	2016/07/18	36.15 ± 1.83	60.95 ± 2.73	37.30 ± 2.25	79.28 ± 1.54
	2016/07/20				
	2016/07/22				
Nadorcott	2016/08/02	39.83 ± 2.05	60.73 ± 2.33	44.07 ± 2.68	74.97 ± 2.19
	2016/08/08				
	2016/08/10				
Late Valencia	2016/09/16	40.33 ± 2.19	61.87 ± 1.56	44.50 ± 1.91	60.74 ± 2.52
	2016/09/21				
	2016/09/23				

Larval development

Larval development of both species was influenced by citrus type. The 1st instars of both fly species developed slower in Eureka compared to Star Ruby and Nadorcott (Figure 3.3.3.9). *Ceratitis rosa* was one day later than *C. quilicii* in Late Valencia (Figure 3.3.3.2D). On day 3, all larvae of both species were at the 1st instar stage in all citrus types (Figure 3.3.3.9). In Nadorcott, no 2nd instars were collected for *C. quilicii* (Figure 3.3.3.10C). The number of 2nd instars of *C. rosa* was lower in Nadorcott compared to other citrus types (Figure 3.3.3.10). For *C. rosa*, development of the 2nd instars was quicker in Star Ruby and for *C. quilicii* development was quicker in Late Valencia fruit compared to the other two citrus types (Figure 3.3.3.10). Development of larvae to 3rd instar for both species was the quickest in Star Ruby (Figure 3.3.3.11). Development of larvae to 3rd instar took one day longer for both species in Late Valencia compared to the other citrus types (Figure 3.3.3.11).

There were differences in larval developmental rates of the two species, depending on citrus type and stage of development. *Ceratitis quilicii* developed quicker to 2nd instar than *C. rosa* in Eureka and Late Valencia but were similar to *C. rosa* in Star Ruby (Figure 3.3.3.10). Development of *C. rosa* to third instar was two days faster than *C. quilicii* in Eureka and two days faster than *C. quilicii* in Nadorcott (Figure 3.3.3.11A and C).

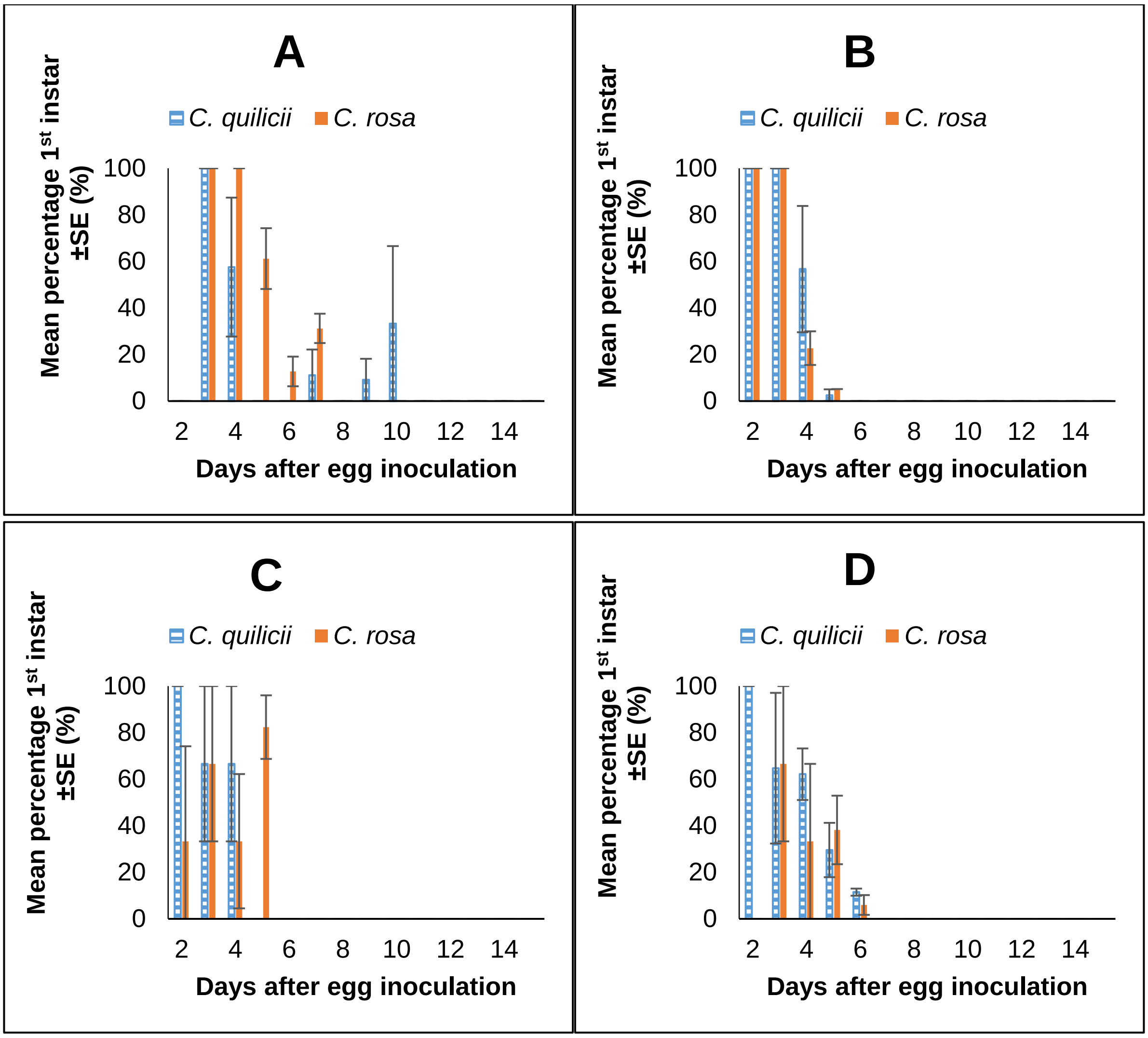


Figure 3.3.3.9. Daily occurrence of first-instars of *Ceratitis quilicii* and *Ceratitis rosa* in fruit of Eureka (A), Star Ruby (B), Nadorcott (C), and Late Valencia (D) for 15 days after inoculation.

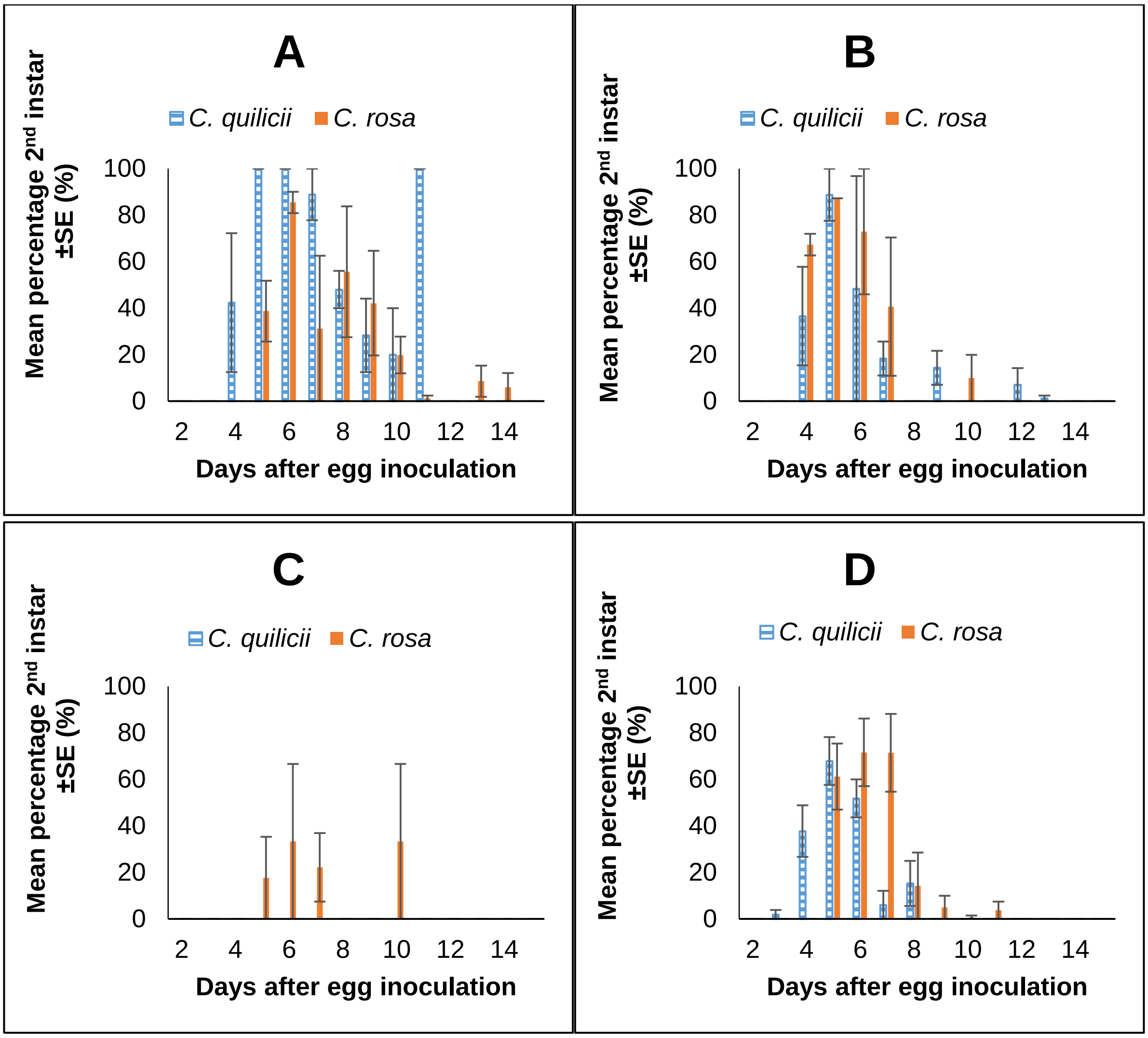


Figure 3.3.3.10. Daily occurrence of second-instars of *Ceratitis quilicii* and *Ceratitis rosa* larvae collected from Eureka (A), Star Ruby (B), Nadorcott (C), and Late Valencia (D) fruit for 15 days after inoculation.

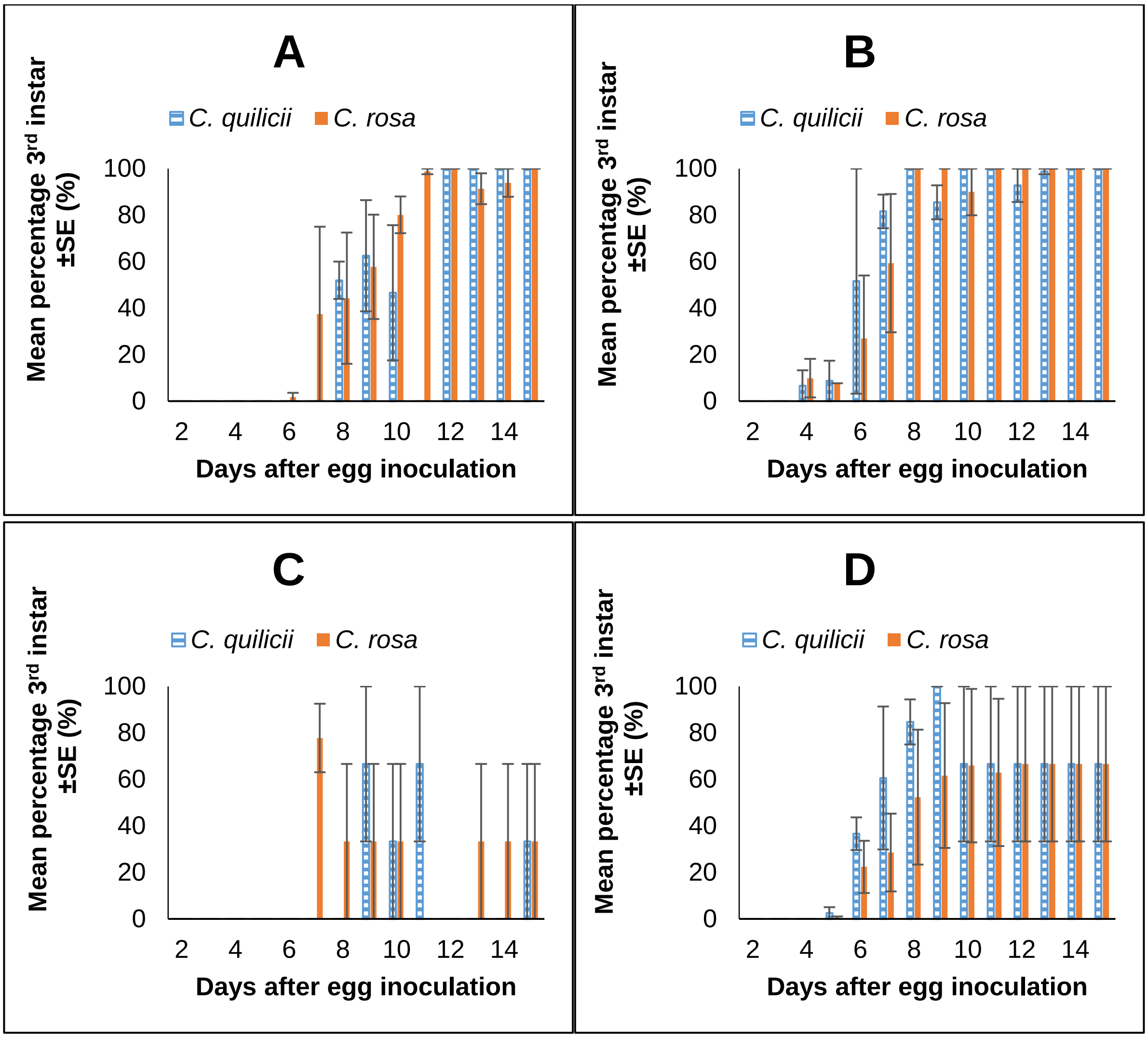


Figure 3.3.3.11. Daily occurrence of third-instars of *Ceratitis quilicii* and *Ceratitis rosa* larvae collected from Eureka (A), Star Ruby (B), Nadorcott (C), and Late Valencia (D) fruit for 15 days after inoculation.

Larvae collected from the dissected fruit were measured to compare the length of the different instars of the two species from each citrus type. The number of larvae measured are represented in Table 3.3.3.8.

Table 3.3.3.8. Total number of larvae of *Ceratitis quilicii* and *Ceratitis rosa* that were measured after they were reared from different citrus types.

		Citrus types		
Species	**Eureka**	**Star Ruby**	**Nadorcott**	**Late Valencia**
C. quilicii	306	378	60	405
C. rosa	664	430	96	639
Total	970	808	156	1044

There were no differences between the lengths of all stages of the larvae of the two fly species ($P < 0.596$) (Figure 3.3.3.12) but larval sizes of both species differed significantly between citrus cultivars ($P < 0.001$) (Figure 3.3.3.12). Longer 1st instars for both species were collected from Star Ruby (Figure 3.3.3.12). Second instars of *C. quilicii* were also longer in Star Ruby (Figure 3.3.3.12) while 2nd instars of *C. rosa* were longer in Eureka (Figure 3.3.3.12). At the third instar stage, larvae of *C. quilicii* were longer when reared in Nadorcott

(Figure 3.3.3.12C). Third instarsof *C. quilicii* were, on the other hand, shorter in Star Ruby (Figure 3.3.3.12B). Third instars of *C. rosa* were longer in Late Valencia (Figure 3.3.3.12D) and shorter in Eureka (Figure 3.3.3.12A).

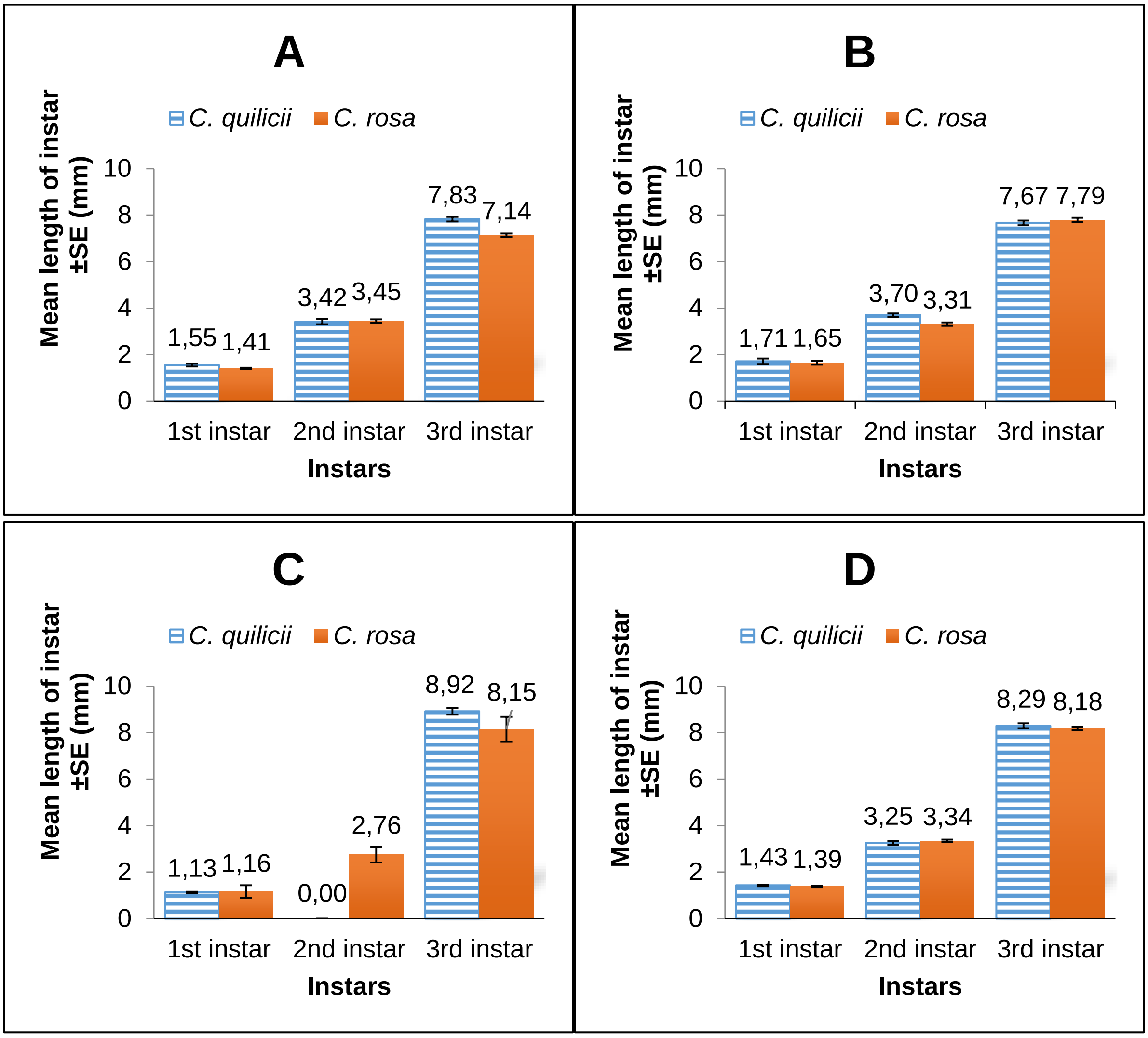


Figure 3.3.3.12. Mean length (mm), indicated above the bars, of *Ceratitis quilicii* and *Ceratitis rosa* larvae at the three development stages collected from Eureka (A), Star Ruby (B), Nadorcott (C), and Late Valencia (D) fruit over 15 days following inoculation.

Larval survival

There were significant differences in survival rates between days after inoculation (P = 0.042), between fly species (P = 0.004) and between citrus types (P < 0.001) but no significant interaction between fruit fly species and citrus types (P = 0.052) and between days, species and citrus type (P = 0.988). Larval survival was lower in Nadorcott for both species (Table 3.3.3.9 and Figure 3.3.3.13). The overall larval survival rates were higher for *C. quilicii* and for *C. rosa* in Late Valencia compared to the other three citrus types (Figure 3.3.3.13). In Eureka and Late Valencia, *C. rosa* performed better than *C. quilicii*.

Table 3.3.3.9. A comparison between the four citrus types (Eureka, Star Ruby, Nadorcott, and Late Valencia) indicating the total number of *Ceratitis quilicii* and *Ceratitis rosa* larvae that were collected over time

	Total number of larvae collected							
	Eureka		Star ruby		Nadorcott		Late Valencia	
Day after inoculation	***C. quilicii***	***C. rosa***	***C. quilicii***	***C. rosa***	***C. quilicii***	***C. rosa***	***C. quilicii***	***C. rosa***
1	0	0	0	0	0	0	0	0
2	0	0	1	3	16	2	30	0
3	21	48	6	11	8	24	42	60
4	18	10	28	75	12	13	53	11
5	12	37	41	39	0	22	61	92
6	10	78	32	43	0	1	38	78
7	6	12	66	54	0	9	17	70
8	30	42	12	29	0	1	19	16
9	45	96	60	28	2	9	22	71
10	28	43	36	35	5	10	22	68
11	1	43	14	31	15	0	17	48
12	29	39	15	30	0	0	22	58
13	40	81	51	43	0	2	30	39
14	27	70	1	4	0	1	16	3
15	39	65	15	5	2	2	16	25
Total gross survival	0.028	0.059	0.033	0.037	0.005	0.007	0.032	0.046
Total net survival	0.052	0.067	0.055	0.046	0.008	0.009	0.051	0.073

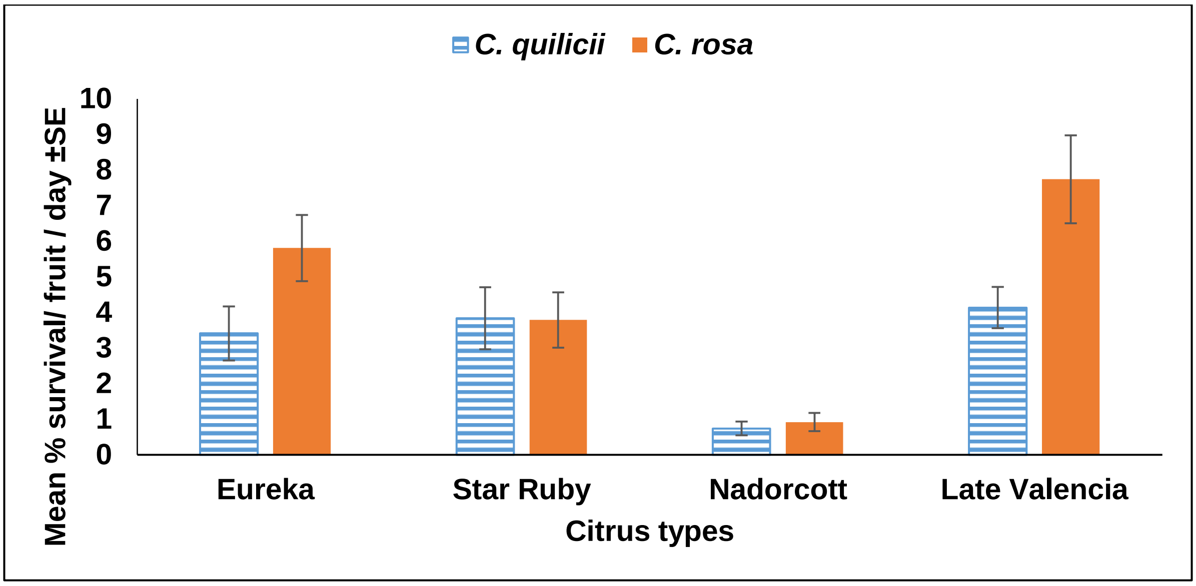


Figure 3.3.3.13. Mean daily larval survival rates of *Ceratitis quilicii* and *Ceratitis rosa* in four citrus types (Eureka, Star Ruby, Nadorcott, and Late Valencia).

Pupal and adult development

The highest number of jumping larvae and pupae were collected from the Late Valencia fruit, followed by Star Ruby, for both fruit fly species (Table 3.3.3.10). For *C. quilicii* the highest number of adults were reared from Eureka and no *C. quilicii* adults were reared from Nadorcott. The highest number of *C. rosa* adults were reared from Late Valencia, however, adult eclosion was the lowest in the same citrus types. Severe fungal growth was observed in the control fruit and might have influenced the number of flies reared. The fruit were infected by green mould (*Penicillium digitatum* (Pers.: Fr.) Sacc.), sour rot (*Galactomyces citri-aurantii* E.E. Butler) and *Aspergillus* spp. (CRI Diagnostic Centre). *Ceratitis rosa* generally had higher pupal survival rates than *C. quilicii*.

Table 3.3.3.10. Total number of jumping larvae, pupae, adults and percentage eclosion recorded from fruit in the control treatments.

Citrus types	Fly Species	No. of jumping larvae	No. of pupae	No. of Males	No. of Females	Total no. adults	% Adult emergence
Eureka	*C. quilicii*	18	14	2	3	5	35.71
	C. rosa	17	16	4	4	8	50.00
Star Ruby	*C. quilicii*	21	15	1	2	3	20.00
	C. rosa	78	72	3	5	8	11.11
Nadorcott	*C. quilicii*	2	2	0	0	0	0.00
	C. rosa	38	37	2	2	4	10.81
Late Valencia	*C. quilicii*	58	44	1	1	2	4.55

C. rosa	148	145	1	8	9	6.21

In contrast to results on the development of larvae to the 3rd instar stage in fruit, the period from inoculation to larval jumping was the shortest in Late Valencia for both fruit fly species followed by Star Ruby (Figure 3.3.3.14). With the control fruit, *Ceratitis quilicii* had a faster larval developmental rate than *C. rosa* in Eureka (Figure 3.3.3.14A) and Star Ruby (Figure 3.3.3.14B), but the two fly species had similar developmental times from inoculation to larval jumping in Late Valencia (Figure 3.3.3.14D). Too few *C. quilicii* larvae were collected from Nadorcott to determine any development rate (Figure 3.3.3.14C).

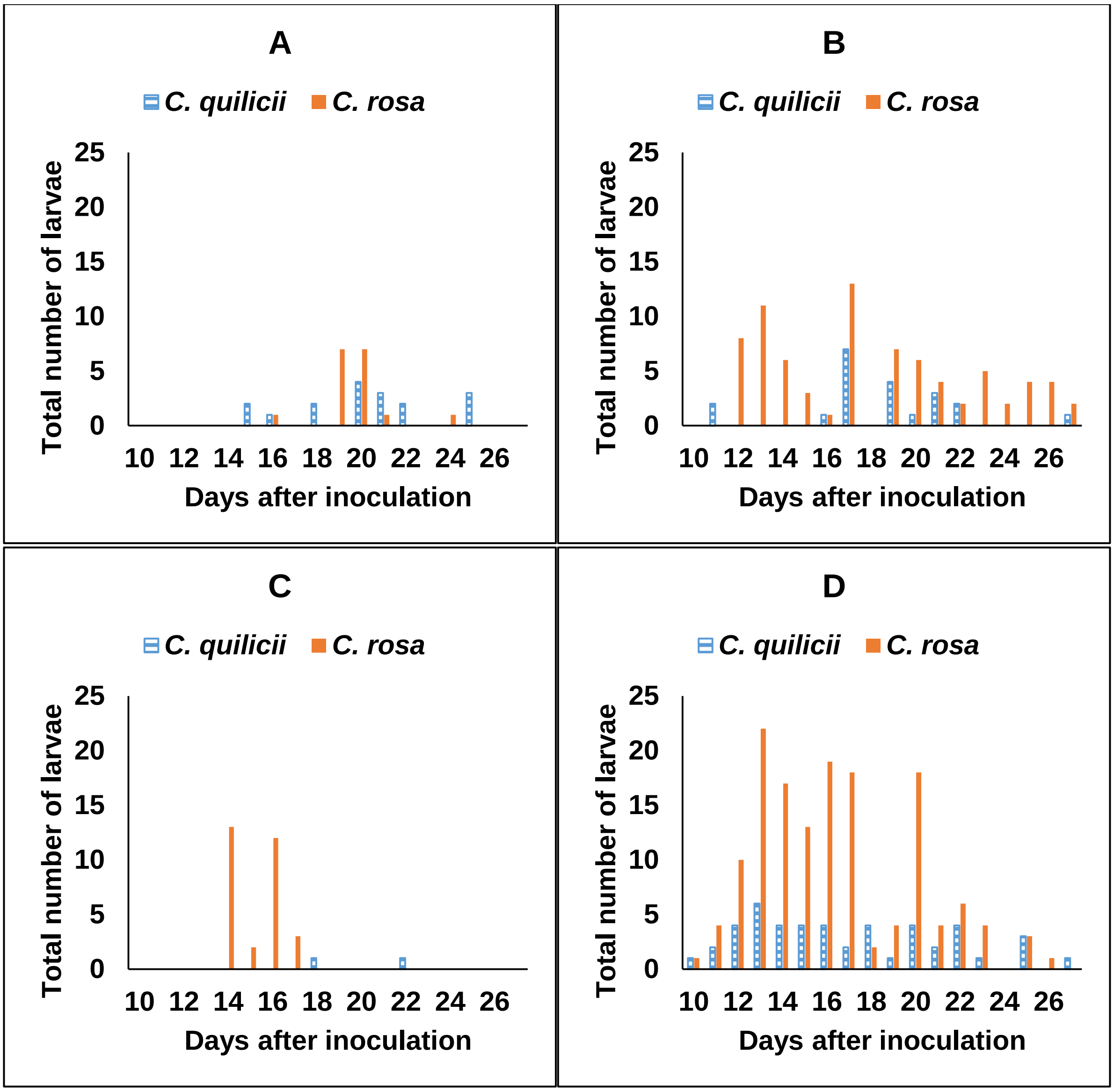


Figure 3.3.3.14. Total number of jumping larvae of *Ceratitis quilicii* and *Ceratitis rosa* collected from Eureka (A), Star Ruby (B), Nadorcott (C), and Late Valencia (D) fruit, each day after inoculation.

There was a significant difference between pupal mass and the different citrus types (P = 0.032) but not between the two fruit fly species (P = 0.118) or between citrus types and species (P = 0.174). While *C. rosa* larvae collected from Nadorcott were the heaviest, *C. quilicii* larvae from Nadorcott fruit were the smallest (Figure 3.3.3.15).

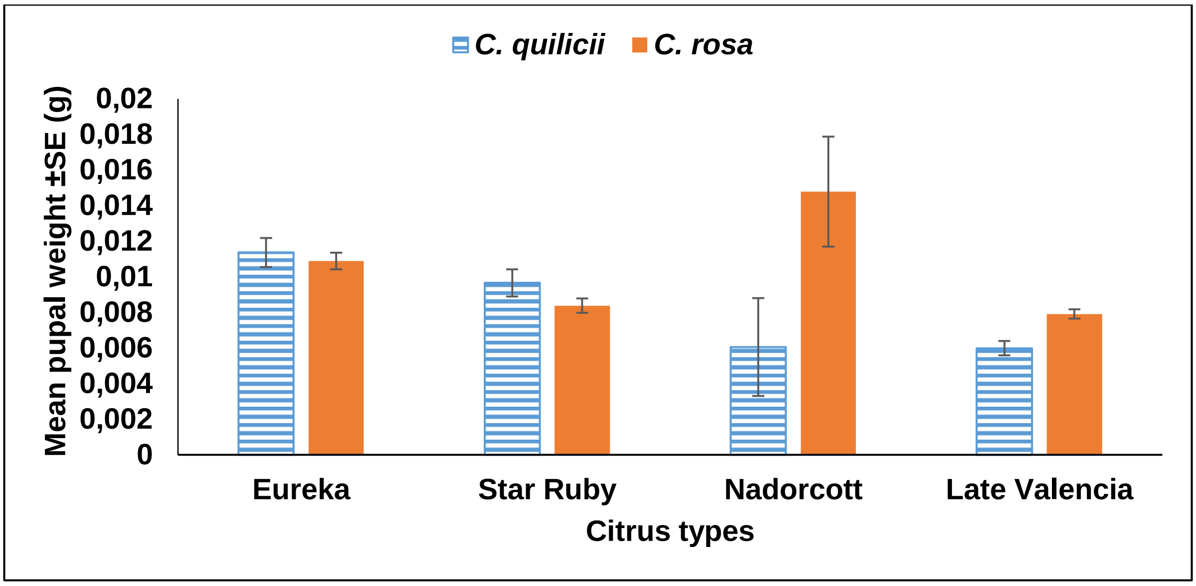


Figure 3.3.3.15. Mean pupal weight of *Ceratitis quilicii* and *Ceratitis rosa* flies collected from the four citrus types (Eureka, Star Ruby, Nadorcott, and Late Valencia).

C. Discussion

In this study, larval development and survival of both species were influenced by citrus type. Survival of *C. quilicii* and *C. rosa* was poor in Nadorcott fruit and no *C. quilicii* adults were reared from this citrus type. Larval performance of polyphagous fruit fly species such as *C. rosa* and *C. quilicii* depend on the host species, fruit size and nutrients in the fruit (Hafsi *et al*. 2016). Hafsi *et al*. (2016) reported that *C. rosa* s.l. larval survival was positively correlated with carbohydrate, lipid, and fibre contents of fruit, but negatively correlated to water content. In this study, Nadorcott had a high juice content together with the highest Brix and ratio. The high sugar content of Nadorcott could have been detrimental to larval development. Hafsi *et al*. (2016) also reported poor larval survival of *C. rosa* s.l. in mandarin, similar to both *C. rosa* and *C. quilicii* in this study. In this study the largest *C. rosa* pupae were recorded from Nadorcott. This heavier weight might be due to the survival of only a few individuals, which had more pulp available, and thus were less exposed to intraspecific competition (Begon *et al.* 2006).

De Lima *et al*. (2007) compared the development of *C. capitata* and *Bactrocera tryoni* (Froggatt) in five citrus types and reported that fewer *C. capitata* survived in lemon fruit. In this study, overall larval survival rates of *C. quilicii* were higher in Late Valencia and *C. rosa* were higher in Eureka and Late Valencia. Lemons have been declared a non-host for fruit flies (Manrakhan *et al*. 2018) and the only reason for the high level of larval survival observed in this study is ascribed to the artificial inoculation of fruit with fly eggs. The mean thickness of lemon rind in this study was 5 mm, which is too thick a barrier for a fruit fly female to penetrate, and similarly a hard peel or pericarp will be difficult to penetrate (Bateman 1972; Díaz-Fleischer & Aluja 2003). Larvae of these species would therefore most likely also survive if they can gain secondary access to fruit pulp, via physical wounds. This emphasized the importance of the conditions/status of collected fruit (physical damage to the peel or splitting of a citrus fruit etc.) when host studies are conducted. This will prevent incorrect reporting of host status of certain plant species for these flies. The initial development of larvae of both *C. rosa* and *C. quilicii* were however slower in Eureka lemon which had the highest acidity and lowest pH among the citrus types tested. It is likely that acidity and pH are limiting factors for larval development of both species. Other studies (Vargas *et al*. 1984; Papadopoulos & Katsoyannos 2002; Papachristos *et al*. 2008) found a similar pattern in that the higher the pH (less acidity) the shorter the development rate of fruit fly larvae. However, for the congeneric species, *Ceratitis capitata*, Papachristos *et al*. (2008) found that acidity, pH and SSC had no influence on survival of the species.

In this study, we found that the development rates of *C. rosa* and *C. quilicii* were more or less similar in all citrus types. However, there were differences in the survival rates of the two species. In citrus types such as Eureka and Late Valencia, *C. rosa* had higher larval and pupal survival rates than *C. quilicii*. Virgilio *et al.*

(2013) reported that *C. rosa* and *C. quilicii* occurred sympatrically in South Africa. The results described in Chapter 3 of this dissertation, confirmed that the distribution of these two species overlap, and that whenever two closely related species occur sympatrically, a difference, such as a temperature adaptation, or morphology adaptation or host utilization, is expected between the two species (Scriven *et al*. 2016; Darwell & Cook 2017). Results on the survival of the two species on four citrus types in this study suggest that the two species may differ in the utilization of fruit depending on certain fruit species. It is important to note that artificial procedures were employed in this study and the results obtained do not confirm the four citrus types as hosts of any of the two fruit fly species.

Conclusion

Ceratitis quilicii is more widely distributed and more abundant in the northern parts of South Africa than *C. rosa*. *Ceratitis rosa* was more abundant in citrus production regions at lower altitude with higher minimum temperatures. All three lures employed in this study attracted both fruit fly species with most of the males caught in Egolure traps.

In areas where *C. quilicii* dominated, the ratio of *C. rosa* and *C. quilicii* males in Egolure was similar to the ratio of *C. rosa* and *C. quilicii* females in Biolure traps. There were however no clear patterns in trap catches in areas where *C. rosa* dominated. Since the females of the two species cannot be distinguished using morphological methods (De Meyer *et al*. 2016), these findings would provide a basis to assume the composition of females of the two species in Biolure traps are similar to Egolure, if Egolure traps are also placed for monitoring of males.

Since this study was conducted in only two provinces it leaves questions on the distribution of the two species in other citrus producing areas. The southernmost distribution of *C. rosa*, along the Indian ocean coast line, as well as its inland distribution along major rivers such as the Limpopo or Orange Rivers are unknown. *Ceratitis quilicii* has the biggest range expansion threat of the two species to fruit production regions, being able to tolerate a wide range of temperatures, and especially its ability to tolerate very cold temperatures.

The results on development of the two species in citrus indicated that there were no differences between the development of the larvae of the two fruit fly species, when artificially inoculated into four citrus types (Eureka, Star Ruby, Nadorcott and Late Valencia. Larval survival in Late Valencia was higher than in the other citrus types for *C. rosa* and *C. quilicii*.

Valencia oranges would the most suitable for further cold disinfestation trials. The results of this study demonstrated that both fruit fly species can be exposed to a cold treatment on the same day, for the 2nd cold disinfestation phase, to determine which life stage is the most cold tolerant.

Future research

It is recommended that to gain a better understanding of the distribution of the two species, a study must be conducted over the rest of South Africa. The extent of the range up to where the two species occur sympatrically is unsure. Especially down the eastern coast line it is not clear where *C. quilicii* occurs as the dominant species and when *C. rosa* becomes more abundant. The culture of *C. quilicii* must be increased to conduct further cold sterilization trials.

Technology transfer

The work has been presented at the follow symposia:

Entomology Society of South Africa (ESSA), 2017, Pretoria – Natal fly versus the Cape fly (Diptera: Tephritidae) in the Limpopo and Mpumalanga provinces, South Africa.

CRI's Research Symposium, 2018, Drakensberg – The biology and ecology of the Natal fly (*Ceratitis rosa*) and the Cape fly (*Ceratitis quilicii*) in citrus in the Limpopo and Mpumalanga provinces of South Africa.

Entomology Society of South Africa (ESSA), 2019, Umhlanga – A comparison of trap catches, larval development in citrus fruit and host utilization between *Ceratitis rosa* and *Ceratitis quilicii* (Diptera: Tephritidae).

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3.3.4 PROGRESS REPORT: Fruit fly rearing

Project 407 (Apr 1999 – Mar 2021) 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, 1245 and 1248. Fruit fly eggs were supplied to Agribiotech Research Consultancies and ExperiCo for post-harvest disinfestation trials on non-citrus fruit crops. In order to ensure supply of good quality fly materials for studies, all fruit fly colonies were refreshed by either crossing wild males reared from fruit with laboratory reared females (for Medfly, Natal fly and marula fly) or establishing founder colonies using males and females reared from wild fruit (for Cape fly and oriental fruit fly). In January 2020, the Medfly colony was refreshed by crossing 462 males reared from peaches collected in Lydenburg with 440 laboratory reared virgin females. Between October and December 2019, the Natal fly colony was refreshed by crossing 291 males reared from various fruit types collected in Nelspruit and Lydenburg with 185 virgin laboratory reared females. Wild flies have continuously been added to the Cape fly colony between April 2019 and February 2020. In total, 2851 wild Cape fly adults originating from Nelspruit, Elands Valley, Bourke's luck, Lydenburg, Pretoria and Stellenbosch were added to the colony. These were mostly flies reared from various fruit types. Between December 2019 and March 2020, the marula fly colony was refreshed by crossing 391 males reared mainly from wild mango and marula with 460 virgin laboratory reared females. Between January and March 2020, 600 oriental fruit fly adults reared mainly from mango collected in Nelspruit and Low's Creek were used to start a founder colony of this species.

Opsomming

Kolonies van vyf vrugtevliegspesies: *Ceratitis capitata* (Mediterreense vrugtevlieg of Medvlieg), *Ceratitis rosa* (Natalse vlieg), *Ceratitis quilicii* (Kaapse vlieg), *Ceratitis cosyra* (maroelavlieg) 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, 1245 en 1248 gebruik. Vrugtevlieg-eiers is aan Agribiotech Research Consultancies en ExperiCo verskaf vir na-oes ontsmettingsproewe op nie-sitrus vruggewasse. Ten einde die verskaffing van goeie kwaliteit vliegmateriaal vir studies te verseker, is alle vrugtevliegkolonies vernuwe deur óf die kruising van wilde mannetjies, geteel vanaf vrugte, met laboratorium-geteelde wyfies (vir Medvlieg, Natalse vlieg en maroelavlieg) óf deur stigterkolonies te vestig deur gebruik te maak van mannetjies en wyfies wat vanaf wilde vrugte geteel is (vir Kaapse vlieg en oosterse vrugtevlieg). In Januarie 2020, is die Medvlieg kolonie vernuwe deur die kruising van 462 mannetjies, geteel vanaf perskes wat in Lydenburg versamel is, met 440 laboratorium-geteelde maagdelike wyfies. Tussen Oktober en Desember 2019, is die Natalse vliegkolonie vernuwe deur die kruising van 291 mannetjies, geteel vanaf verskeie vrugtipes wat in Nelspruit en Lydenburg versamel is, met 185 maagdelike laboratorium-geteelde wyfies. Wilde vlieë is voortdurend, tussen April 2019 en Februarie 2020, tot die Kaapse vlieg kolonie gevoeg. In totaal is 2851 wilde Kaapse vlieg volwassenes, met oorsprong in Nelspruit, Elands Valley, Bourke's luck, Lydenburg, Pretoria en Stellenbosch, tot die kolonie gevoeg. Hierdie was meestal vlieë wat vanaf verskeie vrugtipes geteel is. Tussen Desember 2019 en Maart 2020, is die maroelavlieg kolonie vernuwe deur die kruising van 391 mannetjies, geteel hoofsaaklik vanaf wilde mango en maroela, met 460 laboratorium-geteelde maagdelike wyfies. Tussen Januarie en Maart 2020, is 600 oosterse vrugtevlieg volwassenes, geteel hoofsaaklik vanaf mango wat in Nelspruit en Low's Creek versamel is, gebruik om 'n stigterkolonie vir hierdie spesie te begin.

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

Project 1171 (Apr 2017 - Mar 2021) by Aruna Manrakhan, John-Henry Daneel, Rooikie Beck, Glorious Shongwe, Sean Moore, Vaughan Hattingh (CRI)

Summary

The cold shipping conditions as determined by the False codling moth risk Management Systems for export of citrus produced in South Africa (Citrus FMS) form part of measures for mitigation of risk of fruit fly pests in citrus other than lemons and limes from South Africa under a Fruit Fly Systems Approach. Depending on prescribed shipping conditions in the Citrus FMS, the temperature set points can vary between -1°C and 4°C with estimated resulting pulp temperatures varying between 1°C and 6°C. There are internationally approved cold treatment protocols for fruit flies at temperatures which are at or below 3°C. However, the efficacy of temperatures above 3°C on fruit fly mortality has not yet been quantified. This study therefore aims at

determining the efficacy of a treatment at 3.5°C on fruit fly larval mortality. The relative cold tolerances of third instars of three fruit fly pests of citrus: *Ceratitis capitata*, *Ceratitis rosa* and *Bactrocera dorsalis* were determined in carrot-based medium and in Navel oranges separately at 3.5°C for 18 days. *Ceratitis capitata* was more cold tolerant than *C. rosa* and *B. dorsalis* in trials conducted both in artificial medium and in fruit. Larval mortality in fruit was lower than that in artificial medium after the first six days of cold treatment for all fruit fly species. Based on recorded larval mortality, the estimated exposure periods (95% CI) at 3.5°C required to achieve 95% mortality of third instars of *C. capitata, C. rosa and B. dorsalis* were 8.72 (8.53-8.92), 4.48 (4.35-4.62) and 5.22 (5.05-5.41) days in artificial medium. The estimated exposure periods (95% CI) at 3.5°C required to achieve 95% mortality of third instars of *C. capitata, C. rosa and B. dorsalis* were 17.31 (16.88-17.76), 7.69 (7.51-7.88) and 8.27 (8.08-8.48) days in fruit.

Opsomming

Die koue-verskepingstoestande soos bepaal deur die Valskodlingmot risiko bestuur stelsel (Sitrus *FMS*) vir uitvoer van sitrus in Suid-Afrika geproduseer, vorm deel van maatreëls ten einde die risiko van vrugtevliegplae in sitrus, behalwe vir suurlemoene en lemmetjies vanaf Suid-Afrika, onder 'n Vrugtevlieg Steslselbenadering te verminder. Afhangende van voorgeskrewe verskepingstoestande in die Sitrus *FMS*, kan die temperatuurstellings tussen -1°C en 4°C varieer, met beraamde pulptemperature as gevolg, wat tussen 1°C en 6°C varieer. Daar is internasionaal-goedgekeurde koue-behandelingsprotokolle vir vrugtevlieë teen temperature wat by of onder 3°C is. Die effektiwiteit van temperature bó 3°C op vrugtevlieg mortaliteit is egter nog nie gekwantifiseer nie. Hierdie studie het dus ten doel om die effektiwiteit van 'n behandeling teen 3.5°C op vrugtevlieglarwe mortaliteit te bepaal. Die relatiewe koue-toleransies van derde instar larwes van drie vrugtevliegplae van sitrus: *Ceratitis capitata*, *Ceratitis rosa* en *Bactrocera dorsalis* is in wortel-gebaseerde medium en in Navel lemoene apart teen 3.5°C vir 18 dae bepaal. *Ceratitis capitata* was meer koue-tolerant as *C. rosa* en *B. dorsalis* in proewe wat in beide kunsmatige medium en in vrugte uitgevoer is. Larwe mortaliteit was vir alle vrugtevliegspesies laer in vrugte as in kunsmatige medium ná die eerste ses dae van koue-behandeling. Gebaseer op aangetekende larwe mortaliteit in proewe, was die beraamde blootstellingsperiodes (95% CI) teen 3.5°C nodig ten einde 95% mortaliteit van derde instar larwes van *C. capitata, C. rosa* en *B. dorsalis* te verkry, 8.72 (8.53-8.92), 4.48 (4.35- 4.62) en 5.22 (5.05- 5.41) dae in kunsmatige medium. Die beraamde blootstellingsperiodes (95% CI) teen 3.5°C nodig om 95% mortaliteit van derde instar larwes van *C. capitata, C. rosa* en *B. dorsalis* te verkry, was 17.31 (16.88-17.76), 7.69 (7.51- 7.88) en 8.27 (8.08- 8.48) dae in vrugte.

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

Project 1204 (2018/9 – 2020/1) by T G Grout, P R Stephen, K C Stoltz and V Hattingh (CRI)

Summary

Interruptions of cold treatments can occur for various reasons but apart from the recommendation by APHIS for the cold treatment for false codling moth there are few specific mitigation protocols in the event of one happening. This research is using *Ceratitis capitata* as a test subject to quantify the effect of different interruptions on the mortality of the second instar in Valencia oranges packed in export cartons. A cold treatment of 6 days at an internal pulp temperature of 0.9°C is being used to guarantee some survival of larvae. A single interruption after either 2 days of cold or 4 days of cold where the pulp temperature increased to 2°C above the upper threshold of 1.1°C did not cause consistent differences in mortality from the treatment with no interruption in four replicates conducted. Trials with double interruptions of either 2°C above the threshold or 4°C above the threshold were also similar to the treatment with no interruptions. The mean corrected mortalities of three replicates were 94.25% for two interruptions of +2°C, 95.31% for two interruptions of +4°C and 95.49% for no interruptions. This research will continue with different combinations of interruptions.

Opsomming

Onderbrekings van koue-behandelings kan om verskeie redes gebeur, maar behalwe vir die aanbeveling deur APHIS vir die koue-behandeling vir valskodlingmot, is daar min spesifieke versagtingsprotokolle in geval van so 'n gebeurtenis. Hierdie navorsing gebruik *Ceratitis capitata* as 'n toets voorwerp ten einde die effek van verskillende onderbrekings op die mortaliteit van die tweede instar in Valencia lemoene, wat in uitvoerkartonne verpak is, te kwantifiseer. 'n Koue-behandeling vir 6 dae teen 'n interne pulptemperatuur van 0.9°C word gebruik om 'n mate van oorlewing van larwes te waarborg. 'n Enkele onderbreking na óf 2 dae van koue óf 4 dae van koue, waar die pulptemperatuur na 2°C bó die boonste drempel van 1.1°C verhoog het, het nie beduidende verskille in mortaliteit veroorsaak nie, in vergelyking met die behandeling met geen onderbreking, in vier herhalings. Proewe met dubbele onderbrekings van óf 2°C bó die drempel óf 4°C bó die drempel, was ook soortgelyk aan die behandeling met geen onderbrekings nie. Die gemiddelde reggestelde mortaliteite van drie herhalings was 94.25% vir twee onderbrekings van +2°C, 95.31% vir twee onderbrekings van +4°C, en 95.49% vir geen onderbrekings nie. Hierdie navorsing sal met verskillende kombinasies van onderbrekings voortgaan.

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

Project 1211 (Apr 2018 - Mar 2021) by Aruna Manrakhan, John-Henry Daneel, Sean Moore, Wayne Kirkman (CRI), Mellissa Peyper, Tamryn Marsberg, Sonica Albertyn (RU/CRI)

Summary

The aims of this project were to determine the efficacy of new attract and kill products and optimise their application. Field trials were conducted over three years in citrus orchards to determine the efficacy of registered attract and kill devices on fruit fly suppression. In 2019, two bait stations: M3 and Magnet Med were each tested at two densities in mandarin orchards in Mpumalanga Province. Both M3 and Magnet Med at the different densities tested were equally effective in suppressing fruit fly populations. However, in orchards under high fruit fly prevalence level, none of the treatments were effective enough to maintain fruit fly numbers below trap threshold levels. Fruit fly infestation was recorded on fruit on trees at harvest in blocks treated with M3 at 300 units per ha in one of the study sites. This demonstrates that there is a risk of fruit infestation when M3 bait stations are used at lower than the recommended density in mandarin orchards (400 units per ha). In order to correct high fruit fly numbers in orchards treated with bait stations, application of registered bait sprays is recommended. Phytotoxicity of bait sprays applied on mandarin fruit before colour break was determined. There was a lower incidence of phytotoxic marks on fruit with a mixture of HymLure and Exirel compared to other registered bait mixtures tested (HymLure and malathion and GF-120). The relative attractiveness of Cera Trap, a new product for mass trapping, to three fruit fly pests was quantified in laboratory and semi field tests. In olfactometer tests, Cera trap was more attractive than the standard bait: HymLure at 0.8% (dilution with water) for all species tested. In semi field tests, all baits (Cera trap and three standard baits: HymLure, Biolure and Questlure) were more or less equally attractive to all species.

Opsomming

Die doelwitte van hierdie projek was om die effektiwiteit van nuwe lok en uitwis produkte te bepaal, en hul toediening te optimaliseer. Veldproewe is oor drie jaar in sitrusboorde uitgevoer ten einde die effektiwiteit van geregistreerde lok en uitwis toestelle op vrugtevlieg-onderdrukking te bepaal. In 2019, is twee lokstasies: M3 en Magnet Med elk teen twee digthede in mandarynboorde in Mpumalanga Provinsie getoets. Beide M3 en Magnet Med, teen die verskillende digthede getoets, was ewe effektief in die onderdrukking van vrugtevliegpopulasies. In boorde onder hoë vrugtevlieg voorkomsvlakke, was geen van die behandelings egter effektief genoeg om vrugtevlieggetalle onder lokval drempelvlakke te hou nie. Vrugtevliegbesmetting is op vrugte aan bome by oes aangeteken in blokke wat met M3 teen 300 eenhede per ha in een van die studiepersele behandel is. Dit demonstreer dat daar 'n risiko van vrugbesmetting is wanneer M3 lokstasies gebruik word teen laer as die aanbevole digtheid in mandarynboorde (400 eenhede per ha). Ten einde hoë vrugtevlieggetalle in boorde wat met lokstasies behandel is, reg te stel, word die toedien van geregistreerde lok-aasspuite aanbeveel. Fitotoksisiteit van lok-aasspuite, toegedien op mandarynvrugte vóór kleurbreek, is bepaal. Daar was 'n laer voorkoms van fitotoksiese merke op vrugte met 'n mengsel van HymLure en Exirel, in vergelyking met ander geregistreerde lok-aasmengsels wat getoets is (HymLure en malathion en GF-120). Die relatiewe aantrekkingskrag van Cera Trap, 'n nuwe produk vir massa-vangstelsel, vir drie vrugtevliegplae, is in laboratorium en semi-veld toetse gekwantifiseer. Cera trap was meer aantreklik as die standaard lok-aasmiddel in olfaktometertoetse: HymLure teen 0.8% (verdunning met water) vir alle getoetste spesies. In

semi-veld toetse, was alle lok-aasmiddels (Cera lokval en drie standaard lok-aasmiddels: HymLure, Biolure en Questlure) min of meer ewe aantreklik vir alle spesies.

3.3.8 PROGRESS REPORT: Development of new cold disinfestation treatments for fruit fly pests on citrus

Project 1245 (2019/20 – 2020/21) by A Manrakhan, J-H Daneel, R Beck and G Shongwe (CRI)

Summary

The original aim of the project was to determine the equivalence of an existing cold disinfestation treatment at 1°C for 14 days for an important pest of citrus in South Africa: *Ceratitis capitata* (Wiedemann), Mediterranean fruit fly (Medfly), on a newly described fruit fly species - *Ceratitis quilicii* De Meyer, Mwatawala & Virgilio which is also present in the country. This would have been a proactive study in case *C. quilicii* was considered a pest of citrus by our export markets despite no existing evidence. Trials however could never be initiated due to low numbers of flies in the *C. quilicii* colony. In March 2020, the focus species in the project was changed to *Ceratitis cosyra* (Walker), marula fly. The marula fly is becoming problematic to citrus in southern Africa. There are previous records of the species on citrus from South Africa in online databases and in fruit surveys in East and West Africa. The following new specific objectives were therefore set: (1) Comparison of the in vitro cold tolerance of *C. capitata* and *C. cosyra*.; (2) Determination of treatment conditions for complete mortality of the third larval stage of *C. cosyra* at 1°C for 14 days, and (3) Confirmation of treatment schedule at 1°C for 14 days for disinfestation of *C. cosyra*. The cold tolerance of the third larval stages of *Ceratitis capitata* and *Ceratitis cosyra* was compared in a carrot-based medium at 3.47°C ± 0.00°C at ten exposure periods over 18 consecutive days. There were generally lower mortality rates for *C. capitata* than for *C. cosyra* during the cold treatment. The period to achieve 99% mortality at 3.5°C was estimated at 13.35 days for *C. capitata* compared to 9.25 days for *C. cosyra*. These results indicate that cold treatment schedules for disinfestation of *C. capitata* would also be efficacious against *C. cosyra*.

Opsomming

Die oorspronklike doel van die projek was om ‘n gelykwaardige behandeling van ’n bestaande koue-ontsmettingsbehandeling teen 1°C vir 14 dae vir ’n belangrike plaag van sitrus in Suid-Afrika: *Ceratitis capitata* (Wiedemann), Mediterreense vrugtevlieg (Medvlieg), teen ’n nuut-beskryfde vrugtevliegspesie, *Ceratitis quilicii* De Meyer, Mwatawala & Virgilio, wat ook in die land teenwoordig is, te bepaal. Dit sou ’n pro-aktiewe studie wees vir ingeval *C. quilicii* deur ons uitvoermarkte as ’n plaag van sitrus beskou word, ten spyte van geen bestaande bewyse nie. Proewe kon egter nooit begin word nie weens lae getalle vlieë in die *C. quilicii* kolonie. In Maart 2020, is die fokus spesie in die projek na *Ceratitis cosyra* (Walker), maroelavlieg, verander. Die maroelavlieg is besig om problematies vir sitrus in suider-Afrika te raak. Daar is vorige rekords van die spesie op sitrus vanaf Suid-Afrika in aanlyn-databasisse en in vrug-opnames in Oos- en Wes-Afrika. Die volgende nuwe spesifieke doelwitte is dus gestel: (1) Vergelyking van die *in vitro* koue-toleransie van *C. capitata* en *C. cosyra;* (2) Vasstel van behandelingstoestande vir volledige mortaliteit van die derde larwe stadium van *C. cosyra* teen 1°C vir 14 dae, en (3) Bevestiging van behandelingskedule teen 1°C vir 14 dae vir ontsmetting van *C. cosyra*. Die koue-toleransie van die derde larwe stadia van *Ceratitis capitata* en *Ceratitis cosyra* is in ‘n wortel-gebaseerde medium teen 3.47°C ± 0.00°C by tien blootstellingsperiodes oor 18 opeenvolgende dae vergelyk. Daar was oor die algemeen laer mortaliteittempo’s vir *C. capitata* as vir *C. cosyra* gedurende die koue-behandeling. Die tydperk om 99% mortaliteit teen 3.5°C te bereik, is op 13.35 dae vir *C. capitata* geskat, in vergelyking met 9.25 dae vir *C. cosyra*. Hierdie resultate dui daarop dat koue-behandelingskedules vir ontsmetting van *C. capitata* ook teen *C. cosyra* effektief sal wees.

3.3.9 PROGRESS REPORT: Redefining dispersal potential for adequate fruit fly pest management (Diptera, Tephritidae)

Project 1254 (2019/20 – 2021/22) by A. Manrakhan, L. Serfontein (CRI), S. K. Tsatsu, M. Karsten, P. Addison (SU), M. De Meyer and M. Virgilio (Royal Museum for Central Africa).

Summary

The dispersal potential of two cryptic fruit fly pest species in South Africa: *Ceratitis rosa* and *C. quilicii*, will be quantified by determination of their distribution and host ranges in the country and by an assessment of their demography. The distribution of the two species will be determined by trapping using EGO lure. Traps are being placed in three commercial citrus orchards in each of seven provinces (Eastern Cape, Western Cape, Northern Cape, North West, Limpopo, KwaZulu-Natal and Mpumalanga). In selected provinces, traps will also be set in non-commercial fruit production areas such as home gardens, botanical gardens and private reserves. Trapping will be carried out over a month during two time periods: autumn (April-June) and spring (September-November) in 2020 and 2021. Ripe fruit from trees and on ground, whenever available, will be collected from trapping sites and incubated at the Citrus Research Centre (CRC), CRI Nelspruit, for host range determination. Demographic studies of the two species will be carried out on the following fruit species: *Citrus sinensis*, *C. reticulata*, *Mangifera indica, Psidium guajava* and *Prunus persica*. Laboratory reared flies of the two species will be used. The colony of *C. rosa* is well established at CRC. The colony for *C. quilicii* is currently being augmented. On each fruit species, data on development and survival of immature stages as well as survival and reproduction of adult stages of the two fruit fly species will be collected. Demographic parameters of *C. rosa* and *C. quilicii* will be calculated for each fruit species. Demographic results and literature will be used to develop a model using the Insect Life Cycle Modelling (ILCYM) software to determine the dispersal potential of each species in South Africa. Risk level mapping will be constructed in QGIS using the results from ILCYM.

Opsomming

Die verspreidingspotensiaal van twee kriptiese vrugtevliegplaagspesies in Suid-Afrika: *Ceratitis rosa* en *C. quilicii*, sal gekwantifiseer word deur die vasstel van hul verspreiding en gasheerreekse in die land, en deur 'n bepaling van hul demografie. Die verspreiding van die twee spesies sal met behulp van lokvalle bepaal word, deur van EGO lokmiddel gebruik te maak. Lokvalle word in drie kommersiële sitrusboorde in elk van sewe provinsies geplaas (Oos-Kaap, Wes-Kaap, Noord-Kaap, Noordwes, Limpopo, KwaZulu-Natal en Mpumalanga). In geselekteerde provinsies, sal lokvalle ook in nie-kommersiële vrugproduksie-areas opgestel word, soos huistuine, botaniese tuine en private reservate. Lokvalstelling sal oor 'n maand, gedurende twee tydperiodes, uitgevoer word: herfs (April-Junie) en lente (September-November) in 2020 en 2021. Ryp vrugte vanaf bome en op die grond sal, wanneer ook al beskikbaar is, vanaf lokvalpersele versamel word, en by die *Citrus Research Centre* (*CRC*), CRI Nelspruit, vir die bepaling van gasheerreeks geïnkubeer word. Demografiese studies van die twee spesies sal op die volgende vrugspesies uitgevoer word: *Citrus sinensis*, *C. reticulata*, *Mangifera indica, Psidium guajava* en *Prunus persica*. Laboratorium-geteelde vlieë van die twee spesies sal gebruik word. Die kolonie van *C. rosa* is goed gevestig by *CRC*. Die kolonie van *C. quilicii* word tans vergroot. Data rakende die ontwikkeling en oorlewing van onvolwasse stadia, asook oorlewing en reproduksie van volwasse stadia van die twee vrugtevliegspesies sal, vir elke vrugspesie, versamel word. Demografiese parameters van *C. rosa* en *C. quilicii* sal vir elke vrugspesie bereken word. Demografiese resultate en literatuur sal gebruik word om 'n model te ontwikkel deur gebruik te maak van die *Insect Life Cycle Modelling* (*ILCYM*) sagteware, ten einde die verspreidingspotensiaal van elke spesie in Suid-Afrika te bepaal. Risikovlak-kartering sal in QGIS gebou word deur gebruik te maak van die resultate vanaf ILCYM.

3.3.10 PROGRESS REPORT: In-silico boosted, pest prevention and off-season focussed IPM against new and emerging fruit flies

Project 1261 (Apr 2019 - Mar 2021) by A. Manrakhan, L. Serfontein, R. Beck (CRI), D. Nestel (Agricultural Research Organisation, Israel) and D. Kriticos (CSIRO, Australia)

Summary

The aims of the project are to support prevention, enhance early detection and optimize IPM approaches for new and emerging fruit fly pests. This is a collaborative project under the European Union funded Horizon 2020 programme which involves multiple institutions across the world including Citrus Research International (CRI). *Bactrocera dorsalis* is one of the targeted fruit fly pests. At CRI, a *B. dorsalis* detection system which is optimised both spatially (detection area) and temporally (detection time) will be developed. Climatic models will be used to optimise detection trapping. In the optimised *B. dorsalis* detection system, electronic traps (e-traps) will be used. Three sites for evaluation of e-traps and the optimised detection system were selected in

Mpumalanga Province. The sites selected were at different altitudes and have different levels of prevalence of *B. dorsalis* (based on historical trapping records). The Nelspruit site was considered as high prevalence, Schoemanskloof as medium prevalence and Ermelo as low prevalence. Orthophotos for each of these sites were obtained from the Department of Agriculture, Rural Development and Land Reform. The characteristics of each site are now being mapped based on the orthophotos. E-traps will first be compared to conventional traps in one of the selected sites. The development of an optimised detection system will commence thereafter and will be based on new trapping data generated in each of these sites.

Opsomming

Die doelwitte van die projek is om voorkoming te ondersteun, vroeë opsporing te verbeter, en *IPM* benaderings vir nuwe en ontluikende vrugtevliegplae te optimaliseer. Dit is ŉ gesamentlike projek onder die Europese Unie se befondsde Horizon 2020 program, wat veelvuldige instellings oor die wêreld betrek, insluitende Citrus Research International (CRI). *Bactrocera dorsalis* is een van die geteikende vrugtevliegplae. ŉ *B. dorsalis* opsporingsisteem, wat beide ruimtelik (opsporingsarea) en tydelik (opsporingstyd) geoptimaliseer word, sal by CRI ontwikkel word. Klimaatsmodelle sal gebruik word om opsporing in lokvalle te optimaliseer. In die geoptimaliseerde *B. dorsalis* opsporingsisteem, sal elektroniese lokvalle (e-lokvalle) gebruik word. Drie persele vir evaluasie van e-lokvalle en die geoptimaliseerde opsporingsisteem is in die Mpumalanga Provinsie geselekteer. Die geselekteerde persele was by verskillende hoogtes bo seevlak en het verskillende vlakke van voorkoms van *B. dorsalis* (gebaseer op historiese lokvalrekords) gehad. Die Nelspruit perseel is as hoë voorkoms beskou. Schoemanskloof as medium voorkoms en Ermelo as lae voorkoms. Ortofoto's vir elk van hierdie persele is vanaf die Departement van Landbou, Grondhervorming en Landelike Ontwikkeling verkry. Die kenmerke van elke perseel word nou, gebaseer op die ortofoto's, gekarteer. E-lokvalle sal eers met konvensionele lokvalle in een van die geselekteerde persele vergelyk word. Die ontwikkeling van ŉ geoptimaliseerde opsporingsisteem sal daarna begin, en sal op nuwe lokvaldata gebaseer word wat in elk van hierdie persele gegenereer word.

3.3.11 PROGRESS REPORT: Field performance of a new AVIMA bait station for fruit fly control in citrus.

Project 1285 (Feb 2020 - Oct 2020) by Aruna Manrakhan, John-Henry Daneel, Rooikie Beck (CRI)

Summary

AVIMA developed a new fruit fly bait station which was found to be highly effective in laboratory assays against two important fruit fly pests of citrus: *Ceratitis capitata* (Mediterranean fruit fly or Medfly) and *Bactrocera dorsalis* (Oriental fruit fly). The field performance of this promising bait station is being evaluated in a mandarin orchard. Trials were initiated in February 2020. The AVIMA bait station is being evaluated at two densities: 100 units per ha and 200 units per ha and compared to the standard M3 bait station applied at 400 units per ha. Efficacy of treatments is being determined by trapping. A fruit damage assessment will also be carried out at harvest to compare fruit fly infestation between treatments. Fruit fly catches in blocks treated with AVIMA bait stations were comparable to those in blocks treated with the M3 bait station for 11 weeks after start of treatment.

Opsomming

AVIMA het ŉ nuwe vrugtevlieg lokstasie ontwikkel, wat hoogs effektief in laboratoriumproewe teen twee belangrike vrugtevliegplae van sitrus was: *Ceratitis capitata* (Mediterreense vrugtevlieg of Medvlieg) en *Bactrocera dorsalis* (Oosterse vrugtevlieg). Die prestasie van hierdie belowende lokstasie in die veld, word in ŉ mandarynboord geëvalueer. Proewe is in Februarie 2020 begin. Die AVIMA lokstasie word teen twee digthede geëvalueer: 100 eenhede per ha en 200 eenhede per ha, en met die standaard M3 lokstasie, toegedien teen 400 eenhede per ha, vergelyk. Effektiwiteit van behandelings word deur lokvalstelling bepaal. ŉ Vrugbeskadiging bepaling sal ook by oes uitgevoer word ten einde vrugtevliegbesmetting tussen behandelings te vergelyk. Vrugtevliegvangste in blokke wat met AVIMA lokstasies behandel is, was vergelykbaar met daardie in blokke wat met die M3 lokstasie behandel is, vir 11 weke ná die begin van die behandeling.

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

Project 1177 (2017/8 – 2020/21) by M Gilbert and C Love (CRI)

Summary

The timing of M3 bait station application is being investigated for a second season at the same soft citrus sites. The first season's results indicated that there was no significant justification for the early application of M3s in October in reducing fruit fly populations or damage later in the season. In the second season of this study, one site selected was not under the fruit fly sterile insect release (SIT) programme. The other site was under the SIT programme. *Ceratitis capitata* (Medfly) males caught in this SIT site were identified as sterile or wild. Sterile Medfly males had been treated with an orange fluorescent powder that allowed them to be differentiated under UV black light. Any possibly infested fruit observed while doing fruit inspections in 2019 prior to harvest were brought back to the laboratory and incubated. No adult fruit flies were reared from this fruit. Adult Medfly were reared from fallen fruit collections, but these numbers at both sites were very low. Fruit fly numbers were monitored with Capilure baited Sensus traps and Biolure baited bucket traps throughout the year. Almost all flies caught were Medfly. The early application blocks in both sites were treated with M3s in early to mid-November 2019. The late M3 treatment was applied to the appropriate blocks in late January in the SIT site and early February in the non-SIT site. Fruit inspections on the tree began again in January 2020. At the non-SIT site, both the Biolure and Capilure traps showed two Medfly peaks in January and early February and these were lower in the early application compared to the control. After the application of the late treatment and refreshing of the early treatment, the FTD (flies/trap/day) dropped, for both trap types in all three replicates, to almost zero by the end of March. At SIT site, the Capilure and Biolure results showed no difference in FTD between the early and control blocks after the early treatment was applied in November. The FTD had declined to less than 1 before the late treatment was applied, resulting in no difference between the three treatments. Fallen fruit collected from both sites are currently incubating. Additional fruit collections will be done prior to harvest.

Opsomming

Die tydsberekening van M3 lokstasie uithang was, vir 'n tweede seisoen by dieselfde sagtesitrus proefpersele, nagegaan. Die eerste jaar se resultate het aangedui dat daar geen regverdering is om M3s uit te sit in Oktober om vrugtevlieg getalle of vrugskade later in die jaar te verminder. Die eerste proefperseel is nie deel van die vrugtevlieg steriele insek vrylating program nie. Die tweede perseel is wel binne die program en hier, in vangstes, is onderskeid tussen steriele en wilde *Ceratitis capitata* (Medvlieg) gemaak. Steriele Medvlieg mannetjies is met oranje fluoresserende poeier behandel, wat onder UV swart lig onderskei kon word. Enige moontlike besmette vrugte wat, gedurende voor-oes vrug inspeksies in 2019, opgemerk is, is terug na die laboratorium gebring en ge'inkubeer. Geen volwasse vlieë is van dié vrugte uitgebroei nie. Vrugtevlieg getalle is met beide Capilure Sensus, en Biolure geel emmer, valletjies regdeur die jaar gemonitor; amper net Medvlieg is gevang. Die vroeër herhalings is in vroeg tot Middel-November 2019 met M3 lokstasies behandel. Die laat M3 behandeling is in laat Januarie by proefperseel een, en vroeg in Februarie by proefperseel twee, toegedien. Op-boom vruginspeksies is in Januarie 2020 begin. By proefperseel een, het beide Biolure en Capilure valletjies in Januarie en in vroeë Februarie twee pieke van Medvlieg gewys. Dié pieke is heelwat laer in die blokke wat vroeg behandel is in vergelyking met die kontrole. Na toediening van die laat-, en herhaling van die vroeë, behandeling, die VPD (vlieë/valletjie/dag) het, vir al die herhalings, en vir albei valletjie tipes, tot omtrent nil teen einde Maart gedaal. By die tweede proefperseel (ingesluit by SIT), het Capilure en Biolure resultate geen verskil gewys nie in VPD tussen die vroeë en kontrole blokke na toediening van die vroeë behandeling in November. The VPD het afgeneem na minder as 1 voor die laat M3s toegedien is, met geen verskil tussen die drie behandelings. Vrugte wat geval het is versamel en word tans ge'inkubeer. Verderer versameling van vrugte wat geval het sal voor oes uitgevoer word.

3.3.13 PROGRESS REPORT: Understanding fruit fly trap efficiency: the role of physical and biotic variables

Project 1229 (Apr 2019 - Mar 2021) by Prof. Christopher Weldon (UP)

Summary

A systems approach for quarantine pests is a phytosanitary risk mitigation measure that can be used to achieve quarantine security. At least two independent risk mitigating measures should be implemented in a fruit fly systems approach. These measures can include areas of low fruit fly prevalence, fruit fly control and monitoring measures in the field, inspection and cold storage. In the citrus industry, risk of fruit fly infestation in the field is measured by trapping. This project establishes the role of temperature, relative humidity and fly physiology on the efficiency of fruit fly lures. Milestones indicated in the project proposal have been delayed due to new climate rooms at the University of Pretoria not being fit for purpose and late receipt of custom-made field cages required to pursue semi-field trials. We were eventually able to begin experiments to test loss of lure weight in relation to temperature and relative humidity in March 2020, but these needed to be suspended when the University and then South African national government implemented shutdowns to prevent the spread of COVID-19. No progress has since been possible due to research at universities not being recognised as an essential service that contributes to the agriculture sector. We request an extension to the project, with no additional financial implications for CRI, to produce results promised in our proposal once our research is permitted to continue.

Opsomming

ʼn Stelselbenadering vir kwarantynplae is ʼn fitosanitêre risiko verminderingsmaatreël wat gebruik kan word om kwarantyn sekuriteit te bereik. Ten minste twee onafhanklike risiko verminderingsmaatreëls moet in ʼn vrugtevlieg stelselbenadering geïmplimenteer word. Hierdie maatreëls kan areas van lae vrugtevlieg voorkoms insluit, vrugtevlieg beheer en moniteringsmaatreëls in die veld, inspeksie en koue-opberging. In die sitrus-industrie word die risiko van vrugtevlieg besmetting deur middel van lokvalle in die veld gemeet. Hierdie projek bepaal die rol van temperatuur, relatiewe humiditeit en vlieg fisiologie op die effektiwiteit van vrugtevlieg lokase. Mylpale wat in die projek voorstel aangedui is, is vertraag as gevolg van nuwe klimaat kamers by die Universiteit van Pretoria wat nie toegerus was vir die doel nie, en laat ontvangs van hand-gemaakte veld hokke wat benodig was vir semi-veld proewe. Ons kon uiteindelik in Maart 2020 met eksperimente begin om die verlies van lokaas gewig in verhouding met temperatuur en relatiewe humiditeit te toets, maar dit moes opgeskort word nadat die Universiteit, en daarna die Suid-Afrikaanse nasionale regering, sluitings geïmplementeer het om die verspreiding van COVID-19 te verhoed. Geen vordering was sederdien moontlik nie aangesien dat navorsing by universiteite nie as ‘n essensiële diens wat tot die landbou sektor bydra, erken word nie. Ons versoek ‘n verlenging van die projek, met geen addisionele finansiële implikasies vir CRI nie, om die resultate toe produseer wat beloof is in ons voorstel sodra ons toestemming het om met ons navorsing voort te gaan.

3.4 PROGRAMME: OTHER PESTS

Programme coordinator: Tim G Grout (CRI)

3.4.1 Programme summary

This programme covers research on pests that have not reached the status of the internal fruit pests but may cause increasing losses due to management costs, or be considered phytosanitary pests for specific markets. Mealybugs, especially species other than the citrus mealybug, are phytosanitary pests for some markets and their late season control has become more difficult after the EU MRL for buprofezin was changed. Entomopathogenic fungi may provide a late season solution for mealybug infestations if formulations can be developed that protect against UV irradiation (3.4.2, 3.4.5). Sequential treatments with entomopathogenic nematodes are also being evaluated (3.4.11). Some chemicals that were recently registered for mealybug on citrus are also being investigated (3.4.9). Mealybug pest status appears to increase in citrus trees grown under net. The reason for this is under investigation (3.4.6) but it should be considered by growers with orchards under net. Shade netting can also influence other pest populations and this is receiving attention in the Western Cape (3.4.10) and Mpumalanga (3.4.12). Apart from bud mite on lemons, mites are usually not considered to be serious pests. However, research had to be conducted on the best means of controlling the flat mite *Brevipalpus californicus* because it was found to be vectoring orchid fleck virus that resulted in a form of leprosis (3.4.3). Invasive mite species may also be encountered on budwood imported into the country and treatments to control these on arrival without damaging the budwood need to be developed. Research with this objective was hindered by the inability to find an orchard with widespread bud mite infestation (3.4.7). Proactive research is being conducted to screen systemic insecticides against the vector of HLB, *Diaphorina citri,* in Kenya, but numbers of infested citrus plants are still too low to start the trial (3.4.4). Surveys are also being conducted in different South African production regions for oleander scale, *Aspidiotus nerii,* that is considered a phytosanitary pest by some Asian countries (3.4.8).

Programopsomming

Hierdie program dek navorsing op plae wat nog nie die status van die interne vrugteplae bereik het nie, maar wat toenemende verliese weens bestuurskostes kan veroorsaak, of wat as fitosanitêre plae vir spesifieke markte beskou word. Witluise, veral spesies anders as die sitrus witluis, is fitosanitêre plae vir sommige markte, en hul laat seisoen beheer het moeiliker geword nadat die EU MRL vir buprofezin verander het. Entomopatogeniese swamme kan ʼn laat seisoen oplossing vir witluisbesmettings verskaf indien formulasies ontwikkel kan word wat teen UV bestraling beskerming gee (3.4.2, 3.4.5). Opeenvolgende behandelings met entomopatogeniese aalwurms word ook geëvalueer (3.4.11). Sommige chemikalieë wat onlangs vir witluis op sitrus geregistreer is, word ook ondersoek (3.4.9). Dit lyk of witluis plaagstatus in sitrusbome wat onder net groei, toeneem. Die rede hiervoor word ondersoek (3.4.6), maar dit moet deur produsente met boorde onder net, in ag geneem word. Skadunet kan ook ander plaagpopulasies beïnvloed, en dit ontvang in die Wes-Kaap (3.4.10) en Mpumalanga (3.4.12) aandag. Behalwe vir knopmyt op suurlemoene, word myte normaalweg nie as ernstige plae beskou nie. Navorsing moes egter uitgevoer word om die beste wyse van beheer vir die platmyt, *Brevipalpus californicus*, te vind, aangesien daar gevind is dat dit ʼn vektor vir die orgideeflekvirus is, wat ʼn vorm van leprose veroorsaak het (3.4.3). Indringer mytspesies kan ook op enthout, wat in die land ingevoer word, voorkom, en behandelings om hulle met aankoms te beheer sonder om die enthout te beskadig, moet ontwikkel word. Navorsing met hierdie doelwit is belemmer omdat daar nie ʼn boord gevind kon word met wydverspreide knopmyt-besmetting nie (3.4.7). Pro-aktiewe navorsing word gedoen om sistemiese insekdoders teen die vektor van HLB, *Diaphorina citri,* in Kenia, te toets, maar die aantal besmette sitrusplante is nog te laag om die proef te begin (3.4.4). Opnames word ook in verskillende Suid-Afrikaanse produksiestreke vir oleander dopluis, *Aspidiotus nerii,* gedoen, wat as ʼn fitosanitêre plaag deur sommige Asiatiese lande beskou word (3.4.8).

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

Project 1143 (Apr 2016 – Mar 2019) by Mavis Acheampong, Martin Hill, Candice Coombes (RU) and Sean Moore (CRI)

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/m^2) 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, but results were not promising.

This study was undertaken as a doctoral degree.

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 teen12-98% RH) en UV-sensitiwiteit (ontkieming na UV-bestraling vir 15-120 min teen 0.3 W/m^2) 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-beskermende 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 x 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 & 2016), but also laboratory trials against citrus mealybug (*Planococcus citri*) and citrus thrips (*Scirtothrips aurantii*) (Chartier-Fitzgerald, 2014; Chartier-Fitzgerald *et al*., 2016), and red scale (*Aonidiella aurantii*) (Upfold *et al*., 2016) and field trials against mealybug and thrips (Grout *et al*., 2016). Although results from field trials against FCM have been extremely positive (Coombes *et al*., 2016), efficacy against mealybug and thrips in field trials has been disappointing (Grout *et al*., 2016). 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.

Stated 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 Metarhizium 69 a.i. *M. anisopliae* ICIPE 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 two-week old 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 a Pairwise t-test with Bonferroni adjustment.

B. UV sensitivity trial

Three fungal isolates were irradiated simultaneously 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 H_2O), adjusted to 1×10^5 conidia/mL using a haemocytometer and immediately used for inoculation. Conidial suspensions of the mycoinsecticide products were also prepared using sdt H_2O and adjusted to 1×10^5 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 lamps in a Q-SUN® Xe-3-HC xenon test chamber (Q-Lab Corporation, Westlake, OH, USA) at 0.3 W/m^2 for 15, 30 and 60 min. The corresponding total doses for these periods were 0.7, 1.3 and 2.6 KJ/m^2, 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/m^2 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 Quaite-weighted noon irradiance of 350 mW/m^2 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 Quaite-weighted irradiance in the Q-SUN® Xe-3-HC at 0.6 W/m^2 (unweighted irradiance set point), was found to be 1335 mW/m^2, and equally approximated noon summer irradiance (1300 mW/m^2) 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 the southern citrus producing region in South Africa. Control plates were covered with aluminium foil to block UV radiation. 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 test ($P < 0.05$).

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 (1×10^5 and 1×10^7 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 instars 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 a glass vial, corked with cotton wool and transferred to sealed containers with respective saturated salt 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**
July-Dec 2016 A: Conduct temperature tolerance trials	A: Completed. Results presented in Table 3.4.2.1.
Apr-Jun 2017 C: Conduct humidity trials D_1: Prepare for endophytism trials	C: Completed. Results presented in Table 3.4.2.3 and 3.4.2.4. D: Completed. Results presented in Table 3.4.2.5.
Jul-Sep 2017 D_2: Initiate endophytism trials	D: Completed. Results presented in Table 3.4.2.5.
Oct-Dec 2017 D_3: Continue with endophytism trials	D: Completed. Results presented in Table 5.
Apr-Jun 2018 B_1: Initiate UV sensitivity trials D_3: Continue with endophytism trials	B: Completed. Results presented in Table 3.4.2.2 D: Completed. Results presented in Table 3.4.2.5
Jul-Sep 2018 E_1: Initiate formulation trials B_2: Continue with UV trials	E: Not undertaken. B: Completed. Results presented in Table 3.4.2.2
Oct-Dec 2018 E_2: Continue formulation trials	E: Not undertaken.
Jan-Mar 2019 None provided	Thesis submitted for examination; Manuscripts prepared

A: Temperature tolerance trials (Table 3.4.2.1)

In agreement with 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 (Topt), 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 (TYopt), (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, Tmax, ranged between 32 to 33°C and 33 to 34°C for *B. bassiana* and *M. anisopliae* isolates, respectively (Table 3.4.2.1). 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 in Coombes *et al.*, 2016) revealed their average annual air temperature to be 17.7°C (https://www.wunderground.com). The average air 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 a.,* 2016). Temperatures within orchards in warmer provinces are slightly higher. For instance, the annual mean air temperature in Olifants River Estate, Limpopo, where the EPF field trials against citrus thrips and mealybugs were conducted (Grout *et al.,* 2016) 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, Naturol® (Mola & Afkari, 2012; Paixão *et al*., 2017; Oliveira *et al.,* 2018) could be considered as adjuvants in future formulations should studies for an improved thermotolerance be considered. Although when Grout *et al.* (2016) used *Metarhizium* with and without mineral oil against citrus thrips in Limpopo, treatments with mineral oil (0.3%) were significantly inferior to those without.

B: UV tolerance trials (Table 3.4.2.2)

Two hours' exposure to simulated solar radiation at UV irradiance of 0.3 W/m^2 (5.2 KJ/m^2) 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/m^2) and 30 min (1.3 KJ/m^2) 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/m^2), 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 3.4.2.5). 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). Thus, some UV protectants which protected fungal propagules in various EPF formulations studies such as vegetable oils, mineral oils, sunscreens should be considered in future formulation studies.

Table 3.4.2.1. Estimated parameters (±standard error) from the modified β function[1] (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]**
B. bassiana	G Ar 17 B3	28.31 (0.83) a	0.69 (0.02) f	32.07 (0.05) e	0.38 (0.16) b
B. bassiana	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
B. bassiana	G 14 2 B5	27.44 (0.59) abc	1.81 (0.03) d	32.91 (0.40) bcd	1.32 (0.45) a
B. bassiana	FCM 10 13 L1	26.91 (0.58) abc	1.46 (0.05) e	32.43 (0.17) cde	0.96 (0.19) ab
B. bassiana	Bb PPRI 5339	26.19 (0.20) c	1.72 (0.02) d	32.07 (0.03) de	0.61 (0.05) ab
M. anisopliae	G 11 3 L6	26.33 (0.11) bc	3.42 (0.10) a	33.93 (0.14) a	0.98 (0.05) ab
M. anisopliae	FCM Ar 23 B3	26.90 (0.11) abc	3.15 (0.08) a	32.98 (0.16) bc	0.74 (0.07) ab
M. anisopliae	G OL R8	26.27 (0.10) c	3.23 (0.06) a	33.45 (0.16) ab	0.93 (0.07) ab
M. anisopliae	Ma ICIPE 69	27.27 (0.21) abc	2.55 (0.03) b	32.50 (0.12) cde	0.48 (0.06) b

[1]The generalized β function is given by Y (T) = TYopt ⁄ ((T -Tmin) / (Topt - Tmin)) ^ (Tb3 * ((Topt - Tmin) / (Tmax -Topt))) *((Tmax - T) / (Tmax -Topt)) ^ Tb3, where V (T) is the fungal growth in mm/day (dependent variable) and T is the incubation temperature (independent variable). Tmin is the lowest temperature for fungal growth, and was fixed at 6°C.
[2]Topt is the optimal temperature for fungal growth.
[3]TYopt is the fungal growth at the optimal temperature.
[4]Tmax is the highest temperature for fungal growth.
[5]Tb3 is the shape parameter which influences the temperature range around Topt in which the curve stays near TYopt.
[6]Means within columns with the same letter are not significantly different (Pairwise t-test, P > 0.05 with Bonferroni correction factor).

Table 3.4.2.2. Relative percentage germination (±standard error) of *M. anisopliae* and *B. bassiana* isolates/products after exposure to simulated solar radiation[1] 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
M. anisopliae	G 11 3 L6	99.09 (0.32) Aa	96.43 (0.77) Aa	4.68 (0.75) Bb
M. anisopliae	FCM Ar 23 B3	98.59 (0.39) Aa	97.02 (0.49) Aa	1.41 (0.83) Bc
M. anisopliae	G OL R8	98.36 (0.39) Aa	97.59 (0.54) Aa	7.51(1.01) Bb
M. anisopliae	Ma ICIPE 69	98.90 (0.25) Aa	99.21 (0.39) Aa	1.79 (0.41) Bc
M. anisopliae	Ma ICIPE 69*	99.25 (0.16) Aa	97.83 (0.63) Aa	92.79 (0.69) Aa
B. bassiana	G Ar 17 B3	99.64 (0.14) Aa	99.49 (0.19) Aa	93.06 (0.99) Aa
B. bassiana	G B Ar 23 B3	90. 86 (0.87) Ab	60.19 (2.93) Ab	5.91 (0.71) Bb
B. bassiana	G 14 2 B5	97.33 (0.36) Aa	98.39 (0.44) Aa	97.77 (0.39) Aa
B. bassiana	FCM 10 13 L1	93.45 (0.64) Ab	86.21 (1.28) Aa	5.82 (0.91) Bb
B. bassiana	Bb PPRI 5339	56.39 (1.43) Ac	10.32 (1.84) Bc	2.02 (0.25) Cc
B. bassiana	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/m^2, 28.44 ± 0.61°C and 44.49 ± 3.19% RH.
[2]Means 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 3.4.2.3 and 3.4.2.4)

High mortalities were recorded for the *M. anisopliae* isolates and the two commercial isolates, regardless of relative 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 Metarhizium 69 (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, G B 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 conform to 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 mycoinsectides. 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 3.4.2.3. Mean pupal mortality (±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.

		Mean mortality (%) at the four humidity levels tested[1]			
Species	**Isolate/Treatment**	**12%**	**43%**	**75%**	**98%**
M. anisopliae	G 11 3 L6	77.50 (2.50) Bab	85.83 (2.21) ABa	91.67 (0.83) Aa	85.83 (0.83) ABa
M. anisopliae	FCM Ar 23 B3	83.33 (3.01) Aa	90.00 (2.89) Aa	91.67 (2.21) Aa	90.83 (1.67) Aa
M. anisopliae	G OL R8	80.00 (2.50) Aa	82.50 (1.44) Aa	80.83 (2.21) Aab	82.50 (2.50) Aa
M. anisopliae	Ma ICIPE 69	75.83 (2.21) Aabc	83.33 (1.67) Aa	82.50 (1.44) Aa	85.83 (2.21) Aa
B. bassiana	G Ar 17 B3	40.00 (4.33) Ad	47.50 (3.82) Ab	46.67 (5.07 Ad	45.83 (2.21) Ab
B. bassiana	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
B. bassiana	G 14 2 B5	60.00 (1.44) Abd	57.50 (1.44) Ab	59.17 (1.67) Acd	60.83 (2.21) Ab
B. bassiana	FCM 10 13 L1	50.83 (2.21) Ad	55.83 (2.21) Ab	55.00 (2.89) Ad	58.33 (2.21) Ab
B. bassiana	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 3.4.2.4. Mean pupal mortality (±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.

		Mean mortality (%) at the four humidity levels tested[1]			
Species	**Isolate/Treatment**	**12%**	**43%**	**75%**	**98%**
M. anisopliae	G 11 3 L6	62.50 (6.61) Bab	69.17 (5.83) ABa	80.00 (2.89) Aa	80.83 (3.01) Aa
M. anisopliae	FCM Ar 23 B3	67.50 (6.61) Aa	73.33 (3.01) Aa	71.67 (2.21) Aa	72.50 (1.44) Aa
M. anisopliae	G OL R8	66.67 (3.01) Aa	64.17 (4.41) Aab	71.67 (3.33) Aa	64.17 (3.01) Aab
M. anisopliae	Ma ICIPE 69	66.67 (6.01) Aa	75.83 (2.21) Aa	71.67 (3.33) Aa	75.00 (1.44) Aa
B. bassiana	G Ar 17 B3	25.00 (2.89) Bd	31.67 (3.63) ABc	38.33 (3.63) ABb	40.83 (3.01) Ac
B. bassiana	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
B. bassiana	G 14 2 B5	45.00 (4.33) Abc	44.17 (3.63) Abc	45.00 (5.78) Ab	47.50 (1.44) Abc
B. bassiana	FCM 10 13 L1	40.00 (5.77) Acd	40.83 (4.17) Ac	39.17 (3.01) Ab	37.50 (1.44) Ac
B. bassiana	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 3.4.2.5)

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. G 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 3.4.2.5. Percentage (± standard error) of plant parts colonised 14-15 days after inoculation with four *M. anisopliae* isolates at 24.85 ± 0.06°C, 35.85 ± 0.34 % RH at a 12 h photoperiod.

	Mean colonisation (%) of the various plant parts[1]			
Inoculation method	**Isolate**	**Leaves**	**Stems**	**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 relative 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 radiation, 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.

Technology transfer

Talks or presentations:

Acheampong, M.A., Hill, M.P., Moore, S.D. and Coombes, C.A. 2019. *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 (oral presentation, presenter: C. Coombes).

Acheampong, M., Hill, M.P., Moore, SD. & Coombes, CA. 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).

Titles for refereed papers:

Acheampong, MA., Hill, MP., Moore, SD. & Coombes, CA. 2020. UV sensitivity of *Beauveria bassiana* and *Metarhizium anisopliae* isolates under investigation as potential biological control agents in South African citrus orchards. *Fungal Biology* 124(5): 304-310. (doi: 10.1016/j.funbio.2019.08.009).

Accepted with minor revision:

Acheampong, MA., Coombes, CA, Moore, SD. & Hill, MP. Temperature tolerance and humidity requirements of select entomopathogenic fungal isolates for future use in citrus IPM programmes. *Journal of Invertebrate Pathology*.

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3.4.3 **FINAL REPORT: Controlling flat mite as a vector of Leprosis**

Project 1224 (April 2019 – March 2020) by Sean Moore, Wayne Kirkman, Mellissa Peyper (CRI), Tammy Marsberg (RU)

Summary

Leprosis-N was recently discovered infecting citrus in the Sundays River Valley, which is a first record of this disease on citrus in South Africa. This disease is caused by the orchid fleck virus and is vectored by the flat mite, *Brevipalpus californicus*. As the disease can only be spread by the vector, good control of the vector is extremely important and can even lead to eradication of the disease. Consequently, the efficacy of several registered miticides were tested, as well as other products which are widely used against other pests, but could also have an effect on the mites. One field trial and two detached fruit trials were conducted during the reporting period. Of the products registered for the control of flat mite on citrus, fenpyroximate, spirodiclofen and fenbutatin-oxide were the most effective. Abamectin also resulted in high levels of mortality in all three trials. Due to low levels of flat mite, the proposed trials to determine a new threshold for flat mite in orchards showing symptoms of leprosis could not be conducted.

Opsomming

Sitrus-Leprose is onlangs in die Sondagsriviervallei ontdek. Dit is 'n eerste rekord van hierdie siekte op sitrus in Suid-Afrika. Hierdie siekte word deur die orgideevlek virus veroorsaak en word deur die platmyt, *Brevipalpus californicus*, versprei. Aangesien die siekte slegs deur die vektor versprei kan word, is goeie beheer van die vektor uiters belangrik en dit kan selfs tot die uitwissing van die siekte lei. Gevolglik is die effektiwiteit van verskeie geregistreerde mytdoders getoets, asook ander produkte wat wyd teen ander plae gebruik word, maar wat ook 'n uitwerking op die myte kan hê. Een veldproef en twee afsonderlike vrugproewe is gedurende die verslagtydperk uitgevoer. Van die produkte wat vir die beheer van platmyte op sitrus geregistreer is, was fenpiroksimaat, spirodiklofen en fenbutatien-oksied die doeltreffendste. Abamektien het ook hoë vlakke van mortaliteit in al drie proewe getoon. As gevolg van die lae vlakke van platmyt, kon die voorgestelde proewe om 'n nuwe drempelwaarde vir platmyt in boorde wat simptome van leprose toon te bepaal, nie uitgevoer word nie.

Introduction

Citrus Leprosis-N was recently discovered infecting citrus in the Sundays River Valley, which is a first record of this disease on citrus in South Africa (Cook et al., 2019; Kirkman et al., 2018). This disease is caused by the orchid fleck virus and is vectored by the flat mite, *Brevipalpus californicus* (Fig 3.4.3.1). As the disease can only be spread by the vector, good control of the vector is extremely important and can even lead to eradication of the disease. Consequently, the efficacy of the full range of miticides available for use on citrus must be tested. Additionally, where leprosis is present, the threshold for intervention against flat mite must be lowered. Currently, the recommended threshold, in the absence of leprosis, is 1 mite per fruit or stalk (Grout, 2011). However, during June 2018, in an orchard where infestation was lower than this level, conspicuous occurrence of leprosis disease symptoms were still present. Consequently, a new threshold must be determined for such situations.

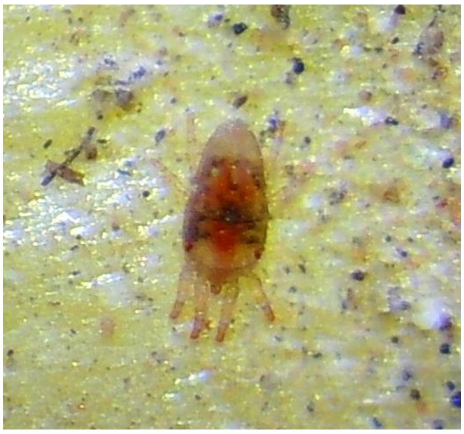

Figure 3.4.3.1. Flat mite, *Brevipalpus californicus*.

Stated objectives

- Compare the efficacy of all available miticides against flat mite
- Determine an appropriate threshold for intervention against flat mite in orchards where leprosis is present.

Materials and methods

One field trial and two detached fruit trials were conducted during the reporting period. The most commonly used products registered for use against flat mite on citrus were applied in the trials, as well as abamectin, spirotetramat and mancozeb, which are widely used against other pests or diseases, but could also have an effect on the mites.

<u>Field trial</u>

The field trial was conducted in a 28-year-old Delta Valencia orchard on Elim East farm in the Sundays River Valley. The trial was laid out in a randomised block format, replicated 10 times. Treatments were applied on 12 August 2019 as full cover applications at an average volume of 13.8 L of spray mixture per tree for all treatments. Once the trees had dried, 20 fruit per tree were covered with brown paper bags to avoid them being harvested. The bags were then removed one day before the scheduled evaluation. Unfortunately, hawkers harvested the fruit before evaluation could take place. In order to salvage some results, 20 leaves per tree were collected to determine the number of live and dead mites. Abbott's correction was applied to mortalities.

<u>Detached fruit trials</u>

Two detached fruit assay trials were conducted during the reporting period. Treatments for the first trial were applied on 21 August 2019, and the second on 23 March 2020. For both trials, harvested infested fruit were treated. Treatments were applied as a fine mist using a hand spray bottle. An average of 4.5 ml was applied per fruit for the first trial, and 3.9 ml for the second trial. Treatments were applied to 10 fruit each in the first trial, and 5 fruit each in the second trial. Fruit were then evaluated after 5 days. Live and dead mites were recorded for each fruit.

Results and discussion

Table 3.4.3.1. Task table

	Objective / Milestone	**Achievement**
A	Compare the efficacy of all available miticides against flat mite	Complete

B	Determine an appropriate threshold for intervention against flat mite in orchards where leprosis is present.	Not possible due to lack of mites and leprosis symptoms

Field trial

In the first trial, spirodiclofen and abamectin were the most effective of all the products used, both resulting in 100% mortality of mites (Table 3.4.3.2). These were followed by fenpyroximate, which provided 59.26% control. Mortality for treatments was corrected according to Abbott (2004) for the 18.18% mortality in the untreated control. There was no significant difference between any of the treatments or the untreated control (Kruskal-Wallis test, H (5, N=6) = 5, p = 0.4159).

Table 3.4.3.2. Corrected mortality of flat mite for treatments in a field trial applied on 12 August 2019, and evaluated 3 days later.

Treatment and rate per 100L water		Mites dead	Mites alive	Corrected mortality (%)
1	Untreated control	2	9	
2	Fenpyroximate (Lesson) 150 ml	2	1	59.26
3	Spirodiclofen (Envidor) 15 ml	7	0	100.00
4	Spirotetramat (Movento) 20 ml	3	3	38.89
5	Abamectin 20 ml	9	0	100.00
6	Mancozeb (Dithane) 210 g	7	5	40.07

Detached fruit trials

In the first detached fruit trial, fenpyroximate, spirodiclofen and fenbutatin-oxide were the most effective registered products tested (Table 3.4.3.3). Abamectin also once again caused a reasonable level of mortality. There were no significant differences between treatments or the untreated control (Kruskal-Wallis test: H (7, N=8) = 7, p = 0.4289).

Table 3.4.3.3. Corrected mortality of flat mite for treatments applied on 21 August 2019, and evaluated 5 days later.

Treatment and rate per 100L water		Mites dead	Mites alive	Corrected mortality (%)
1	Untreated control	27	41	
2	Fenpyroximate (Lesson) 150 ml	36	5	79.77
3	Spirodiclofen (Envidor) 15 ml	18	5	63.94
4	Spirotetramat (Movento) 20 ml	33	38	11.23
5	Fenbutatin-oxide 10 ml	61	16	65.54
6	Fenproprathin (Meothrin) 30 ml	38	40	14.95
7	Abamectin 20 ml	78	58	57.47
8	Mancozeb (Dithane) 210 g	71	62	22.68

In the second detached fruit trial, all the registered products were effective (Table 3.4.3.4). Abamectin again caused a high level of mortality, as did mancozeb and spirotetramat. It is possible that the low level of mites in this trial led to an inflated level of efficacy. Once again there were no significant differences between treatments or the untreated control (Kruskal-Wallis test, H (6, N=7) = 6, p = 0.4232).

Table 3.4.3.4. Corrected mortality of flat mite for treatments applied on 23 March 2020, and evaluated 5 days later.

Treatment and rate per 100L water	Mites dead	Mites alive	Corrected mortality (%)

1	Untreated control	5	32	
2	Fenpyroximate (Lesson) 150 ml	10	0	100
3	Spirodiclofen (Envidor) 15 ml	12	7	57.40
4	Spirotetramat (Movento) 20 ml	10	2	80.73
5	Fenbutatin-oxide 10 ml	19	6	72.25
6	Abamectin 20 ml	16	1	93.20
7	Mancozeb (Dithane) 210 g	17	1	93.58

Conclusion

Of the products registered for the control of flat mite on citrus, fenpyroximate, spirodiclofen and fenbutatin-oxide were the most effective. Abamectin also resulted in high levels of mortality in all three trials. Due to low levels of flat mite, the proposed trials to determine a new threshold for flat mite in orchards showing symptoms of leprosis could not be conducted.

Future research

Another field trial may be attempted if a suitable site can be found.

Technology transfer

None as yet.

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3.4.4 PROGRESS REPORT: New systemic insecticides for citrus

Project 1148 (2016/7 – 2019/20) by T G Grout, P R Stephen (CRI), and S Mohammed (*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. This research is currently focused on research with *D. citri* in Kenya. Collaborators there at *icipe* erected an insect-proof screenhouse at their Muhaka field station in SE Kenya where *D. citri* has established. More than 100 potted citrus plants within this screenhouse have been infested with *D. citri* but this number is still inadequate for planned screening trials. Surprisingly, attempts to use *Murraya paniculata* seedlings as alternative host plants have been unsuccessful. Once sufficient infested citrus trees can be used in screening trials, 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 kwekerye en vir nie-draende bome gebruik kan word. Hierdie navorsing fokus tans op navorsing met *D. citri* in Kenia. Medewerkers by *icipe* het 'n insek-bestande evaluasietonnel by hul

Muhaka veldstasie in SO Kenia opgerig, waar *D. citri* gevestig is. Meer as 100 gepotte sitrusplante binne hierdie evaluasietonnel is met *D. citri* besmet, maar hierdie getal is steeds onvoldoende vir beplande evaluasieproewe. Pogings om *Murraya paniculata* saailinge as alternatiewe gasheerplante te gebruik, was verbasend onsuksesvol. Sodra voldoende besmette sitrusbome in evaluasieproewe gebruik kan word, sal navorsing na jong-geplante sitrusbome uitgebrei word.

3.4.5 PROGRESS REPORT: The efficacy of commercial entomopathogenic fungi products for control of citrus pests

Project 1174 (April 2017 – April 2020) by 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. Previous trials showed no significant efficacy 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 infestation 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 fumororosea* 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. The product was first tested in an orchard with an extremely high mealybug infestation. Unfortunately, no significant efficacy was recorded. During the course of last season two trials were conducted with three different rates of *Isaria fumororosea* on aphids and psylla. Aphid infestation was reduced by 93%, 59% and 10% for the three rates, only being a significant reduction for the highest rate. However, there was very little persistence in control, as recorded in two subsequent evaluations. Consequently, it appears that more than one spray would be required in practice. The trial against psylla showed no significant efficacy for any of the treatments. However, infestation was patchy and the trial warrants repeating. A third trial was to be conducted, but lockdown prevented this from happening. We will aim to conduct another trial next season.

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 ʼn reeks sitrusplae word al hoe meer algemeen, maar hierdie bewerings bots gereeld met die ondervindinge van wetenskaplikes in die bedryf. Vorige proewe het geen beduidende doeltreffendheid getoon teen rooidopluis, witluis of blaaspootjie met ʼn voorkomende program deur die gebruik van beide *Beauveria bassiana* en *Metarhizium anisopliae* gebaseerde produkte. Gevolglik is ʼn korrektiewe proef toegedien met drie produkte by ʼn 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 ʼn nuwe *Isaria fumororosea* produk met berigte doeltreffendheid teen Asiatiese sitrusbladvlooi (ABV), *Diaphorina citri*, is verkry vir proewe. Die doel was om dit te toets teen plae soos sitrusbladvlooi**,** plantluise of wollerige witvlieg, wat kan dui op die doeltreffendheid daarvan teen ABV. Gevolglik is die produk in ʼn boord met ʼn uiters hoë witluis besmetting getoets. Ongelukkig is geen betekenisvolle doeltreffendheid waargeneem nie. Gedurende die afgelope seisoen is twee proewe uitgevoer met drie verskillende dosisse van *Isaria fumororosea* op plantluise en sitrusbladvlooi. Plantluis besmetting is met 93%, 59% en 10% verminder vir die drie dosisse, waar die afname net vir die hoogste dosis statisties betekenisvol was. Daar was egter ʼn baie kort nawerking, aangeteken in nog twee evaluasies daarna.

Gevolglik, blyk dit dat meer as een bespuiting in die praktyk nodig sal wees. Die proef teen bladvlooi het geen beduidende werking vir enige van die behandelings getoon nie, maar besmetting was kol-kol en die proef kan dus herhaal word. Ons was van plan om 'n derde proef hierdie seisoen te spuit maar Covid-19 inperking het die verhoed. Ons sal dus volgende seisoen nog 'n proef spuit.

3.4.6 PROGRESS REPORT: Determine the primary cause for mealybug repercussions under netting

Project 1195 (2018/9 – 2020/1) by T G Grout, P R Stephen and P Cronjè (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 trees placed under the shade net and in the open adjacent to the structures and citrus mealybug cultures initiated on butternuts. Several attempts to transfer citrus mealybug from butternut cultures to the potted citrus plants have been unsuccessful so comparisons of growth rates under the net versus in the open have not yet been possible. Leaf samples from trees in adjacent orchards under net and in the open in Limpopo and Mpumalanga were analysed for total phenolic content in mid-summer and early autumn. In most cases rows ran east-west and leaves on the northern side of the tree had higher levels of phenolics than on the southern side. These differences were greater than differences between leaves sampled under net and in the open. It therefore does not appear that phenolic differences as a result of the net would directly influence mealybug populations.

Opsomming

Witluis het in sitrusboorde onder 20% skadunet, in beide Australië en Suid-Afrika, 'n belangrike plaag geword. Hierdie navorsing is uitgevoer ten einde die hoofredes hiervoor vas te stel. Skadunet strukture is by die Sitrus Navorsingsentrum op Nelspruit gebou, gepotte bome is onder die skadunet en in die oopte naasliggend aan die strukture geplaas, en sitrus witluis kulture is op botterskorsies gestig. Verskeie pogings om sitrus witluis vanaf botterskorsie kulture na die gepotte sitrusplante oor te dra, was onsuksesvol, dus is vergelykings van groeitempo's onder die net teenoor in die oopte nog nie moontlik nie. Blaarmonsters vanaf bome in naasliggende boorde onder net en in die oopte in Limpopo en Mpumalanga is vir totale fenoliese inhoud in middel-somer en vroeë herfs geanaliseer. In die meeste gevalle het rye oos-wes geloop en blare aan die noordelike kant van die boom, het hoër vlakke fenole gehad as aan die suidelike kant. Hierdie verskille was groter as verskille tussen blare wat onder net en in die oopte versamel is. Dit blyk dus dat fenoliese verskille as gevolg van die net, nie witluis populasies direk sal beïnvloed nie.

3.4.7 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, some preliminary work with fumigants Vapormate (ethyl formate) and carbon dioxide was conducted last year. However, no suitable sources of citrus bud mite have been found to continue this work in 2019/20 and evaluate acaricide dips. Once a suitable source of mites has been found, this research will continue.

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 sagte wyse van beheer, is 'n bietjie voorlopige werk met berokingsmiddels Vapormate (etielformaat) en koolstofdioksied laas jaar uitgevoer. Geen geskikte bronne van sitrus knopmyt is egter gevind om hierdie werk in 2019/20 voort te sit en mytdoder doopmiddels te evalueer nie. Sodra 'n geskikte bron van myte gevind word, sal hierdie navorsing voortgesit word.

3.4.8 **PROGRESS REPORT: Surveying for Oleander scale, *Aspidiotus nerii***

Project 1243 (Sep 2018 - Aug 2020) by Sean Moore, Tim Grout, Martin Gilbert, Aruna Manrakhan, Wayne Kirkman, Peter Stephen, John-Henry Daneel, Kim Stoltz, Mellissa Peyper, Elma Carstens, Vaughan Hattingh (CRI), Tammy Marsberg (RU)

Summary

Oleander scale, *Aspidiotus nerii*, is listed as a pest on citrus, including in South Africa. However, on citrus in South Africa, *A. nerii* has only been collected on four occasions. Additionally, this infestation was recorded on branches, not on fruit. None of these detections were in commercial citrus orchards, all being from lemons in home gardens. Although this insect has a relatively cosmopolitan distribution, Philippines and Vietnam list *A. nerii* as a quarantine pest. Consequently, this listing serves as an impediment to the export of South African citrus to these countries. Both countries have stated a requirement for a scientific survey in South African citrus orchards in order to demonstrate low phytosanitary risk and to remove this hurdle to continued exports to these countries. Consequently, surveys for *A. nerii* are being conducted in several citrus orchards, including lemons, in which any other CRI field trials are being conducted. This is in Western Cape, Eastern Cape and Mpumalanga provinces. These surveys were conducted for a full season during 2018/19 and are continuing during the 2019/20 season. Thus far no infestation of *A. nerii* has been recorded.

Opsomming

Oleander dopluis, *Aspidiotus nerii*, is as ‘n plaag op sitrus gelys, insluitend in Suid-Afrika. Op sitrus in Suid-Afrika is *A. nerii* egter net op vier geleenthede versamel. Daarbenewens is hierdie besmetting net op takke aangeteken en nie op vrugte nie. Geen van hierdie onderskeppings is in kommersiële sitrusboorde nie; almal is van suurlemoen bome in huistuine. Alhoewel hierdie insek ‘n relatief kosmopolitaanse verspreiding het, word dit deur die Filippyne en Viëtnam gelys as ‘n kwarantyn plaag. Gevolglik dien hierdie notering as ‘n struikelblok tot die uitvoer van Suid-Afrikaanse sitrus tot hierdie lande. Albei lande vereis ‘n wetenskaplike opname in Suid-Afrikaanse sitrusboorde om te demonstreer dat daar ‘n lae fitosanitêre risiko bestaan en om hierdie struikelblok tot uitvoere in die toekoms te verwyder. Gevolglik word opnames vir *A. nerii* in verskeie sitrusboorde gedoen, insluitend suurlemoene, waar enige ander CRI veldproewe gedoen word. Hierdie is in Wes-Kaap, Oos-Kaap en Mpumalanga provinsies. Hierdie opnames is vir ‘n volle seisoen in 2018/19 gedoen en word in die 2019/20 seisoen voortgesit. Geen besmetting van *A. nerii* is egter tot dusver opgelet nie.

3.4.9 **PROGRESS REPORT: Chemical control of mealybug on citrus**

Project 1257 (April 2020 - March 2021) by Sean Moore, Wayne Kirkman, Mellissa Peyper (CRI), Tammy Marsberg and Luke Cousins (RU)

Summary

Organophosphates and buprofezin were for years the mainstays of the spray programme for mealybug control on citrus in South Africa. Several years ago, the most effective organophosphates were restricted to only being used before petal drop. Consequently, the only organophosphates usable correctively on the fruit were chlorpyrifos and mevinphos, neither of which are as effective as profenofos, prothiofos and methidathion. Fortunately, buprofezin could be used up until 45 days before harvest. This was changed recently, due to a reduction in the MRL for most markets, including the EU. Buprofezin can now be used no later than mid-October for oranges and grapefruit and no later than 90% petal fall for lemons and soft citrus. Consequently, we have a suite of newly registered mealybug products, namely Closer (sulfoxaflor), Lesson (fenpyroximate) and Tivoli (spirotetramat), and others that are not renowned for their efficacy against mealybug, namely methomyl and Wetcit. Additionally, none of these products are registered specifically for corrective (late season) control or even recommend such an option on the label. Consequently, we conducted two late season corrective trials, using these products against high levels of mealybug infestation. In one trial Closer was the most effective product, followed by Tivoli. In the other trial, this was reversed. In both trials Closer was more effective when applied with BreakThru compared to mineral oil.

Opsomming

Organofosfate en buprofezin was vir jare die belangrikste produkte in die spuitprogram vir witluis beheer op sitrus in Suid-Afrika. ‘n Paar jaar gelede is die gebruik van die mees doeltreffende organofosfate beperk tot voor blomblaarval. Gevolglik, was chlorpirifos en mevinfos die enigste organofosfate wat korrektief op vrugte gebruik kon word, en die profukte is nie so doeltreffend soos profenofos, prothiofos en methidathion nie. Gelukkig kon buprofezin nogsteeds tot 45 dae voor oes gebruik word, maar hierdie het nou verander, met ‘n vermindering in MRL vir meeste markte, insluitend die EU. Buprofezin mag nou nie later as middel Oktober op lemoene en pomelos gebruik word nie en nie later as 90% blomblaarval op suurlemoene en sagtesitrus. Gevolglik het ons ‘n klomp nuwe produkte beskikbaar vir witluis, naamliks Closer (sulfoksaflor), Lesson (fenpiroksimaat) en Tivoli (spirotetromat), en ander produkte wat nie so bekend is vir hulle doeltreffendheid teen witluis nie, byvoorbeeld metomiel en Wetcit. Bonop is geen een van hierdie produkte spesifiek vir laat seisoen korrektiewe beheer van witluis geregistreer of so op die etiket aanbeveel nie. Daarom het ons laas seisoen twee laat seisoen korrektiewe proewe gedoen met hierdie produkte teen hoë vlakke van witluis besmetting. In een van die proewe het Closer die beste beheer gegee, gevolg deur Tivoli en in die ander proef was dit anders om gewees. In albei proewe was Closer met BreakThru meer doeltreffend as Closer met minerale olie.

3.4.10 **PROGRESS REPORT: IPM under Nets in the Western Cape**

Project 1228 (2019/20 – 2021/22) by M Gilbert and C Love (CRI)

Summary

The use of netting may change the citrus orchard environment. Thus it is essential to determine what effect, if any, nets may have on different citrus pest populations as well as those of predators and parasitoids. Both netted and open orchards, of the same cultivar at the same site, were required in order to make a valid comparison. Two suitable sites in the Western Cape were identified. The first site, with Nadorcott mandarins, was just outside Porterville and is not part of any sterile insect release (SIT) programmes. The second site, in the Breede River Valley, had Nules Clementines and this site is part of both the FCM and fruit fly SIT programmes. Traps were set out for: FCM (yellow Delta traps with FCM lures); fruit fly (Capilure in Sensus traps and Biolure in yellow bucket traps), thrips and other pests, predators and parasitoids (yellow sticky traps). Three traps of each type were placed in the netted and the open blocks in mid-November at the first site and late November at the second site. Fruit inspections began in December. While still in the early stages of monitoring, some initial results can be mentioned. No FCM were trapped at the first site, but both sterile and wild FCM were trapped at the second site. Trap catches tended to be higher in the open areas for both wild and sterile FCM, but did vary. Only *Ceratitis capitata* has been caught to date in the fruit fly traps, with numbers generally higher in the open areas, but Medfly is also present in the netted blocks at both sites. The most commonly trapped pests on the yellow sticky traps were citrus thrips and green leafhoppers at both sites. Low numbers of citrus leafhoppers, red scale males, whiteflies, as well as green lacewing predators were recorded. Fruit inspections primarily showed wind damage at this stage, with more damage in the open blocks. Fruit collections and leaf inspections will continue.

Opsomming

Die gebruik van nette mag dalk die sitrusboord omgewing verander. Dit is dus noodsaaklik om te bepaal of daar enige effekte op sitrusplaag populasies is, sowel as predatore en parasitoeide. Dit is nodig om boorde onder- en sonder nette, van dieselfde kultivaar en by dieselfde perseel, te identifiseer, om ‘n geldige vegelyking te maak. Twee geskikte perseële in die Wes-Kaap is geindentifiseer, een buitekant Porterville wat nie deel is van enige steriele insek loslating program nie. Die tweede perseël, naby die Breederivier Vallei, het Nules Clementine gekweek, en hier is albei steriele VKM en vrugtevlieë losgelaat. Lokvalle is uitgesit vir die volgende insekte: VKM (geel Delta met Chempac lokmiddels); vrugtevlieg (Sensus met Capilure lokmiddel en geel emmer met Biolure); blaaspootjies, bladspringer, predatore en parasitoeide (geel kleefvalle). Drie valletjies van elke tipe onder nette en in oop boorde is in middel November by die eerste perseel en laat November by die tweede perseel uitgesit. Alhoewel die proef in ‘n vroeë stadium is, die volgende resultate is tot dus ver aangeteken. By Porterville is geen VKM gevang nie, maar wel albei wilde VKM en steriele VKM by die twede perseel. Vangstes is geneig om hoër te wees in oop boorde vir albei wilde en steriele VKM, maar het gewissel.

Wat vlieë aanbetref, is net *Ceratitis capitata* tot op datum gevang, met hoër getalle in die ope boorde, alhoewel Medvlieg ook in boorde met nette op albei proefpersele gevang is. Die mees algemene plae, wat op geel kleefvalle verskyn het, is sitrublaaspootjie en groen bladspringer by albei proefpersele. Lae getalle van sitrus bladspringer, rooidopluis mannetjies, witvlieë, sowel as goudogie predatore is aangeteken. Vruginspeksie het tot hede hoofsaaklik wind skade gewys, met meer skade in ope bloke. Vrug versameling en blaarinspeksies sal voortgesit word.

3.4.11 PROGRESS REPORT: Synergism and formulation of entomopathogenic fungi for foliar control of various citrus pests

Project 1188 (Feb 2019 – Dec 2020) by Samantha Prinsloo (RU – MSc), Candice Coombes (RU – Entomology), Martin Hill (RU - Entomology), Sean Moore (CRI) and Antoinette Malan (SU - Nematology)

Summary

False codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) (FCM), is a major insect pest of citrus in South Africa. Owing to restrictions on the use of insecticides, non-chemical control options, including the use of entomopathogenic fungi (EPF) and entomopathogenic nematodes (EPN) have been investigated. Previous research has shown that a synergistic relationship might occur between EPN and EPF, when they are combined for the management of late instar lepidopteran larvae. This study aims to investigate the control potential of three EPN species alone (*Steinernema yirgalemense*, *S. jeffreyense* and *Heterorhabditis noenieputensis*) and in combination with one EPF isolate, *Metarhizium anisopliae* FCM Ar 23 B3, on late fifth instar FCM larvae, using standardised laboratory bioassays. Dose response bioassays for the individual nematode agents have been conducted to determine the LC_{50} values (*S. yirgalemense*: 4.38 IJs/50 µl; *S. jeffreyense*: 4.46 IJs/50 µl; *H. noenieputensis*: 7.11 IJs/50 µl). Trials assessing the effect of EPF and EPN application timing required to achieve optimal larval mortality have also been undertaken. These results show that all three EPN species should be applied 72 hours after EPF application. EPF and EPN combination trials using LC_{50} values still need to be completed.

In previous laboratory and field trials *M. anisopliae* FCM Ar 23 B3 has been shown to be a successful control agent of FCM. However, when targeting foliar citrus pests, field results were not promising. This was attributed to the negative effects that environmental conditions impose on conidia. Solar radiation in particular was found in a laboratory study, to be extremely detrimental to *M. anisopliae* FCM Ar 23 B3 viability. Thus, a further component of this research aims to investigate UV protectant formulations to improve control of foliar pests. Three formulations, namely Nu Film 17, a liposomal formulation and a Pheroid formulation, will be assessed. These trials will be conducted using the Q-SUN® Xe-3-HC (Q-Lab Corporation, Westlake, OH, USA) sunlight chamber at CSIR following a similar protocol to that outlined in Acheampong et al. (2020) (section 3.4.2).

Opsomming

Valskodlingmot, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) (VKM), is 'n belangrike insekplaag van sitrus in Suid-Afrika. Weens die beperkings op die gebruik van insekdoders, is nie-chemiese beheermaatreëls, insluitend die gebruik van entomopatogene swamme (EPF) en entomopatogeniese aalwurms (EPN), ondersoek. Vorige navorsing het getoon dat 'n sinergistiese verhouding tussen EPN en EPF kan voorkom, wanneer hulle gekombineer word vir die bestuur van laat stadium Lepidoptera larwes. Hierdie studie se doel was om die beheerpotensiaal van drie EPN-spesies alleen te ondersoek, (*Steinernema yirgalemense, S. jeffreyense* en *Heterorhabditis noenieputensis*) en in kombinasie met een EPF-isolaat, *Metarhizium anisopliae* FCM Ar 23 B3, op die laat vyfde instar VKM, met behulp van gestandaardiseerde laboratorium-biotoetse. Dosisrespons-biotoetse vir die aalwurms op hulle eie is uitgevoer om die LC_{50}-waardes te bepaal (*S. yirgalemense*: 4.38 IJs/50 µl; *S. jeffreyense*: 4.46 IJs/50 µl; *H. noenieputensis*: 7.11 IJs/50 µl). Proewe om die effek van die EPF en EPN toedieningstyd te bepaal wat nodig is om optimale larwe mortaliteit te bereik, is ook onderneem. Hierdie resultate toon dat al drie EPN-spesies 72 uur na EPF-toediening toegedien moet word. EPF- en EPN-kombinasieproewe met LC_{50}-waardes moet nog voltooi word.

Vorige laboratorium- en veldproewe het *M. anisopliae* FCM Ar 23 B3, het bewys dat dit 'n doeltreffende beheermiddel van VKM is. Wanneer blaarbespuitings vir sitrusplae toegedien is, was die veldresultate egter nie belowend nie. Dit is toegeskryf aan die negatiewe gevolge wat omgewingstoestande op konidia plaas. Veral sonstraling is in 'n laboratoriumstudie gevind om uiters nadelig te wees vir die lewensvatbaarheid van *M. anisopliae* FCM Ar 23 B3. 'n Verdere komponent van hierdie navorsing se doel was om UV-beskermende formulasies te ondersoek om die beheer van bo-grondse plae te verbeter. Drie formulasies, naamliks Nu Film 17, 'n liposomale formulasie en 'n ”Pheroid” formulasie, sal getoets word. Hierdie proewe word uitgevoer word met ’n Q-SUN® Xe-3-HC (Q-Lab Corporation, Westlake, OH, VSA) sonligkamer by die WNNR, volgens 'n soortgelyke protokol as wat in Acheampong et al. (2020) beskryf is (afdeling 3.4.2).

3.4.12 **PROGRESS REPORT: IPM under nets in Mpumalanga Province**

Project 1205 (Apr 2018 – Dec 2020) by Karlien Grobler, Martin Hill (RU) and Sean Moore (CRI)

Summary

The incidence and severity of citrus thrips throughout the citrus growing season were found to be less under 20% enclosed shade net in comparison to the open citrus orchard. Mealybug populations were higher and more concentrated under the citrus netting than in the open orchard, contradicting the recordings in the previous growing season. *Coccidoxenoides perminutus* and *Anagyrus* spp. were released in equal quantities simultaneously under citrus netting and in the open orchard. Mealybug infestation declined, while percentage mealybug parasitized increased six weeks after release in both the enclosed and open orchard. Bollworm and orange dog populations did not seem to be any different in the two citrus orchards. FCM total trap catches were higher under citrus shade net in comparison to the open orchard, while fruit fly species were caught in higher quantities in the open orchard. However, these have not been compared statistically yet. Little fruit infestation by both FCM and fruit flies was recorded during harvesting. Mummified mealybugs were collected in citrus sites and placed in emergence boxes. Only *Coccidoxenoides perminutus* emerged. Red scale was collected and inspected for parasitism by *Aphytis* spp. None have been detected to date. Citrus fruit sampled for residue tests resulted in higher concentrations of dithiocarbamates and pyraclostrobin under the enclosed netting in comparison to the open orchard. Seychelles scale and pink wax scale infestation was higher in litchi orchards under shade net in comparison to the control. Mango scale infestation seems to be similar in litchi orchards in both scenarios. No litchi moth adults were trapped and no larval infestation was detected. False codling moth trap counts and larval infestation were higher under the litchi netted orchard. Fruit fly trap catches and larval infestation were found to be higher in the open orchard than under shade net. *Cryptolaemus montrouzieri* was released in equal quantities simultaneously in the litchi netted orchard and open orchard for the control of Seychelles scale and pink wax scale. Both pest populations were reduced to acceptable IPM levels before harvest. *Rodolia cardinalis* and *Chilocorus* spp. were found to occur naturally under litchi shade net, but none in the open orchard.

Opsomming

Die intensiteit en populasie druk van sitrus blaaspootjie deur die seisoen was laer op vrugte onder 20% skadu net teenoor die oop boord. Witluis populasies was meer oplettend en teen hoër getalle teenoor die oop kontrole boord, wat teenstrydig is met data van die vorige seisoen. *Coccidoxenoides perminutus* and *Anagyrus* spp. was vrygelaat op dieselfde dag in dieselfde hoeveelhede onder, asook buite die net. Witluis populasies het verminder en parasitisme vlakke het toegeneem in beide sitrus boorde ongeveer ses weke na vrylating. Bolwurm en sitrus skoenlapper populasies het nie groot verskille getoon tussen die twee sitrus boorde nie. VKM se totale mot vangste was hoër onder net teenoor die oop boord terwyl meer vrugtevlieë in die oop boord gevang was. Minimale vrug besmetting was opgemerk tydens oes. Geparasiteerde witluis van beide boorde was geneem en in bokse geplaas vir parasiet ontwikkeling. *Coccidoxenoides perminutus* het uitgebroei. Rooidopluis monsters op vrugte was geneem en geinspekteer met n mikroskoop vir parasitisme deur *Aphytis* spp. Geen parasitisme was opgemerk nie. Drie verskeie sitrus vrug monsters van beide boorde was geneem vir residu analise. Die aktiewe bestandele ditiokarbamaat asook piraklostrobin was in hoër konsentrasies onder die net teenoor vrugte uit die kontrole boord. Seychelles dopluis en pienk wasdopluis was in hoër populasies gevind onder die lietjie net teenoor die kontrole boord. Die net het nie n effek getoon op mangodopluis se voorkoms nie aangesien vlakke soortgelyk opgemerk was. Geen lietjie motte of larwes was onder die net

gevind nie. VKM mot tellings en infestasie vlakke was meer in die oop lietjie boord as onder net. *Cryptolaemus montrouzieri* was vrygelaat in gelyke getalle op dieselfde dag onder die nette asook in die kontrole boord vir die beheer van Seychelles dopluis en pienk wasdopluis. Beide peste se populasies het verlaag tot aanvaarbare IPM vlakke voor oes. *Rodolia cardinalis* en *Chilocorus* spp. kom natuurlik voor onder net maar geen kewer was tot op hede in die kontrole boord gevind nie.

4 PORTFOLIO: DISEASE MANAGEMENT

4.1 PORTFOLIO SUMMARY

By Jan van Niekerk (Portfolio Manager: DM, CRI)

Sustainable production of high quality citrus fruit is dependent on successful management of various pre- and postharvest pathogens. In order to focus on all of these, the Disease Management portfolio of CRI is divided into different research programmes. These are preharvest (Fruit and Foliar with CBS and Soilborne diseases), Graft Transmissible Diseases (GTD) and postharvest diseases programmes. Within each programme, specific industry research needs are addressed while proactive research is done to prepare the industry for any future challenges pertaining to disease management.

Within the GTD programme, great strides were made in the study of CTV and specifically the identification and characterization of single strain sources. This has enabled project 1173 to evaluate the performance of different single strains in various citrus types with the aim to identify the best strains for cross protection. It also aims to find strains suitable for infectious clone production within project 1160. The development of the infectious clone within 1160 is a novel way to target HLB and its vector. This is done in preparation for the eventuality that HLB and its vector arrives in South Africa. Within project 1160 the mechanism of stem pitting caused by CTV is also studied to better understand this disease and how to manage it. Early results from a field trial in project 1074 are showing that certified plant material from the Citrus Improvement Scheme (CIS) performs horticulturally (growth and tree health) compared to field cut material. Viroids remain a problem in the industry despite the use of certified material. New orchards planted with sensitive rootstocks can specifically suffer from viroids and in project 1155 a trial is being done to determine the viroid sensitivity of new and existing rootstock selections to viroid infections. Plant material is cleaned from all the GTD pathogens through shoot tip grafting (STG) and at this stage successful elimination of pathogens is determined through molecular and laborious biological screening. In an attempt to speed up this evaluation, project 1241 is investigating if high throughput sequencing (HTS) can be used to successfully determine the GTD pathogen status of material, thereby potentially leading to quicker release of material to the industry. Due to the HLB pathogen,'*Candidatus*' Liberibacter asiaticus (Las), and its vector, *Diaphorina citri*, being confirmed to be present in Africa, detection capabilities are essential to allow for early detection and control measures. Therefore, within project 1200, a real-time diagnostic test was developed to detect and identify the different citrus Liberibacters in one test and to also test insects from yellow sticky traps for the presence of the vector and pathogen.

On the postharvest front the industry is constantly faced with challenges regarding the future of certain postharvest chemicals. Project 123 is on a continuing basis investigating new potential alternative products in comparison with existing actives. Most of the products investigated were sanitation products, many of them based on copper as an anti-microbial. However, none of these were effective. Two phenolic based products also proved to be ineffective. Other products had moderate results that were variable. However, certain GRAS chemicals and a known food preservative gave very good and promising results. For the control of sour rot a UV-C treatment was evaluated that gave very good results. Project 1242 is studying the phytochemical relationship between the CBS pathogen, *Phyllosticta citricarpa*, and five different citrus types with varying degrees of CBS susceptibility. Analysis of the phytochemicals of the fruit rinds indicated that there are differences in phytochemicals between the different types and that this is correlated with the CBS susceptibility. It was furthermore seen that the phytochemistry in the rind changes with fruit development. Results from this study could assist in the development of better CBS management strategies. The use of OPP in the treatment of pallet bases was in project 1165 identified as the likely source of SOPP residues on fruit. In this project alternatives for pallet base treatment are investigated and also the fungi involved in causing fungal decay on pallet bases and bottom layer boxes. A number of fungal species were identified based on molecular identification while some very promising alternative treatments were found that will eliminate this problem. In 2019 the industry was notified that the MRL of imazalil in the EU might be lost in the near future. This threat initiated two projects, 1250 and 1251. Within 1250 five postharvest fungicides already allowed for use on citrus were evaluated further for *Penicillium digitatum* control and also to optimize their application in terms of concentration, solution temperature and pH. Some of the fungicides provided good curative control of the abovementioned pathogen while varying results were obtained for all in terms of sporulation inhibition. Further trials are being done to further optimize the aqueous applications of the most promising fungicides. On the other hand project 1251 is looking at the application of these fungicides in wax and the optimization of this application. Again, some of the fungicides performed very well in wax applications in terms of curative, protective and sporulation inhibition, while others were not as good. Some combinations of the fungicides provided even better results.

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 are softer, more environmentally friendly control options, disease epidemiology, fungicide application technology and better chemical spray programmes.

Project 762 and 1030 are focused on finding alternative control measures for soilborne pests and diseases and the long term effect of preplant soil fumigation. Results from 762 indicate that the preplant fumigation treatments are having the best effect on tree growth. In 1030 commercial biological control agents were evaluated for *in vitro* inhibition of soilborne pathogens. Promising results were obtained for the inhibition of oomycete pathogens while the inhibition of *Neocosmospora* spp. was not as good. Further evaluation will be done in glasshouse trials. In the Sunday's River Valley and Gamtoos River valley a decline of citrus trees has been observed for a number of years. Work in the last three years indicated that this is a decline syndrome and that many factors are contributing to this decline. It was named Valley Bushveld Decline and more work will be done to determine possible soil allelochemicals and non-culturable factors that might be involved in the decline. A rootstock trial will be planted in 2020 while in-arching on decline trees was done to determine if this could be a solution for existing decline orchards. Biological control of citrus replant pathogens is the topic of project 1215. A number of bacterial and *Trichoderma* strains were isolated from citrus tree roots and used in screening for *in vitro* inhibition of replant pathogens. Good results were obtained for the inhibition of oomycete pathogens but the inhibition of *Neocosmospora* spp. was not good. In this project tools for the detection of these pathogens were developed and validated successfully.

Citrus black spot (CBS) and Alternaria brown spot (ABS) are major pathogens negatively impacting on fruit quality and export. Several projects are therefore focused on the epidemiology and chemical management of these two diseases. In project 750 it was found that alternative chemicals and management programmes are available for ABS management while some promising new chemicals were identified in project 970 along with spray mixture additives that could replace mineral oil in strobilurin applications. In spray application work (Project 1132) the use of Tree Row Volume (TRV) as a basis for spray calibration was tested and promising results were obtained that showed this concept could work in citrus. Some further work on the biological efficacy of TRV based spray volumes will be done. CBS epidemiology is receiving much attention in the research project with five projects devoted to various aspects of the epidemiology. Project 1186 is studying the period of fruit susceptibility for CBS infection and found that fruit susceptibility declines with increasing fruit age. Results from project 1187 indicated that in netted orchards the likelihood of pycnidiospore infection periods is slightly higher than in open orchards while no difference in ascospore infection periods were seen. However, in the case of ABS and Botrytis, infection potential in netted orchards was higher compared to open orchards. Project 1223 has some practical implication for CBS management and is looking at the role of pruning debris as a source of ascospores. However, no results are available yet for this project. The infection parameters of CBS ascospores are the topic of project 1244. In a breakthrough, ascospores were produced in culture and this will allow infection studies with these spores. Results of this study will then be used in project 1238 for the improvement of the CBS prediction models in CRI-PhytRisk. In the latter project ABS and Botrytis prediction models were added to PhytRisk with the aim of improving chemical management of these two diseases. However, some refinement of specifically the Botrytis model is still needed and this is done in project 1236. This project is also looking at chemical control options for Botrytis as currently there are no chemical products registered for this disease. The last project on CBS is 1235 which is using high throughput sequencing (HTS) methods to study the population structure of CBS populations in South Africa and internationally. Findings from this study will further help develop better management strategies for CBS.

In conclusion the Disease Management portfolio is currently addressing the most important grower research needs. The availability of additional levy funding in future will enable an increased research scope and also allow for more proactive research.

PORTEFEULJE-OPSOMMING

Volhoubare produksie van hoë kwaliteit sitrusvrugte is afhanklik van suksesvolle bestuur van verskeie voor- en na-oes patogene. Ten einde op dit alles te fokus, is die Siektebestuur-portefeulje van CRI in verskillende navorsingsprogramme verdeel. Hierdie is voor-oes (Vrug- en Blaar- met SSV, en Grondgedraagde siektes), Ent-oordraagbare Siektes (*GTD*) en na-oes siektes programme. Spesifieke navorsingsbehoeftes van die industrie word in elke program aangespreek, terwyl pro-aktiewe navorsing gedoen word ten einde die industrie vir enige uitdagings rakende siektebestuur in die toekoms, voor te berei.

Binne die *GTD* program is groot vordering gemaak in die studie van CTV, en spesifiek identifisering van die identifikasie en karakterisering van enkel ras bronne. Dit het projek 1173 in staat gestel om die optrede van verskillende enkel rasse in verskeie sitrustipes te evalueer met die doel om die beste rasse vir kruisbeskerming te identifiseer. Dit het ook ten doel om rasse te vind wat geskik is vir aansteeklike kloonproduksie binne projek 1160. Die ontwikkeling van die aansteeklike kloon binne 1160 is ŉ nuwe manier om HLB en sy vektor te teiken. Dit word gedoen ter voorbereiding vir wanneer HLB en sy vektor in Suid-Afrika aankom. Binne projek 1160 word die meganisme van stam-uitputting / stam-insinking, veroorsaak deur CTV, bestudeer ten einde die siekte en sy bestuur beter te verstaan. Vroeë resultate van ŉ veldproef in projek 1074 toon dat gesertifiseerde plantmateriaal vanaf die Sitrusverbeteringskema (SVS) hortologies goed vaar (groei en boomgesondheid), in vergelyking met veldgesnyde materiaal. Viroïedes bly ŉ probleem in die industrie ten spyte van die gebruik van gesertifiseerde materiaal. Nuwe boorde wat met sensitiewe onderstamme geplant word kan spesifiek weens viroïedes swaar trek, en in projek 1155 word ŉ proef gedoen om die sensitiwiteit van nuwe en bestaande onderstamseleksies vir viroïed-infeksies vas te stel. Plantmateriaal word deur groeipunt-enting (*STG*) van alle *GTD* patogene skoon gemaak, en op hierdie stadium word suksesvolle uitwissing van patogene deur molekulêre en moeisame biologiese toetsing bepaal. In ŉ poging om hierdie evaluasie te bespoedig, ondersoek projek 1241 of hoë deurset DNA volgordebepaling [*high throughput sequencing* (*HTS*)] gebruik kan word om die *GTD* patogeenstatus van materiaal suksesvol te bepaal, en daardeur moontlik tot vinniger vrystelling van materiaal na die industrie kan lei. Weens die feit dat dit bevestig is dat die HLB patogeen,'*Candidatus*' Liberibacter asiaticus (Las), en sy vektor, *Diaphorina citri*, in Afrika teenwoordig is, is die vermoë om op te spoor noodsaaklik ten einde vroeë opsporing en beheermaatreëls moontlik te maak. Binne projek 1200 is dus ŉ *real-time* diagnostiese toets ontwikkel om die verskillende sitrus Liberibacters in een toets op te spoor en te identifiseer, en ook om insekte vanaf geel kleeflokvalle vir die teenwoordigheid van die vektor en patogeen te toets.

Op die na-oes front word die industrie konstant met uitdagings rakende die toekoms van sekere na-oes chemikalieë in die gesig gestaar. Projek 123 ondersoek op ŉ voortdurende basis nuwe potensiële alternatiewe produkte in vergelyking met bestaande aktiewe. Die meeste van die produkte wat ondersoek is, was sanitasie produkte, en baie gebaseer op koper as ŉ teen-mikrobiese produk. Geen van hierdie was egter effektief nie. Twee fenolies-gebaseerde produkte was ook oneffektief. Ander produkte het matige resultate gelewer wat variërend was. Sekere GRAS chemikalieë en ŉ bekende voedselpreserveermiddel het baie goeie en belowende resultate gelewer. ŉ UV-C behandeling is vir die beheer van suurvrot geëvalueer wat baie goeie resultate gelewer het. Projek 1242 bestudeer die fitochemiese verhouding tussen die SSV patogeen, *Phyllosticta citricarpa*, en vyf verskillende sitrustipes met verskillende grade van SSV vatbaarheid. Analise van die fitochemikalieë van die vrugskille het aangedui dat daar verskille in fitochemikalieë tussen die verskillende tipes was, en dat dit met SSV vatbaarheid korreleer. Daar is verder gesien dat die fitochemie in die skil met vrug-ontwikkeling verander. Resultate van hierdie studie kan tot die ontwikkeling van beter SSV bestuurstrategieë bydra. Die gebruik van OPP in die behandeling van palletbasisse is in projek 1165 geïdentifiseer as die moontlike bron van SOPP residue op vrugte. Alternatiewe vir palletbasis behandeling word in hierdie projek ondersoek, asook die swamme wat betrokke is om swamverval van palletbasisse en onderlaagbokse te veroorsaak. ’n Aantal swamspesies is op grond van molekulêre identifikasie geïdentifiseer, terwyl ’n paar baie belowende alternatiewe behandelings gevind is wat die probleem sal uitskakel. In 2019 is die industrie laat weet dat die MRL van imazalil in die EU in die nabye toekoms mag opgeskort word. Hierdie bedreiging het tot twee projekte aanleiding gegee, 1250 en 1251. Binne 1250 is vyf na-oes fungisiedes, wat alreeds vir gebruik op sitrus toegelaat word, verder vir *Penicillium digitatum* beheer geëvalueer, en ook om hul toediening te optimaliseer in terme van konsentrasie, oplossingstemperatuur en pH. Sommige van die fungisiedes het goeie uitwissende beheer van bogenoemde patogeen verskaf, terwyl variërende resultate vir almal in terme van sporulasie inhibisie verkry is. Verdere proewe word gedoen om die waterige toedienings van die mees belowende fungisiedes verder te optimaliseer. Aan die ander kant kyk projek 1251 na die toedien van hierdie fungisiedes in waks en die optimalisering van hierdie toediening. Sommige van die fungisiedes het weereens baie goed in wakstoedienings, in terme van uitwissende, beskermende en sporulasie inhibisie, gevaar, terwyl ander nie so goed was nie. Sommige kombinasies van die fungisiedes het selfs beter resultate gelewer.

Die navorsingsfokus in die voor-oes navorsingsprogram val op die bestuur van grondgedraagde plae en siektes, asook vrug- en blaarsiektes, insluitende Sitrus swartvlek (SSV). Aspekte wat ondersoek word, sluit in: sagter, meer omgewingsvriendelike beheer-opsies, siekte-epidemiologie, fungisied toedieningstegnologie en ’n beter chemiese spuitprogram.

Projekte 762 en 1030 fokus op die vind van alternatiewe beheermaatreëls vir grondgedraagde plae en siektes en die langtermyn-effek van voor-plant grondberoking. Resultate van 762 dui daarop dat die voor-plant berokingsbehandelings die beste effek op boomgroei het. Kommersiële biologiese beheer-agente is in 1030 vir *in vitro* inhibisie van grondgedraagde patogene geëvalueer. Belowende resultate is vir die inhibisie van oömiseet-patogene verkry, terwyl die inhibisie van *Neocosmospora* spp. nie so goed was nie. Verdere evaluasie sal in glashuisproewe gedoen word. In die Sondags- en Gamtoosriviervallei is ’n agteruitgang van sitrusbome vir ’n aantal jare waargeneem. Werk die laaste drie jaar het aangedui dat dit ’n agteruitgang-sindroom is, en dat baie faktore tot hierdie agteruitgang bydra. Dit is *Valley Bushveld Decline* genoem en verdere werk sal gedoen word om moontlike grond allelochemikalieë en nie-verbouingsfaktore te bepaal wat in die agteruitgang betrokke kan wees. ’n Onderstamproef sal in 2020 geplant word, terwyl brug-enting op agteruitgangbome gedoen is om vas te stel of dit ’n oplossing vir bestaande agteruitgangboorde kan wees. Biologiese beheer van sitrusherplantpatogene is die onderwerp van projek 1215. ’n Aantal bakteriese en *Trichoderma* isolate is vanaf sitrusboomwortels geïsoleer en in toetsing vir *in vitro* inhibisie van herplantpatogene gebruik. Goeie resultate is vir die inhibisie van oömiseet-patogene verkry, maar die inhibisie van *Neocosmospora* spp. was nie goed nie. In hierdie projek is hulpmiddels vir die opsporing van hierdie patogene ontwikkel en suksesvol gevalideer.

Sitrus swartvlek (SSV) en Alternaria bruinvlek (ABV) is belangrike patogene met ’n negatiewe impak op vrugkwaliteit en uitvoer. Verskeie projekte fokus dus op die epidemiologie en chemiese bestuur van hierdie twee siektes. In projek 750 is gevind dat alternatiewe chemikalieë en bestuursprogramme vir ABV bestuur beskikbaar is, terwyl ’n paar belowende nuwe chemikalieë in projek 970 geïdentifiseer is, tesame met spuitmengsel bymiddels wat minerale olie in strobilurien-toedienings kan vervang. In spuittoedieningswerk (Projek 1132) is die gebruik van Boomry Volume (*TRV*) as basis vir spuitkalibrasie getoets, en belowende resultate is verkry wat aandui dat hierdie konsep in sitrus kan werk. Verdere werk op die biologiese effektiwiteit van *TRV*-gebaseerde spuitvolumes, gaan gedoen word. SSV epidemiologie kry baie aandag in die navorsingsprojek, met vyf projekte wat aan die verskeie aspekte van die epidemiologie gewy word. Projek 1186 bestudeer die periode van vrugvatbaarheid vir SSV infeksie, en het gevind dat vrugvatbaarheid met toenemende vrugouderdom afneem. Resultate van projek 1187 het aangedui dat die moontlikheid van piknidiospoor infeksieperiodes effens hoër is in boorde onder skadunet, as in oop boorde, terwyl geen verskil in askospoor infeksieperiodes gesien is nie. In boorde onder skadunet was die ABV en Botrytis infeksiepotensiaal egter hoër in vergelyking met oop boorde. Projek 1223 het praktiese implikasie vir SSV bestuur en kyk na die rol van snoeisels as bron van askospore. Geen resultate is egter al beskikbaar vir die projek nie. Die infeksieparameters van SSV askospore is die onderwerp van projek 1244. In ’n deurbraak is askospore in kultuur geproduseer, en dit sal infeksiestudies met hierdie spore moontlik maak. Resultate van hierdie studie sal dan in projek 1238 gebruik word vir die verbetering van die SSV voorspellingsmodelle in

CRI-PhytRisk. In laasgenoemde projek is ABV en Botrytis voorspellingsmodelle tot PhytRisk gevoeg met die doel om chemiese bestuur van hierdie twee siektes te verbeter. Verfyning van spesifiek die Botrytis model word egter nog benodig, en dit word in projek 1236 gedoen. Hierdie projek kyk ook na chemiese beheermoontlikhede vir Botrytis aangesien daar tans geen chemiese produkte vir hierdie siekte geregistreer is nie. Die laaste projek op SSV is 1235 wat *high throughput sequencing* (*HTS*) metodes gebruik om die populasiestruktuur van SSV populasies in Suid-Afrika en internasionaal te bestudeer. Bevindinge vanuit hierdie studie sal verder help om beter bestuurstrategieë vir SSV te ontwikkel.

Ten slotte, die Siektebestuur-portefeulje spreek tans die belangrikste navorsingsbehoeftes van produsente aan. Die beskikbaarheid van addisionele befondsing in die toekoms sal uitbreiding van die navorsingsomvang moontlik maak, en ook meer pro-aktiewe navorsing op probleme wat in die toekoms voorsien word, insluit.

4.2 PROGRAMME: GRAFT TRANSMISSIBLE DISEASES

Programme coordinator: G. Cook (CRI)

4.2.1 Programme summary

Cross-protection is a management strategy applied to mitigate the damaging effects of citrus tristeza virus (CTV). An ongoing 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. Field trials evaluating performance of single-strain CTV sources in various citrus types are continuing (Project 1173). These trials are done with the aim of evaluating them as potential cross-protection sources, but also to test the suitability of isolates for application in CTV infectious clone construction (Project 1160). The development of an infectious CTV clone to combat HLB is a novel approach whereby CTV is used as a vehicle to systemically deliver antimicrobial peptides or RNA ‘signals’ to arrest the insect vector or the Liberibacter pathogen of HLB. The construction of a full-length clone was completed and the challenge is now to achieve infectivity. In the interim, RNAi gene targets in the insect vectors were identified and a test strategy is in preparation. Investigation to determine the stem pitting determinants of CTV is also underway in project 1160.

Early results of a field trial comparing the horticultural performance of field-cut propagation material to budwood supplied by the Citrus Improvement Scheme (CIS) show that better growth and tree health is achieved using CIS propagation material (Project 1074). Despite the use of certified budwood, viroids remain problematic in the industry due to their mechanical transmissibility. Newly planted orchards are especially vulnerable if susceptible rootstocks are used. To better understand which commercial and potentially important rootstock selections are sensitive, a field trial was prepared which was planted this season (Project 1155).

Shoot-tip grafting is the technique used to establish all new citrus accessions entering the CIS and is used to render the material pathogen free. Confirmation of the successful elimination of pathogens is done using both molecular and biological screening. Project 1241 is aimed to investigate the use of high throughput sequencing (HTS) based detection to potentially fast-track diagnostic processes in the STG pipeline and enable quicker release of cultivars to the industry.

The confirmed presence of both ‘*Candidatus*’ Liberibacter asiaticus (Las) and *Diaphorina citri* on the African continent necessitates preparation for an incursion event. Detection capabilities are vital for early detection and control interventions. A real-time diagnostic assay was developed to detect and differentiate citrus Liberibacters in a single assay and will be further validated on insect specimens from sticky traps (Project 1200).

Programopsomming

Kruisbeskerming is een van die bestuurstrategieë wat toegepas word om die skadelike effekte van sitrus tristeza-virus (CTV) te verminder. Navorsing, binne die GTD-program (Graft Transmissible Diseases), is daarop gerig om die effek van CTV-rasse te ondersoek en om te bepaal watter 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 en veldproewe van verkillende sitrustiepes word gedoen om die prestasie van verskillende enkel CTV rasse te toets (projek 1173). Hierdie proewe word gedoen met die doel om rasse as moontlike kruisbeskermingsbronne te evalueer, maar ook om die geskiktheid van isolate vir toediening in CTV- kloonkonstruksie te toets (Projek 1160). Hierdie is ‘n unieke benadering om ‘n CTV kloon te gebruik om antimikrobiese peptiedes of RNA ‘seine’ in die plant te lewer, om die insekvektor of die Liberibacter-patogeen van HLB te beheer. Die konstruksie van 'n vollengte kloon is voltooi en die uitdaging is nou om infektiwiteit te bewerkstellig. In die tussentyd is RNAi-gene-teikens in die insekvektore geïdentifiseer en 'n toetsstrategie is in voorbereiding. In projek 1160 word ondersoek ook ingestel na die bepaling van die stamgleufdeterminante van CTV.

Vroeë resultate van 'n veldproef waarin die tuinbouprestasies van veldgesnyde voortplantingsmateriaal vergelyk word met voortplantingsmateriaal verskaf deur die Sitrusverbeteringsskema (SVS), toon dat beter groei en boomgesondheid verkry word van CIS-voortplantingsmateriaal (Projek 1074). Ten spyte van die gebruik van gesertifiseerde enthout, is viroïede steeds problematies in die industrie vanweë hul meganiese oordraagbaarheid. Nuut aangeplante boorde is veral kwesbaar as vatbare onderstamme gebruik word. Om beter te verstaan watter kommersiële en potensieel belangrike onderstokkeuse sensitief is, is 'n veldproef daargestel wat hierdie seisoen geplant is (Projek 1155).

Groeipuntenting (GPE) word gebruik om sitrus materiaal te vrywaar van ent-oordraagbare patogene voor vrystelling van nuwe kultivars. Bevestiging van die suksesvolle eliminasie van patogene word gedoen met behulp van beide molekulêre en biologiese tegnieke. Projek 1241 is daarop gemik om die gebruik van diagnostiek, gebaseer op hoë-deurset volgordebepaling gebaseerde opsporing (HTS), te ondersoek om die diagnostiese prosesse van die GPE-pyplyn te bespoedig en vinniger vrystelling van kultivars aan die bedryf te bewerkstellig.

Die bevestigde teenwoordigheid van beide 'Candidatus' Liberibacter asiaticus (Las) en Diaphorina citri op die Afrika-kontinent, noodsaak voorbereiding vir die moontlike inbeweeg. Diagnostiese vermoëns is noodsaaklik vir vroeë opsporing en beheer ingryping. 'n diagnostiese toets is ontwikkel om sitrus-Liberibakters op te spoor en te onderskei in 'n enkele toets en sal verder bevestig word op insekmonsters vanaf klewerige lokvalle (Project 1200).

4.2.2. **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. De Bruyn and C. Steyn (CRI)

Summary

Sectors in the citrus industry claim 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 assertion is investigated in a trial using two navel 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, production and fruit characteristics of CIS supplied material with that of field-cut and viroid infected material. Budwood was collected from original field sources of the cultivars, which contained various populations of CTV strains and citrus viroids. These were budded to ‘Swingle’ citrumelo, ‘Carrizo’ citrange and ‘C35’ citrange rootstocks. The same was done for budwood obtained from the CFB and for CFB budwood to which HSVd-IIa was additionally inoculated. A field trial was established at Burgersfort in 2016. After four years, significant differences in tree growth were observed between treatments. Reduced canopy volumes were associated with field-cut material of all three cultivars on all rootstocks. Trees made from CFB supplied budwood were consistently larger in all treatments. The first fruit were harvested from the trial, but yields were low. Only the Valencia cultivar yielded sufficient fruit for internal quality determination and differences in maturity status were observed between the different treatments. These are, however, early trial results and future harvests will determine if these differences persist.

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 geïnokuleer 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, produksie en vrugeienskappe van veld-gesnyde materiaal, wat verskeie CTV-rasse en sitrusviroïede bevat, te vergelyk met dié van SVS materiaal asook met SVS materiaal waarop HSVd-IIa addisioneel geïnokuleer is. Okuleerhout is op 'Swingle' citrumelo, 'Carrizo' citrange en 'C35' citrange onderstamme ge-okuleer en 'n veldproef is in 2016 op Burgersfort geplant. Na vier jaar is beduidende verskille in boomgroei waargeneem. Verminderede boom volumes is geassosieer met veld-gesnyde materiaal van al drie kultivars op alle onderstamme. Bome van SVS materiaal was deurgans groter in alle behandelings. Die eerste vrugte is geoes vanaf die proef, maar die opbrengs was laag. Slegs die Valencia kultivar het voldoende vrugte gelewer vir interne kwaliteit bepaling. Verskille in vrug rypwording is tussen die verskillende bronne waargeneem, maar toekomstige oeste sal toon of hierdie verskille voortduur.

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

Project 1155 (2016/7 – 2025/6) by G. Cook, J.H.J. Breytenbach, C. Steyn, R. De Bruyn 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 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 USA. 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 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 and transmission confirmation tests will be repeated. The trial was planted in October 2019 at Crocodile Valley, Nelspruit and the trees are well-established.

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 plae insluit. Viroïede is oordraagbare entiteite wat verskeie simptome kan veroorsaak op sensitiewe bo- en onderstamme. Hulle is selde problematies as siektevrye, geserifiseerde 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 siektetoestande, 'Exocortis' en 'Cachexia', kan viroïede ook simptome soos baskraak en verdwerging 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 is voorberei om die sensitiwiteit van hierdie nuwer kommersiële of potensieel kommersiële onderstamme teen 'citrus dwarfing viroid' en 'hop stunt viroid-IIa' te toets. 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. Elke proefplant

sal weer getoets word om viroïed oordraging te bevestiging. Die proef is in Oktober 2019 by 'Crocodile Valley' plaas in Nelspruit geplant en die bome het goed gevestig.

4.2.4 **PROGRESS REPORT: Application of CTV infectious clones to combat HLB.**
Project 1160 (2016/17 – 2020/2021) by R Bester (CRI), D Aldrich (SU), G. Cook, Kobus Breytenbach (CRI), Prof Johan T Burger (SU), Prof William O Dawson (University of Florida, USA), H.J. Maree (CRI/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 silencing targets (payloads) that would form part of a management strategy to contain HLB and limit its impact. CTV infectious clones of strain T36 were obtained from 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 the Dawson clone from a T36 strain into the local RB (asymptomatic) strain. The construction of a full-length clone based on the CTV-RB strain was completed. However, no systemic infection in *N. benthamiana* could be observed after multiple infiltrations. Plasmid DNA was sent for low level high-throughput sequencing to detect the presence of potential mutations that might be affecting systemic infection. Two mutations in critical open reading frames were identified that likely influence virus infectivity and spread. These mutations will be repaired by replacing fragments of the RB strain. A dual reporter infectious clone containing an additional PDS silencing cassette was also constructed and evaluated in citrus. The clone proved to be an effective expression and silencing vector. This clone will be used as a test platform to screen different 'payloads' while the RB clone is under construction. Four RNAi gene targets in *Diaphorina citri* were identified from literature and homologues for *Trioza erytreae* were identified using the HTS data generated from *Trioza erytreae* RNA. CTV vectors will be constructed to contain payloads directed towards both *Trioza erytreae* and *Diaphorina citri* for each gene target. CTV vectors targeting all 4 genes per insect will also be constructed. To try and limit the effect of Covid-19 on progress, the assembly strategy was adapted to include the use of gblocks that we believed will save time during the assembly phase of the various vectors.

Additionally, the project objectives include the identification of CTV-induced stem pitting determinants. The full complement of fourteen infectious clone mutants proposed for studying CTV-induced stem pitting were successfully assembled. Four additional hybrid clones were also constructed that serve as follow-up experiments to the GFMS12 differential stem pitting work from Project 1100. The infectivity of all CTV mutants was confirmed in *N. benthamiana* and infected material was sampled and used for infiltration of Mexican lime plants. Four rounds of citrus infiltration were attempted following the first successful citrus infiltration in April 2019. A full-panel RNA extraction and PCR screening was performed to validate CTV infection status and clone composition in all infiltrated greenhouse citrus plants. Outstanding clones will be re-infiltrated as soon as good systemic spread is observed in *N. benthamiana*, and the facilities at the University of Cape Town are available again. We conducted a trial study to evaluate scanning electron microscopy to characterise plant tissues impacted by stem pitting, which generated promising images. The sectioning procedure will be optimized to allow clear, high resolution study of phloem and xylem tissue in pitted areas of citrus plants, and to combine it with fluorescence microscopy to determine GFP-tagged virus localisation in these areas.

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 uiteindelike inval van Asiatiese vergroening (HLB). Die doel van hierdie projek is om 'n paneel van CTV infektiewe klone te vestig met 'n verskeidenheid 'silencing' teikens (payloads) wat sal deel uitmaak van 'n beheerstrategie om die impak van HLB te beperk. Infektiewe klone van die T36 ras is verkry van Prof W.O. Dawson (Florida University). Protokolle vir die infiltrasie van hierdie klone in *N. benthamiana* is geoptimiseer. Rekombinante viruspartikels

is daarna gesuiwer vanuit sistemies-geinfekteerde *N. benthamiana* plante deur middel van ultra-sentrifugasie (by die Universiteit van Kaapstad). Sitrussaailinge is intussen suksesvol besmet met die deels-gesuiwerde viruspartikels. ŉ Strategie is ontwerp om die T36-klone om te skakel na ŉ plaaslike RB (asimptomaties) ras. Die konstruksie van ŉ vollengte kloon gebaseer op die CTV-RB ras is wel voltooi, maar geen sistemiese infeksie in *N. benthamiana* kon na verskeie infiltrasies waargeneem word nie. Plasmied DNA is gestuur vir 'n lae-vlak hoë-deurvloei-volgorde bepaling (HTS) om die teenwoordigheid van potensiële mutasies wat sistemiese verspreiding kan beïnvloed, te evalueer. Twee mutasies in kritieke leesrame is geïdentifiseer wat virus infektiwiteit en verspreiding kan beïnvloed. Hierdie mutasies sal herstel word deur fragmente van die RB-genotipe te vervang. Daar is ook 'n dubbel-rapporteerder infektiewe kloon gebou, gebasseer op die T36-kloon (Dawson), wat addisioneel 'n PDS 'silencing cassette' bevat. Hierdie kloon is reeds geëvalueer in sitrus en het bewys dat die kloon 'n doeltreffende uitdrukking en 'silencing' vector is. Hierdie kloon sal dien as 'n toetsplatform om die verskillende 'payloads' te toets terwyl die RB-kloon gefinaliseer word. Vier RNAi-teikens in *Diaphorina citri* is uit die literatuur gekies en homoloë vir *Trioza erytreae* is geïdentifiseer met behulp van die HTS-data wat gegenereer is uit *Trioza erytreae* RNA. CTV-vektore met 'payloads' wat na beide Trioza erytreae en *Diaphorina citri* gerig is, word beplan. CTV-vektore wat op al 4 die gene per insek gerig is, sal ook gemaak word. Om die effek van Covid-19 op die vordering te probeer beperk, is die strategie aangepas om die gebruik van 'gblocks' in te sluit wat tyd met die samestellingsfase van die verskillende vektore sal bespaar.

Verder sluit hierdie projekdoelwitte die bepaling van CTV-geinduseerde stamgleuf determinante in. Die volle komplement van viertien mutante CTV-infektiewe-klone wat aanvanklik voorgestel is vir die studie van CTV-geinduseerde stamgleuf is suksevol gebou. Vier bykomstige hibriedklone is instussen ook gebou en dien as addisionele eksperimentele werk tot die GFMS12 diffierensiele stamgleuf-induskie bevindinge vanuit Project 1100. Die infektiwiteit van alle CTV-klone van hierdie studie is bevestig in *N. benthamiana* en besmette materiaal van alle klone is intussen gebruik vir die infiltrasie van "Mexican lime" sitrusplante. Vier rondtes van sitrusinfiltrasies is reeds uitgevoer sedert ons eerste suksesvolle infiltrasie van CTV-klone tot in sitrus in April 2019. ŉ Volledige RNA-ekstraksie en PCR-toetsing van alle geinfiltreerde sitrus is onalngs uitgevoer om infeksiestatus en kloonsamestelling vas te stel. Die infiltrasie van enige uitstaande CTV-klone sal uitgevoer word sodra daar goeie sistemiese verspreiding in *N. benthamiana* waargeneem word, en die fasiliteite by die Universiteit van Kaapstad weer beskikbaar gestel word. Ons het ook ŉ grondslagstudie geloots om die nut van "scanning electron microscopy" te toets om die effek van CTV-geinduseerde stamgleuf op sitrus stamweefstel te bestudeer. Hierdie tegnologie het baie belowende beelde gelewer. Die volgende stap in hierdie proses is om die prosedure om dunsnitte van sitrus te genereer te optimiseer om sodoende die hoë-resolusie studie van die betrokke stamweefsels toe te laat, en dit te kombineer met fluoressensie-mikroskopie, vir die bepaling van virus-lokalisering van GFP-gekoppelde CTV klone in hierdie areas.

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. De Bruyn (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. Yield differences between treatments were observed for the first harvest from the two soft citrus cultivars, but yields were low at this early stage. The first harvest for the navels is expected in the 2020 season. Tree canopy volumes were determined for the second year. Significant differences were observed between treatments of the Clementine and also for one navel cultivar. The trees are still young and it is too soon to draw any conclusions regarding the impact of the different strains.

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 ʼn Mandaryn is in Limpopo geplant. Die Kakamas-proef is beëindig aangesien talle proefbome gevrek het weens 'n tekort aan water kort na plant. Opbrengsverskille tussen behandelings van die sagte sitrus kultivars is waargeneem vir die eerste oes van die twee sagte sitrus kultivars, maar die oes was nog klein. Die eeste oes van die navels word in in 2020 seisoen verwag. Boomvolumes van die Navels-, Clementine- en Mandaryn-proefbome is bepaal, twee jaar na plant. Beduidende verskille is waargeneem tussen behandelings van die Clementine asook een navel kultivar. Die bome is nog te jonk om enige afleidings te kan maak aangaande die impak van die CTV rasse.

4.2.6 PROGRESS REPORT: Validation of primer regions used for the differentiation of Asian HLB, African greening and its subspecies.

Project 1200 (2018/19 –2019/20) by R Roberts (ARC-PHP) and G. Cook (CRI)

Summary

The presence on the African continent of both *Diaphorina citri* and *‘Candidatus* Liberibacter asiaticus’ (Las), the bacterium associated with Huanglongbing (HLB), poses an imminent threat to the southern African citrus industry. In preparation for incursion events, it was necessary to develop diagnostic assays to detect and differentiate Las from ‘*Ca*. L. africanus’ (Laf), the bacterium associated with African Greening. A real-time diagnostic assay was developed in the initial phase of this project which can detect citrus Liberibacters and also differentiate Las from Laf in a single assay. Further verification is however required to validate the assay on *Trioza erytreae* and *Diaphorina citri* specimens from sticky traps. An optimal DNA extraction method is required which can yield high quality DNA and which is time and cost efficient. A *T. erytreae* colony is ideally necessary for this task. However, the past season was extremely hot and triozids were not found in orchards for colony establishment. A few specimens, previously collected and stored, were used to test three extraction methods, but further validation is required. The project will be extended for another year in order to achieve the outstanding objectives.

Opsomming

Die teenwoordigheid op die Afrika-kontinent van beide *Diaphorina citri* en '*Candidatus* Liberibacter asiaticus' (Las), die bakterie wat met Huanglongbing (HLB) geassosieer word, hou 'n dreigende gevaar in vir die Suider-Afrikaanse sitrusbedryf. Ter voorbereiding op invallingsgebeurtenisse, was dit nodig om diagnostiese toetse te ontwikkel om Las op te spoor en te onderskei van ‘*Ca*. L. africanus’ (Laf), die bakterie wat met African Greening geassosieer word, 'n Diagnostiese toets is ontwikkel in die aanvangsfase van hierdie projek wat sitrus-Liberibakters kan opspoor en ook Las van Laf kan onderskei in 'n enkele toets. Verdere verifiëring is egter nodig om die tegniek te toets op *Trioza erytreae* en *Diaphorina citri*-monsters gevang op klewerige lokvalle. 'n Optimale DNA-ekstraksiemetode is nodig wat DNA van hoë gehalte kan lewer en wat tyd en koste-effektief is. 'n *T.erytreae*-kolonie word ideal benodig vir hierdie taak. Die afgelope seisoen was egter buitengewoon warm en bladvloeie was nie in die boorde gevind vir die vestiging van ‘n kolonie nie. 'n Paar monsters, wat voorheen versamel en geberg is, is gebruik om drie verskillende ekstraksiemetodes te toets, maar verdere validering is nog steeds nodig. Die projek sal nog 'n jaar verleng word ten einde die uitstaande doelstellings te bereik.

4.2.7 **PROGRESS REPORT: Application of high-throughput sequencing (HTS) for routine virus and viroid detection in high value accessions.**
Project 1241 (2019/20 – 2021/22) by R. Bester (CRI/SU), G. Cook, K. Breytenbach, C. Steyn, R. De Bruyn, P. Fourie, H. J. Maree (CRI)

Summary

The primary aim of this project is to validate high throughput sequencing (HTS) based detection of known viruses and viroids of citrus for routine detection and to open up the possibility to fast-track release of accessions in the shoot-tip grafting (STG) pipeline without additional risk. Plant material infected with a range of viruses (positive and negative stranded viruses) and viroids was established. These plants were also inoculated with *Candidatus* Liberibacter africanus (Laf) to determine if this technology and approach could be extended to detect Liberibacters. Total RNA was extracted from three representative samples of each plant (4 plants) using two different methods (CTAB vs Zymo Research RNA kit) and send for HTS (Macrogen, Korea). Data were received 30 March 2020. Data QC analysis showed that 98% of the data has a quality score above Q30 and 95% of the data has a quality score above Q20. One representative sample of each plant was also sent to the Central analytical facility (CAF) in Stellenbosch for HTS on an Ion torrent platform. The complete dataset will allow for the evaluation of the biological and technical variation or variation associated with different RNA extraction methods and different sequencing platforms. The bioinformatic detection pipeline is currently being built. The project will also include a direct comparison between an HTS-based detection assay and the conventional methods as applied in the Citrus Improvement Scheme (CIS). Seven accessions were selected for this comparison. Total RNA was extracted from these samples using two different methods (CTAB vs Zymo Research RNA kit) and send for HTS (Macrogen). Data were received 30 March 2020. One of these samples was selected as source material and was used to deliberately infect healthy seedlings. The infection status of these seedlings will be monitored for seven months at four or five time-points using both HTS and RT-PCR to test the sensitivity of both approaches to detect viruses and viroids. Additionally, one of the objectives of this project is to investigate diseases with unknown aetiology (such as the Psorosis-like diseases) using HTS. Psorosis-like symptomatic plants were identified and subjected to HTS. Citrus virus A (CiVA) was identified in these samples, however the association of the fruit rind symptom and the presence of CiVA requires further investigation. A new detection assay was also developed for citrus coguviruses. This assay is currently being evaluated for sensitivity to detect citrus virus A (CiVA). Positive controls for citrus concave gum-associated virus (CCGaV) were requested from international collaborators. The complete genome of a South African variant of CiVA was assembled using the HTS data. This draft sequence was validated using Sanger sequencing and will be published.

Opsomming

Die hoofdoel van die projek is om die gebruik van hoë-deurset volgordebepaling gebaseerde opsporing (HTS) van virusse en viroïede in sitrus as ŉ roetine toets te valideer. Die metode kan moontlik die vrystelling van plantmateriaal in die Groeipuntenting (GPE) pyplyn bespoedig sonder addisonele risiko. Plant materiaal, geïnfekteer met ŉ verskeidenheid virusse (positiewe en negatiewe string virusse) en viroïede, is voorberei. Hierdie plante is ook geïnfekteer met *Candidatus liberibacter africanus* (Laf) om te bepaal of hierdie tegnologie en metode kombinasie wel Laf ook kan opspoor. Totale RNA is uit drie verteenwoordigende monsters van elke plant (4 plante) met behulp van twee verskillende metodes (CTAB vs Zymo Research RNA kit) uitgehaal en vir HTS (Macrogen, Korea) gestuur. Data is op 30 Maart 2020 ontvang. Die kwaliteitsontleding van data het getoon dat 98% van die data 'n kwaliteitspunt bo Q30 het en 95% van die data 'n gehaltepunt bo Q20 het. Een verteenwoordigende monster van elke plant is ook na die Sentrale analitiese fasiliteit (CAF) in Stellenbosch gestuur vir HTS op die Ion torrent platform. Die volledige datastel maak voorsiening vir die evaluering van die potensiële variasie wat verband hou met verskillende RNA-ekstraksiemetodes, verskillende volgorde bepalingsplatforms, asook moontlike biologiese en tegniese variasie. Die bioinformatiese opspoorpypleiding word tans gebou. Die projek bevat ook 'n direkte vergelyking tussen 'n HTS-gebaseerde opsporingstoets en die konvensionele metodes soos toegepas in die sitrusverbeteringskema (CIS). Sewe monsters is vir hierdie vergelyking gekies. Die totale RNA is met behulp van twee verskillende metodes (CTAB vs Zymo Research RNA kit) uit hierdie monsters onttrek en vir HTS (Macrogen, Korea) gestuur. Data is op 30 Maart 2020 ontvang. Een van hierdie monsters is as bronmateriaal geselekteer en is gebruik om gesonde saailinge doelbewus te

besmet. Die besmettingstatus van hierdie saailinge sal vir sewe maande op vyf tydpunte gemonitor word, met beide HTS en RT-PCR om die sensitiwiteit van albei benaderings om virusse en viroïede op te spoor, te toets. Addisioneel is een van die doelstellings van hierdie projek om siektes met onbekende etiologie (soos die Psorose-agtige siektes) met behulp van HTS te ondersoek. Psorose-agtige simptomatiese plante is geïdentifiseer en aan HTS onderwerp. Sitrus virus A (CiVA) is in hierdie monsters geïdentifiseer, maar die assosiasie van die vrugteskilsimptoom en die teenwoordigheid van CiVA sal verder ondersoek moet word. 'n Nuwe opsporingstoets is ook ontwikkel vir sitrus coguvirusse. Hierdie toets word tans geëvalueer vir sensitiwiteit om sitrusvirus A (CiVA) op te spoor. Positiewe kontroles vir sitrus-konkaaf gom-geassosieerde virus (CCGaV) is van internasionale medewerkers aangevra. Die volledige genoom van 'n Suid-Afrikaanse variant van CiVA is ook saamgestel met behulp van HTS-data. Hierdie konsepvolgorde was gevalideer met behulp van Sanger-volgorde bepaling en gaan gepubliseer word in ‘n eweknie-beoordeelde vaktydskrif.

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 Black spot, 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, commercially available biocontrol products were evaluated *in vitro* for their ability to inhibit citrus replant pathogens. Promising results were obtained with inhibition of oomycete pathogens. However, inhibition of *Neocosmospora* spp. was not as good. In further work biocontrol agents will also be evaluated in the glasshouse for control of *Phytophthora nicotianae*. In a new field trial, a biocontrol agent for the control of citrus nematode will be evaluated.

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. A pathogen complex was identified with all pathogens being known as stress pathogens. Revision of research findings led to the disease being named Valley Bushveld Decline as it had the characteristics of a decline disease. Further work will entail identifying different factors associated with the decline. In-arching of decline trees, with less sensitive rootstocks was done to determine if decline orchards can be saved in this manner. A rootstock trial will also be planted in spring of 2020.

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 and validated. 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 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. Primers were also successfully developed and validated for the detection and quantification of these 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 (≥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. However, some phytotoxicity was observed with this application. Addition of the adjuvants Nufilm-17 and Entreé as replacement for mineral oil in strobilurin applications, also provided excellent CBS control.

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, a trial was done to evaluate tree-row-volume (TRV) as a basis for spray calibration. Spray deposition results showed that reducing volumes based on TRV gave similar deposition values on fruit and leaves compared to higher volume applications. It was furthermore seen that air volume during application had no significant effect on spray deposition. Further trials will be done on other citrus types and to determine if using TRV for spray calibration will be biologically effective.

In the area of CBS epidemiology, several projects are being conducted. In project 1187 it was observed that in netted orchards, more pycnidiospore infection periods are predicted compared to open orchards. However, in terms of ascospore infection periods, no difference was observed between netted and open orchards. In the case of ABS and Botrytis, higher infections were predicted for netted orchards compared to open orchards. Project 1186 focusses on the period of CBS fruit susceptibility. Results from the first season indicated that fruit susceptibility does decline with increasing fruit age and that the fruit protection periods used in control programmes are valid. Results from the first season are being confirmed in a second season while the project was expanded to include CBS susceptibility of mandarin fruit. This project links to project 1242 where it was found that the rind phytochemistry of five citrus types, all differing in CBS susceptibility, changed with fruit maturity. It was also seen that types with different levels of susceptibility, had different rind phytochemical composition. These findings could help explain the interaction between CBS and different fruit types and their level of susceptibility. Project 1223 is aiming to determine how fast chopped pruning debris composts and if it plays a major role as a CBS inoculum source. From a lemon orchard in Hoedspruit, chipped and unchipped pruning debris were collected and analysed for the presence of CBS ascospores. However, the results from the first season are still outstanding. Project 1244 ties in with 1223 and is focusing on the infection parameters of CBS pycnidio and ascospores. This is with the aim of improving CBS prediction models. In a breakthrough, CBS ascospores were produced in culture and this allows further studies on the infection parameters of this important infection source. Results from project 1244 will eventually feed into project 1238 that is aimed at improving the prediction model in CRI-PhytRisk. Apart from improving the CBS prediction model, CRI-PhytRisk was also expanded to include ABS and Botrytis prediction models. However, these two models still need some refinement for South African conditions. To improve our understanding of the CBS pathogen's population structure, project 1235 is looking at this aspect. Using high throughput sequencing (HTS) the clonal nature of the *Phyllosticta citricarpa* population in the USA was confirmed. This tool will now also be used to characterise the South African population in the same manner.

The last project in the programme is 1236 that is investigating the epidemiology of Botrytis on lemons. Work from the first year indicated that lemon blossoms are the primary sources of inoculum in lemon orchards. It was furthermore seen that fludioxonil and fenhexamid provided good control of *Botrytis cinerea in vitro*. Further work will entail correlating the flower infections with climatic data to validate and refine the Botrytis prediction model already incorporated in CRI-PhytRisk.

Programopsomming

Die navorsingsfokus binne die voor-oes siekteprogram val op grondgedraagde siektes van sitrus, en vrug- en blaarsiektes, insluitende Sitrus Swartvlek, van sitrus. Die navorsing fokus op die vind van alternatiewe, sagter bestuursmoontlikhede, studies oor die epidemiologie van die verskillende patogene, en optimalisering van die beheer van hierdie patogene deur beter toedieningstegnologie of beter chemiese beheerprogramme.

Projekte 762 en 1030 het spesifiek ten doel om alternatiewe wyses van beheer vir *Phytophthora* en sitrus-aalwurm te vind. Data word sedert 2011 in projek 762 aangeteken. Dit het duidelik geword dat die verskillende voor-plant grondberokingbehandelings, spesifiek die 1,3 dichloropropeen en metam-natrium behandelings, veroorsaak het dat bome in hierdie behandelings langer met dikker stamme was, in vergelyking met die ander behandelings en die onbehandelde kontrole. Binne projek 1030 is kommersieel-beskikbare biobeheer produkte *in vitro* geëvalueer vir hul vermoë om sitrusherplantpatogene te inhibeer. Belowende resultate is met inhibisie van oömiseet-patogene verkry. Inhibisie van *Neocosmospora* spp. was egter nie so goed nie. In verdere werk sal biobeheer-agente ook in die glashuis vir beheer van *Phytophthora nicotianae* geëvalueer word. In ʼn nuwe veldproef, sal ʼn biobeheer-agent vir die beheer van sitrus-aalwurm geëvalueer word.

ʼn Agteruitgang en afsterwe van sitrusbome is vir ʼn aantal jare vanaf die Gamtoos- en Sondagsriviervallei aangeteken. Opnames van siek bome is as deel van projek 1068 in die Gamtoos- en Sondagsriviervallei produksie-areas gedoen. ʼn Patogeenkompleks is geïdentifiseer met alle patogene bekend as strespatogene. Hersiening van navorsingsbevindinge het gelei tot die benaming van die siekte as *Valley Bushveld Decline* aangesien dit die kenmerke van ʼn agteruitgangsiekte het. Verdere werk sal die identifisering van die verskillende faktore wat met die agteruitgang geassosieer word, behels. Brug-enting van agteruitgangbome met minder sensitiewe onderstamme, is gedoen om vas te stel of agteruitgangboorde op dié manier gered kan word. ʼn Onderstamproef sal in die lente van 2020 geplant word.

Projek 1215 is ʼn nuwe projek wat in April 2019 begin het. Goeie vordering is gemaak in die vind van potensiële biologiese beheer-agente vir grondgedraagde patogene. Komponente van waardevolle hulpmiddels wat gebruik kan word in die bestudering van die interaksies van die verskillende patogene is ook ontwikkel en gevalideer. Tussen verskeie bakteriese isolate wat vanuit die risosfeergrond van siek bome verkry is, is vyf *Bacillus* en *Pseudomonas* isolate verkry wat baie goeie *in vitro* inhibisie van *Pythium irregulare, Phytophthora nicotianae* en *P. citrophthora* getoon het. Ongelukkig was die inhibisie van die *Neocosmospora* spp. wat met sitrus geassosieer word, nie so goed nie. Twaalf *Trichoderma* isolate is ook verkry en wat toon dat hulle nie-vlugtige verbindings produseer wat die verskillende oömiseet-patogene volledig inhibeer. Die inhibisie van die *Neocosmospora* spp. was weereens nie so goed nie. Inleiers vir die opsporing van bogenoemde twee *Phytophthora* spp. is suksesvol ontwikkel. Die inleiers het in validasie getoon dat hulle spesifiek tot die spesie was en nie ander naby-verwante *Phytophthora* spp. of ander grondgedraagde patogene opspoor nie. Verdere optimalisering en validasie van die inleiers is onderweg. Inleiers is ook suksesvol ontwikkel en gevalideer vir die opsporing en kwantifisering van hierdie patogene.

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 insluiting van boscalid en minerale olie aan die begin van ʼn spuitprogram die beste beheer verskaf, hoewel die hoeveelheid beheer nie betekenisvol verskil het van dit wat verkry word met die program bestaande uit mankoseb en koper, toegedien teen die helfte van hul geregistreerde dosisse, in tenkmengsels, nie. In projek 970 is ʼn paar nuwe fungisiedes vir SSV beheer geëvalueer met goeie resultate. Goeie SSV beheer (≥90% skoon vrugte) is met verskeie getoetste fungisiedes verkry, maar die beste beheer (99.8% skoon vrugte) is met die eksperimentele fungisied, GF 3540, verkry wat vier keer teen 6-week intervalle, in ʼn tenkmengsel met minerale olie, toegedien is. ʼn Mate van fitotoksisiteit is egter met hierdie toediening waargeneem. Byvoeg van die bymiddels Nufilm-17 en Entreé, as vervanging van minerale olie, in strobilurien-toedienings, het ook uitstekende SSV beheer verskaf.

Behalwe vir die evaluasie 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 ʼn proef gedoen om boom ry volume (BRV) as basis vir spuitkalibrasie te evalueer. Spuitneerleggingsresultate het getoon dat die vermindering van volumes gebaseer op BRV, soortgelyke neerleggingswaardes op vrugte en blare gegee het in vergelyking met hoër volume toedienings. Daar is verder gesien dat lugvolume gedurende toediening geen betekenisvolle effek op spuitneerlegging gehad het nie. Verdere proewe sal op ander sitrustipes gedoen word, en om vas te stel of die gebruik van BRV vir spuitkalibrasie biologies effektief is.

Op die gebied van SSV epidemiologie word verskeie projekte uitgevoer. In projek 1187 is opgemerk dat in boorde onder skadunet meer piknidiospoor infeksieperiodes voorspel word in vergelyking met oop boorde. In terme van askospoor infeksieperiodes is egter geen verskil waargeneem tussen boorde onder skadunet en oop boorde nie. In die geval van ABV en Botrytis, is hoër infeksies vir boorde onder skadunet voorspel in vergelyking met oop boorde. Projek 1186 fokus op die periode van SSV vrugvatbaarheid. Resultate van die eerste seisoen dui daarop dat vrugvatbaarheid met toenemende vrugouderdom afneem, en dat die vrugbeskermingsperiodes wat in beheerprogramme gebruik word, geldig is. Resultate van die eerste seisoen word in ʼn tweede seisoen bevestig, terwyl die projek uitgebrei is om SSV vatbaarheid van mandarynvrugte in te sluit. Binne projek 1242 is bevind dat skilfitochemie van vyf verskillende sitrustipes, met verskillende vlakke van SSV vatbaarheid, verander soos die vrugte verouder. Dit is verder vasgestel dat die fitochemie tussent die tipes ook verskil. Hierdie bevindinge kan help om die interaksie tussen SSV en vrugte te verstaan en ook die verskille in vatbaarheid tussent sitrustipes. Projek 1223 het ten doel om vas te stel hoe vinnig opgekapte snoeisels komposteer en of dit ʼn belangrike rol as SSV inokulumbron speel. Opgekapte en nie-opgekapte snoeisels is vanaf ʼn suurlemoenboord in Hoedspruit versamel en geanaliseer vir die teenwoordigheid van SSV askospore. Die resultate van die eerste seisoen is egter nog uitstaande. Projek 1244 is met 1223 verbind en fokus op die infeksie-parameters van SSV piknidio- en askospore. Dit is met die doel om SSV voorspellingsmodelle te verbeter. In ʼn deurbraak is SSV askospore in kultuur geproduseer en dit baan die weg vir verdere studies op die infeksie-parameters van hierdie belangrike infeksiebron. Resultate van projek 1244 sal uiteindelik in projek 1238 gebruik word wat ten doel het om die voorspellingsmodel in CRI-PhytRisk te verbeter. Behalwe vir die verbetering van die SSV voorspellingsmodel, is CRI-PhytRisk ook uitgebrei om ABV en Botrytis voorspellingsmodelle in te sluit. Hierdie twee modelle benodig egter nog verfyning vir Suid-Afrikaanse toestande. Projek 1235 kyk daarna om ons kennis van die SSV patogeen se populasiestruktuur te verbeter. Deur die gebruik van *high throughput sequencing* (*HTS*), is die klonale aard van die *Phyllosticta citricarpa* populasie in die V.S.A. bevestig. Hierdie hulpmiddel gaan nou ook gebruik word om die Suid-Afrikaanse populasie op dieselfde manier te karakteriseer.

Die laaste projek in die program is 1236 wat die epidemiologie van Botrytis op suurlemoene ondersoek. Werk van die eerste jaar dui daarop dat suurlemoenbloeisels die primêre bronne van inokulum in suurlemoenboorde is. Daar is verder gesien dat fludioxonil en fenhexamid goeie beheer van *Botrytis cinerea in vitro* gegee het. Verdere werk sal die korrelasie van blom-infeksies met klimaatsdata behels ten einde die Botrytis voorspellingsmodel wat alreeds in CRI-PhytRisk geïnkorporeer is, te valideer en te verfyn.

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

Project 1030 (2008 – 2019/2020) by JM van Niekerk, MC Pretorius, Siyethemba Masikane and C Olivier (CRI)

Summary

Research in this project focusses on finding safer, more sustainable alternatives for the management of *Phytophthora* diseases of citrus and the citrus nematode, *Tylenchulus semipenetrans*. In the current report promising results are presented on the *in vitro* efficacy of commercially available *Trichoderma* and bacteria based biological control products. Through non-volatile action, the *Trichoderma* based products showed good efficacy in inhibiting mycelium growth of oomycete pathogens. However, their efficacy in inhibiting *Neocosmospora solani* was not sufficient. The two bacterial products displayed poor inhibition of oomycete pathogens but showed promising results in inhibiting *N. solani*. New trials aimed at evaluating *Trichoderma* based products in the glasshouse and a fungus based product for nematode control in the field, are also outlined.

Opsomming

Binne hierdie projek fokus navorsing daarop om veiliger, meer volhoubare alternatiewe vir die beheer van *Phytophthora* siektes van sitrus asook die sitrus nematode, *Tylenchulus semipenetrans* te vind. Belowende resultate word rapporteer aangaande die *in vitro* effektiwiteit van kommersieël beskikbare *Trichoderma* en bakteriese biologiese beheer produkte. Die *Trichoderma* produkte het deur nie-vlugtige aksie goeie effektiwiteit getoon om die mycelium groei van oomycete patogene te inhibeer. Hul effektiwiteit in die inhibisie van *Neocosmospora solani* was nie voldoende nie. Die twee bakteriese produkte was swak om die mycelium groei van oomycete patogene te inhibeer, maar het belowende resultate getoon om *N. solani* te inhibeer. Nuwe proewe gemik op die evaluering van *Trichoderma* produkte in 'n glashuis en 'n swamgebasseerde produk vir die beheer van die sitrus nematode in die veld, word bekendgestel.

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 soils and with poor performance of mature citrus trees. The host range of *T. semipenetrans* includes all *Citrus* species and most hybrids of citrus with other members of the rutaceous family such as trifoliate orange (*Poncirus trifoliata* L.Raf*)*. Non-rutaceous plants such as grape (*Vitis vinifera*, L*),* olive (*Olea europea*, L*)* and persimmon (*Diospyros spp.)* are also hosts (Verdejo-Lucas, 2002).

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

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

In the past, the nematicide 1,2 dibromo-3-chloropropane (DBCP) was widely used in the irrigation water against this nematode with great success. The nematode was effectively controlled while yields were also substantially increased (O'Bannon *et al.,* 1963; Philis, 1969). The activity on eggs is the most important difference between the soil fumigants used to control nematodes earlier this century, and today's non-fumigant chemicals. Following the withdrawal of DBCP, non-fumigant post-plant nematicides (carbamate or organophosphate acetylcholinesterase inhibitors) were introduced. These chemicals, however, could not eliminate, or greatly reduce, nematode populations even if applied every year. Fenamiphos is translocated systemically in the vascular system of plants, whilst the other nematicides are non-systemic and reduce nematode populations through their initial contact action only. This explains the quick recovery of nematode populations once the nematicide has been degraded in soil and emphasizes the adverse effect of enhanced degradation, as eggs hatching after the nematicide has been degraded can continue the nematode's life-cycle. The following nematicides are currently registered on citrus in South Africa: aldicarb, cadusafos, fenamiphos, terbufos, ethoprophos, fosthiazate and furfural (Nel *et al*., 2002). When multiple nematicide applications were introduced on a commercial scale to citrus orchards in South Africa, situations occurred where growers were not successful in disrupting the nematode's life cycle despite adhering strictly to prescribed procedures. In an investigation to determine the efficacy of cadusafos in soil where aldicarb and fenamiphos failed as a result of accelerated degradation, it was found that in the absence of sufficient irrigation water none of the nematicides were distributed thoroughly through the soil profile and they consequently failed to eliminate the citrus nematode (Le Roux *et al*., 1998).

Due to safety, environmental concerns and market pressure, only a few registered chemical nematicides remain worldwide for utilization by farmers, and the use of these products is highly restricted. Developing alternatives to chemical nematicides is essential and a great concern to researchers worldwide. Recent attempts to develop alternative methods to manage plant-parasitic nematodes include the use of entomopathogenic nematodes and various biologically derived nematicides and other organic compounds. The aim of this project 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.

Objective

1. 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

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 orchard of old Midknight 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 the number of citrus nematode juveniles in the soil were on average 2 068 per 250 cc soil. In the root samples on average 1 532 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/m^2. For evaluation purposes 10 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 CRI Diagnostic Centre (DC) in Nelspruit. Tree height and stem diameter were also measured. The recorded data were subjected to statistical analyses and are reported below.

In vitro *evaluation of commercially available bacterial and* Trichoderma *spp. based biological control agents*

The screening of the *Trichoderma* and bacterial based products for their ability to inhibit citrus replant pathogens was done using a non-volatile test. Details of the products are listed in Table 4.3.2.1.

Trichoderma products and three isolates of each of the replant pathogens were grown on PDA in the dark at 28°C for three days. Mycelial plugs (6 mm) colonized by the *Trichoderma* products were placed face down on PDA plates covered with autoclaved 50 µm thick cellophane membranes (Sigma, Germany) (84 mm in diameter). The cellophane PDA plates were then incubated in the dark at 28°C. After 48 hours, the cellophane membranes with *Trichoderma* growth were removed ensuring that the PDA plates were completely free of *Trichoderma*. Then, mycelial plugs (6 mm diameter) of the citrus replant pathogens were inoculated in the center of the PDA plates and incubated in the dark at 28°C for 2, 6, and 10 days for *Pythium irregulare*, *Neocosmospora* spp. and *Phytophthora citrophthora* and *Phytophthora nicotianae* respectively (Figure 4.3.2.1). For the control plates, *Trichoderma* was not inoculated on the cellophane membranes and three technical repetitions were done for each pathogen isolate. At the end of the incubation period, the mycelial diameters of the pathogens from each PDA plate were measured three times and the percentage inhibition was calculated according to [(A-B)/A x 100] where A is the mycelial diameter of the control plates and B is the mean of three mycelial diameters of pathogens.

The bacterial products were prior to plating onto the cellophane, streaked out onto NA and incubated at 28°C in the dark for 48 h. The resulting cultures were then used to streak the bacterial products out onto the cellophane membranes on the PDA plates. These plates were then also incubated for 48 h prior to the removal of the membranes and plating of the replant pathogens. The rest of the evaluation was done as above.

Table 4.3.2.1. Details of commercial *Trichoderma* spp. or bacterial based biological control agents evaluated in a non-volatile test against replant pathogens.

Product	**Biological control agent species**	**Company**
USPP-T1	*Trichoderma atroviride*	Department of Plant Pathology, University of Stellenbosch
TrichoPlus	*Trichoderma asperelloides*	BASF
TriCure-SP	*Trichoderma harzianum*	MBFI
Eco-T	*Trichoderma asperellum*	Plant Health Products
Eco-77	*Trichoderma atroviride*	Plant Health Products
Rizofos Fruit & Veg	*Pseudomonas fluorescens*	MBFI
B-Rus	*Bacillus subtilis*	Stimuplant

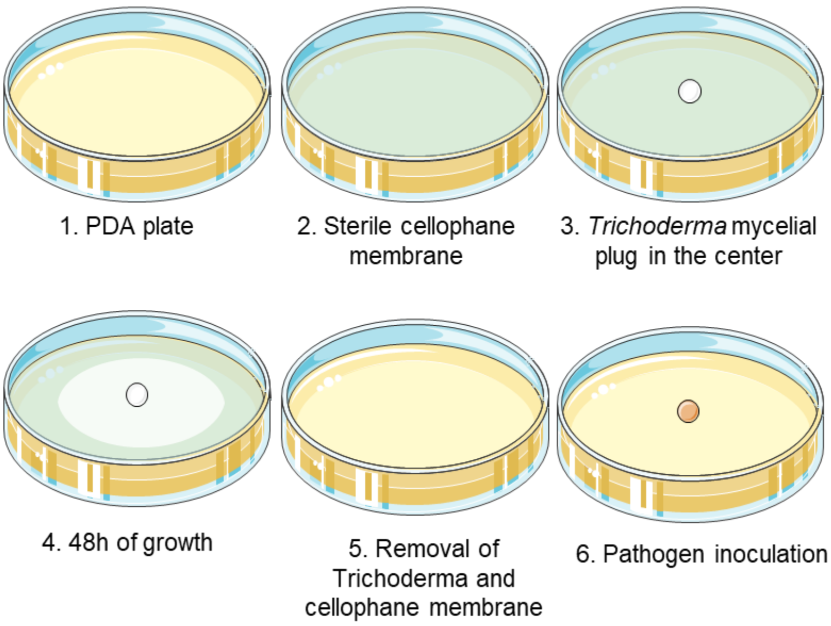

Figure 4.3.2.1. Illustration of the non-volatile test of commercial *Trichoderma* and bacterial biological control products against citrus replant pathogens.

Glasshouse evaluation of commercially available Trichoderma *formulations for the control of Phytophthora root rot*

Swingle citrumelo seedlings were grown in the glasshouse in a mixture of 50:50 composted pine bark and steam sterilized topsoil. Seedlings were grown until they were on average 40 cm tall. Watering was done as needed and insect management and sprays were also done based on weekly scouting.

The *Trichoderma* based products were selected based on them having a registration to control *Phytophthora* spp. on tree and other crops. Details of the products are presented in Table 4.3.2.2.

Table 4.3.2.2. Details of commercial *Trichoderma* spp. biological control agents evaluated in a glasshouse trial for the control of Phytophthora root rot of Swingle citrumelo seedlings.

Product	**Biological control agent species**	**Company**
USPP-T1	*Trichoderma atroviride*	Department of Plant Pathology, University of Stellenbosch
TrichoPlus	*Trichoderma asperelloides*	BASF
TriCure-SP	*Trichoderma harzianum*	MBFI
Eco-T	*Trichoderma asperellum*	Plant Health Products
Eco-77	*Trichoderma atroviride*	Plant Health Products
Rootguard	*Trichoderma harzianum*	Biocult

These products will all be applied as a monthly drench application at 25 g of product per 100 L water. A total of 4 applications will be done prior to inoculation with a *Phytophthora nicotianae* mycelium/zoospore suspension. Inoculations will be done weekly for a period of two months. This is to ensure proper colonization of the growing medium by the pathogen. Included are two control treatments; an untreated, un-inoculated treatment and an inoculated only treatment. Eight weeks after the last inoculation, the trial will be evaluated destructively. Plants will be measured for total wet mass and total wet root mass. The level of *P. nicotianae* infection in the roots of treated seedlings will be determined using a species specific qPCR assay. All data obtained will then be subjected to statistical evaluation. Each treatment is replicated on 8 seedlings, split into 2 blocks with 4 replicates per block.

Evaluation of a Paecilomyces lilacinus *based biological control product for the control of the citrus nematode,* Tylenchulus semipenetrans

This product will be evaluated at two trial sites. One will be located outside of Nelspruit, Mpumalanga and one in Citrusdal, Western Cape. Trial sites will be selected based on pre-determined female nematode counts present in the orchards. Sites will have a count in access of 5000 per 10 g of roots. At each trial site, each treatment programme will be replicated on 8 trees that will be split into 2 tree plots in each of 4 blocks. The different treatment programmes for the two trial sites are given in Tables 4.3.2.3 and 4.3.2.4. These programmes are based on the recommended application intervals of the product and a commercial cadusafos product.

At the Nelspruit site, root and soil samples will be collected in November 2020 and again in March 2021. These will be analysed for the presence of juvenile citrus nematodes in the soil and female nematodes in the roots. Similarly, at the Citrusdal trial site, soil and root samples for analysis will be collected in August and November 2020. Data obtained will be statistically analysed to determine treatment programme efficacy.

Table 4.3.2.3. Treatment programmes for the evaluation of a commercial *Paecilomyces lilacinus* (PL) product in the Nelspruit area of South Africa.

Treatment programme	**Treatment**	**Sept 2020**	**Oct 2020**	**Nov 2020**	**Dec 2020**	**Jan 2021**	**Feb 2021**

1	Untreated	None	None	None	None	None	None
2	PL @ 125 g/ha	PL	PL	PL	PL	PL	PL
3	Cadusafos	Cadusafos		Cadusafos		Cadusafos	
4	PL + cadusafos 1	Cadusafos	PL	PL	PL	PL	PL
5	PL + cadusafos 2	Cadusafos	PL	Cadusafos	PL	Cadusafos	PL

Table 4.3.2.4. Treatment programmes for the evaluation of a commercial *Paecilomyces lilacinus* (PL) product in the Citrusdal area of South Africa.

Treatment programme	Treatment	May 2020	Jun 2020	Jul 2020	Aug 2020	Sept 2020	Oct 2020
1	Untreated	None	None	None	None	None	None
2	PL @ 125 g/ha	PL	PL	PL	PL	PL	PL
3	Cadusafos	Cadusafos		Cadusafos		Cadusafos	
4	PL + cadusafos 1	Cadusafos	PL	PL	PL	PL	PL
5	PL + cadusafos 2	Cadusafos	PL	Cadusafos	PL	Cadusafos	PL

Results and discussion

Objective / Milestone	Achievement
Apr –Jun 2019 1. Annual report	1. Annual report was written and submitted.
Jul – Sept 2019 1. Trial planning	1. Trial was planned and products obtained.
Oct – Dec 2019 1. Complete *in vitro* evaluation of products	1. Trials were completed and results compiled.
Jan – Mar 2020 1. Data analysis and writing of report	1. Data analysis was done and report written.

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

The 2020 evaluation of this trial was scheduled for the end of March 2020. However, due to the COVID-19 national lockdown this had to be postponed to post lockdown. The results obtained will be included in the progress/proposal to be submitted at the end of 2020.

In vitro *evaluation of commercially available bacterial and* Trichoderma *spp. based biological control agents*

Inhibition of mycelium growth of *P. nicotianae* and *P. citrophthora* was 100% for 4 of the five products tested. The one product that performed significantly poorer was the *T. harzianum* based product of MBFI that achieved only 50 to 60% inhibition of the respective *Phytophthora* spp (Figures 4.3.2.2 and 4.3.2.3). However, in the case of *Pythium irregulare*, 100% inhibition of mycelium growth was obtained by all the products tested. But inhibition of *Neocosmospora solani* was poor for all the products tested. The mean inhibition was below 40%. The best performing product was USPP-T1 followed by Eco-77. The inhibition achieved by these two products were between 30% and 40%. Inhibition achieved by the other 3 products was below 20% (Figure 4.3.2.5).

Mycelium growth inhibition achieved by the two tested bacterial biological control products varied greatly. The *Pseudomonas fluorescens* inhibited the mycelial growth by more than 60% while the *Bacillus subtilis* product did not achieve any inhibition (Figure 4.3.2.6). Inhibition of *P. citrophthora* was almost similar to *P. nicotianae*. The *P. fluorescens* product inhibited the mycelium growth by almost 60%, but the *B. subtilis* product again resulted in no inhibition (Figure 4.3.2.7). *Pythium irregulare* mycelium growth was inhibited 100% by *P. fluorescence* while the *B. subtilis* product inhibited the mycelium by less than 30% (Figure 4.3.2.8). However, results obtained by the two bacterial products were good in the case of *N. solani.* The *B. subtilis* product inhibited this pathogen by more than 80% while the *P. fluorescens* product inhibited the growth significantly less at almost 70% (Figure 4.3.2.9).

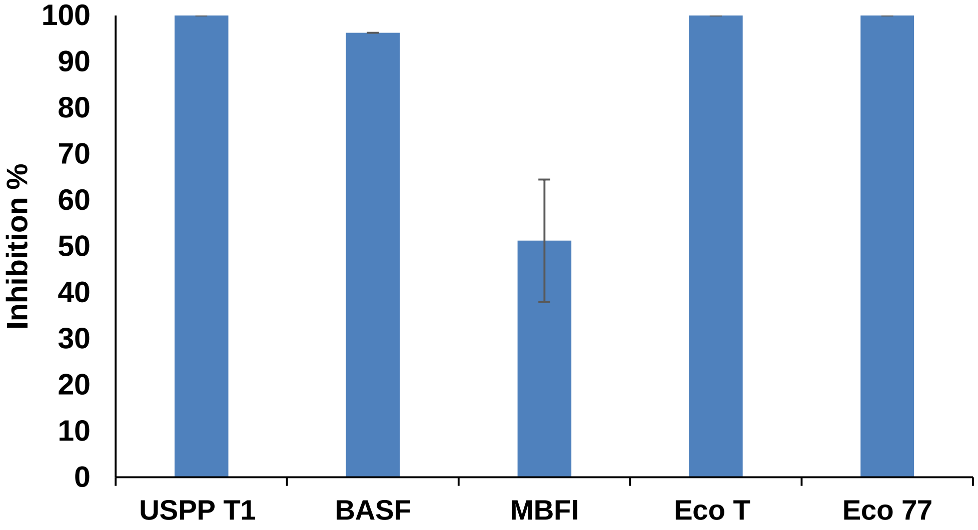

Figure 4.3.2.2. Percentage inhibition of *P. nicotianae* mycelium growth by non-volatile compounds secreted by five commercial *Trichoderma* spp. based biological control agents.

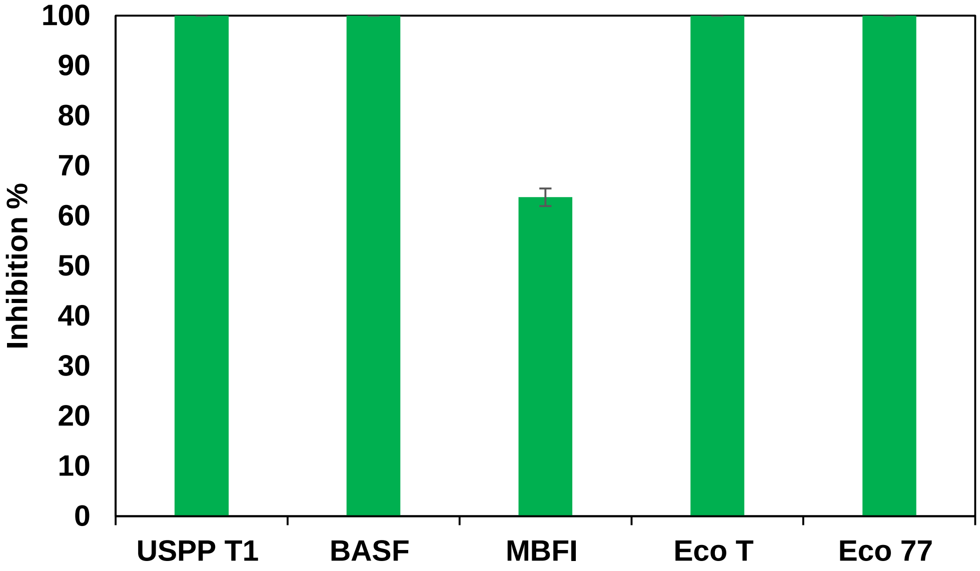

Figure 4.3.2.3. Percentage inhibition of *P. citrophthora* mycelium growth by non-volatile compounds secreted by five commercial *Trichoderma* spp. based biological control agents.

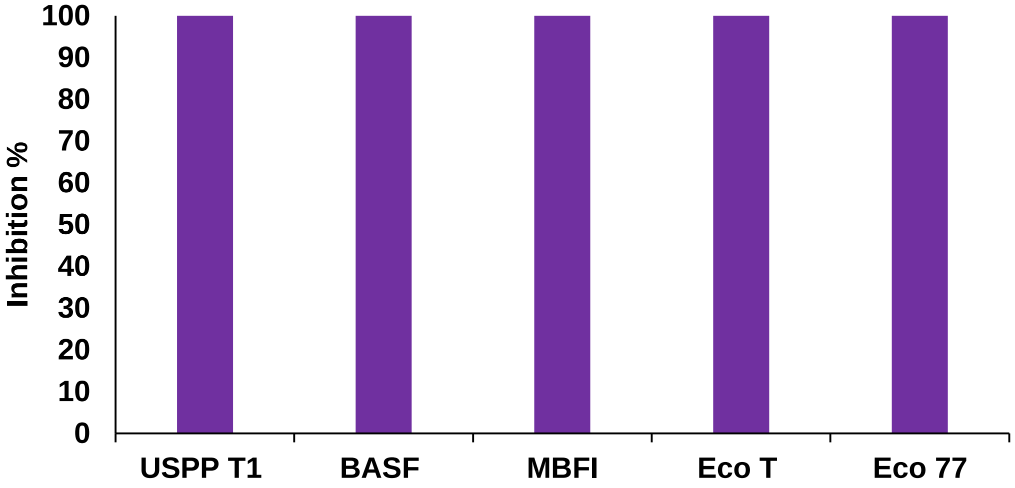

Figure 4.3.2.4. Percentage inhibition of *Pythium irregulare* mycelium growth by non-volatile compounds secreted by five commercial *Trichoderma* spp. based biological control agents.

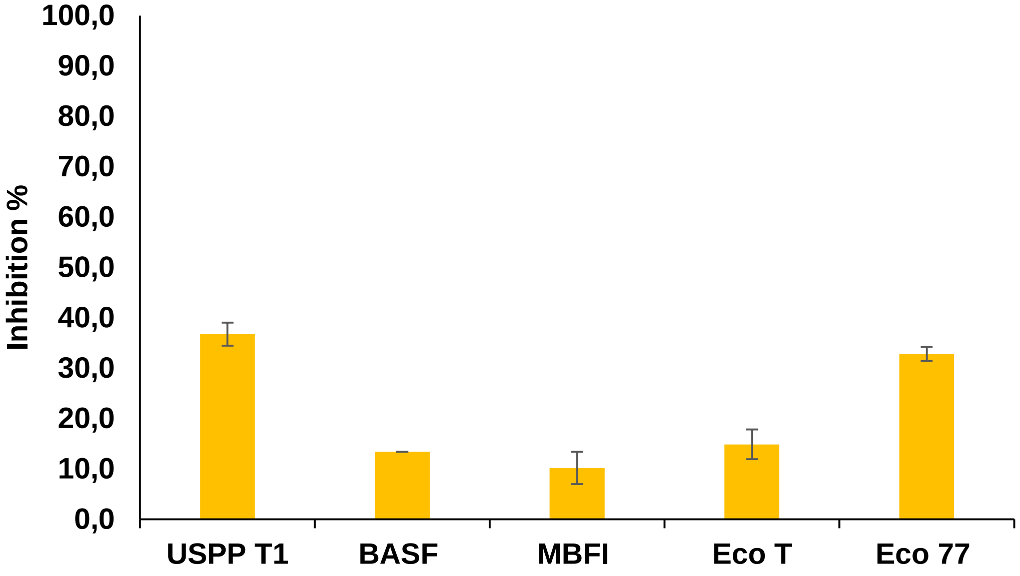

Figure 4.3.2.5. Percentage inhibition of *Neocosmospora solani* mycelium growth by non-volatile compounds secreted by five commercial *Trichoderma* spp. based biological control agents.

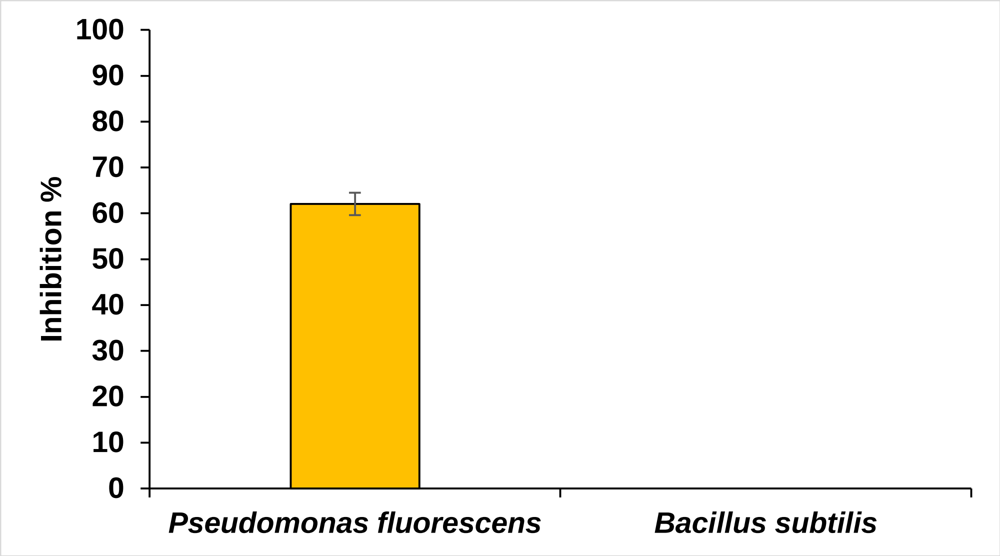

Figure 4.3.2.6. Percentage inhibition of *Phytophthora nicotianae* mycelium growth by non-volatile compounds secreted by two commercial bacterial biological control agents.

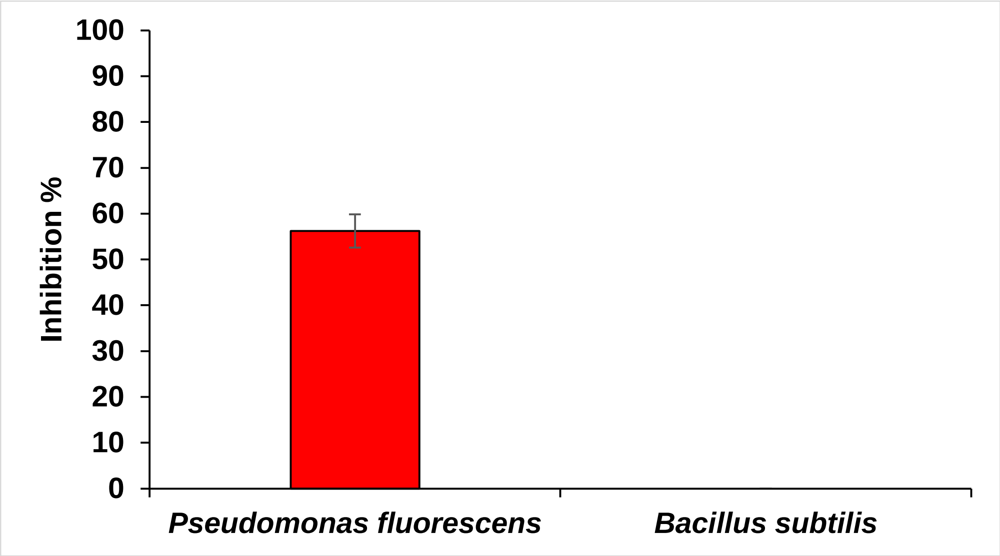

Figure 4.3.2.7. Percentage inhibition of *Phytophthora citrophthora* mycelium growth by non-volatile compounds secreted by two commercial bacterial biological control agents.

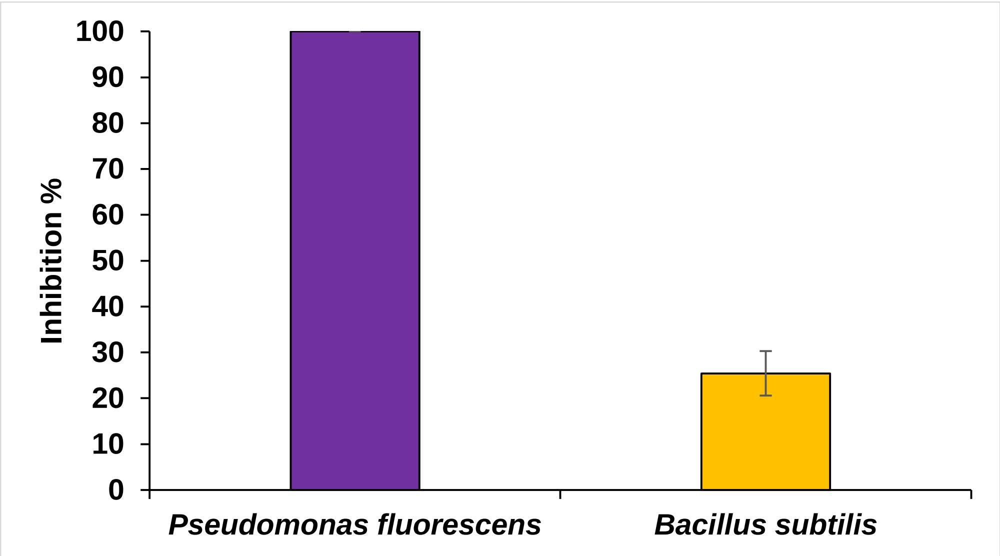

Figure 4.3.2.8. Percentage inhibition of *Pythium irregulare* mycelium growth by non-volatile compounds secreted by two commercial bacterial biological control agents.

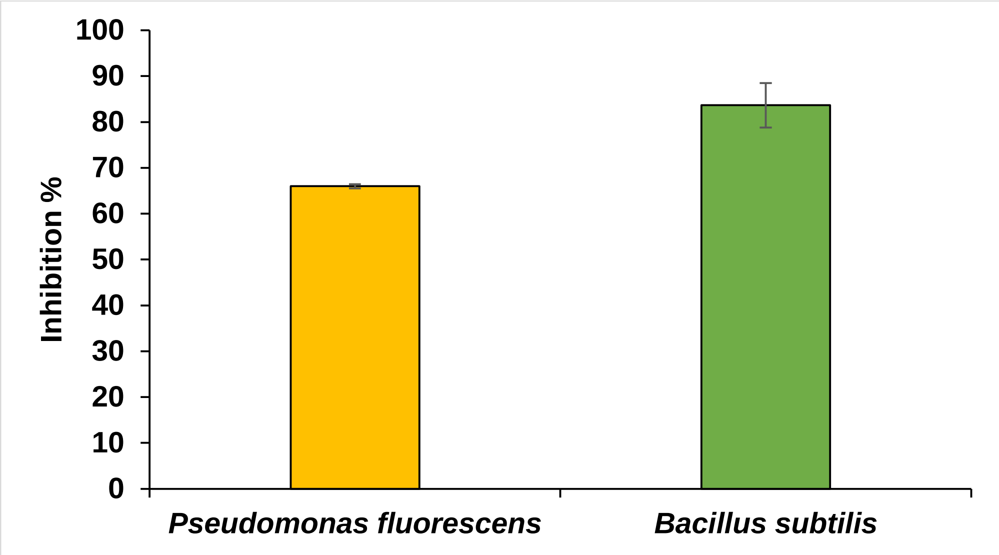

Figure 4.3.2.9. Percentage inhibition of *Neocosmospora solani* mycelium growth by non-volatile compounds secreted by two commercial bacterial biological control agents.

Glasshouse evaluation of commercially available Trichoderma *formulations for the control of Phytophthora root rot*

This trial commenced in March 2020 due to a delay in the production of the seedlings needed. The results will be available by the end of 2020 and will be included in the 2021 report.

Evaluation of a Paecilomyces lilacinus *based biological control product for the control of the citrus nematode,* Tylenchulus semipenetrans

The two trials as described above will also commence in 2020. It was delayed due to difficulties obtaining the product from the holding company in Kenya. Results from these two trials will be included in the 2021 report.

Conclusions to date

The very good *in vitro* results obtained with four of the five *Trichoderma* based products indicate that these products have great potential for the control of *Phytophthora* spp. They performed poorly in inhibiting *N. solani*, however, this pathogen was significantly inhibited by the two bacterial products tested. The performance of the *Trichoderma* products are currently being evaluated in the glasshouse and this will determine if the *in vitro* performance is replicated *in vivo* in citrus seedlings.

Technology transfer

Relevant results from previous work was presented at the 2018 CRI 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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4.3.3 **PROGRESS REPORT: Unravelling the clonal distribution of *Phyllosticta citricarpa* through a Genotyping-By-Sequencing approach**

Project 1235 (2019 - 2021) by Aletta Bester-van der Merwe (SU), Paul Fourie; Elma Carstens (CRI); Megan Dewdney (University of Florida, USA)

Summary

Knowledge of pathogen population genetic structure is critical for a comprehensive understanding of disease epidemiology. Whole-genome sequence data is also a valuable resource to determine the distribution patterns of molecular markers and to help understand whether these sequences have any functional or evolutionary significance. Simple sequence repeats (SSRs) are powerful tools to study population biology. This was the first study to use high throughput sequencing (HTS) to study the genetic diversity within a *P. citricarpa* population. The fine-scale genetic differences within 17 USA *P. citricarpa* isolates previously genotyped with 15 SSR markers were investigated. The results obtained confirm the clonality of the USA *P. citricarpa* isolates and *in silico* detection of mating types confirmed that only one mating type is present in the USA.

Opsomming

Kennis van patogeenpopulasies se genetiese struktuur is van kritieke belang vir die verstaan van die siekte-epidemiologie. Data oor die totale genoom-volgorde is ook 'n waardevolle hulpbron om die verspreidingspatrone van molekulêre merkers te bepaal en om te help om te verstaan of hierdie volgordes enige funksionele of evolusionêre betekenis het. Eenvoudige volgorde herhalings (SSR's) is kragtige instrumente om populasiebiologie te bestudeer. Hierdie was die eerste studie wat hoë deurvloei-volgordebepaling (HTS) gebruik het om die genetiese diversiteit binne 'n *P. citricarpa*-populasie te bestudeer. Die fynskaalse genetiese verskille binne 17 VSA *P. citricarpa* isolate wat voorheen met 15 SSR-merkers genotipeer is, is ondersoek. Die resultate wat verkry is, bevestig die klonaliteit van die VSA *P. citricarpa* isolate en die *in silico* opsporing van paringstipes het bevestig dat slegs een paringstipe in die VSA teenwoordig is.

4.3.4 **PROGRESS REPORT: Epidemiology and management of *Botrytis cinerea* in citrus**
Project 1236 by Junré Marais, Julia C. Meitz-Hopkins and Cheryl L. Lennox (SU)

Summary

Infection of citrus blossoms and young fruitlets by *Botrytis cinerea* can result in blossom and fruitlet drop, as well as rind distortion in fruit. To manage the disease, it is important to determine the inoculum sources and find a way to manage the levels of inoculum, to reduce disease. During this project it has been determined that lemon blossoms are the primary inoculum source of *B. cinerea* in lemon orchards. Weeds, twigs, and fruit mummies were also collected and no *B. cinerea* could be extracted from these, even with the use of a Botrytis selective media. Fungicide sensitivity trials on amended media have been conducted to determine which fungicides can be used to control *B. cinerea* in the orchard. Preliminary data indicate that the two fungicides to which the pathogen showed the highest sensitivity were fludioxonil and fenhexamid. Fruit have also been rated in the orchard as to whether it shows any symptoms of infection by *B. cinerea* and this will be compared to the data of blossom infections recorded in September 2019. To quantify the inoculum levels qPCRs will be conducted in the near future. Weather data still need to be analysed in conjunction with infection periods, and the prediction made by the model on CRI-PhytRisk.

Opsomming

Die infeksie van sitrus bloeisels en onvolwasse vrugte deur *Botrytis cinerea* kan lei tot blomval en vrugval van onvolwasse vrugte. Dit veroorsaak ook skilvervorming. Om die siekte te beheer is dit belangrik om die bron van inokulum te bepaal, asook maniere te vind hoe om die vlakke van inokulum te verlaag sodat die siekte beheer kan word. Tydens hierdie projek is dit bepaal dat suurlemoen bloeisels die hoofbron van *B. cinerea* inokolum in suurlemoen boorde is. Onkruid, takkies, en vrug-mummies was ook versamel, maar geen *B. cinerea* kon vanaf dit geisoleer word nie, selfs nie met die gebruik van 'n Botrytis selektiewe media nie. Swamdoder sensitiwiteits toetse op gewysigde media was gedoen om te bepaal watter swamdoders kan moontlik gebruik word om *B. cinerea* in die boord te beheer. Voorlopige data toon dat *B. cinerea* die hoogste sensitiwiteit toon vir fludioxonil en fenhexamid. Vrugte is ook in die boord gegradeer, gebaseer op die voorkoms van *B. cinerea* simptome. Hierdie data sal vergelyk word met die infeksies van bloeisels wat in September 2019 waargeneem is. Om die inokulum vlakke te kwantifiseer gaan qPCRs uitgevoer word in die nabye toekoms. Weer data moet nog geanaliseer word in vergelyking met die infeksie periodes en die voorspellings gemaak deur die model of CRI-PhytRisk.

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 JM van Niekerk, MC Pretorius and S Masikane (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 ŉ 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 dichloropropeen 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 ŉ 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 – 2019/20) by JM van Niekerk (CRI) and Elodie Stempien (USPP)

Summary

Research done over the last three years has indicated that the Valley Bushveld citrus decline is a disease syndrome caused by the interaction of predisposing, inciting and contributing factors. Rootstocks sensitive to high pH soil and water along with soil chemical characteristics were identified as predisposing factors. To address these, an in-arching trial with rootstocks not sensitive to high pH was started while a rootstock trial will be planted in September 2020. Microbiome and allelochemical analyses in diseased and healthy orchards and the Valley Bushveld will be done to determine if there are any soil chemicals or microorganisms present that predispose the trees to infection by the complex of pathogens identified. Inciting factors are most likely long term factors. Long term data regarding various factors will therefore be gathered to determine if there are any of these factors that incited the development of the decline. Any contributing factors such as the effect of identified allelochemicals or microorganisms on tree growth will be studied. Once all these factors and their interaction is understood, management strategies will be developed.

Opsomming

Navorsing wat in die laaste drie jaar gedoen is, het aangedui dat die “Valley Bushveld citrus decline” sindroom deur die interaksie van predisponerende, aanhitsende en bydraende faktore, veroorsaak word. Onderstamme wat sensitief is vir hoë grond en water pH, tesame met ander chemiese grondeienskappe, is as predisponerende faktore geïdentifiseer. Om hierdie aan te spreek is ŉ brugent proef met pH nie-sensitiewe onderstamme begin terwyl ŉ onderstam proef in September 2020 geplant sal word. Mikrobioom en allelochemie analises in siek en gesonde boorde asook die Vallei Bosveld, sal gedoen word om te bepaal of daar enige chemiese middels of mikroorganismes in die grond is wat bome predisponeer vir infeksie deur die patogeenkompleks wat identifiseer is. Aanhitsende faktore is waarskynlik langtermyn faktore. Langtermyn data van verskeie faktore sal dus versamel word om te bepaal of enige van hierdie faktore die sindroom aanhits. Enige bydraende faktore soos die effek van geïdentifiseerde allelochemikalië of mikrobioom organismes sal bestudeer word. Sodra al hierdie faktore en interaksies verstaan word sal beheerstrategieë ontwikkel word.

4.3.7 **PROGRESS REPORT: Evaluation of reduced volume fungicide and pesticide sprays for control of citrus black spot and false codling moth.**
Project 1132 (2014/15 – 2020/21) by Jan van Niekerk (CRI), Gideon van Zyl (ProCrop) and PH Fourie (CRI)

Summary

Citrus trees are susceptible to a wide range of insect pests and fungal diseases. Spray application of plant protection products (PPPs) in citrus production is commonly done using pre-determined application methodologies without taking target pest, orchard and sprayer characteristics into account This results in the use of common high volume spray application which can be wasteful due to loss of water and PPPs due to run-off and drift. Previous studies showed that the reduced volume spray applications generally achieved higher deposition quantity values on fruit and leaves in comparison with the high volume applications. However, the higher spray volumes achieved better deposition uniformity results (CV%) and had better pest and disease control. This indicated that lower volume applications should be optimised to improve the deposition uniformity (CV%) and quality (ICD%). A trial was therefore done in a mature Nules Clementine orchard with a tree row volume (TRV) of 3700 L/ha. The TRV was used to determine the spray volumes of the trial which were 1233 L/ha (1/3 TRV), 1850 L/ha (1/2 TRV), 3700 L/ha (TRV) and 7400 L/ha. These volumes were applied at two air volumes of 48 000 m^3/h and 36 000 m^3/h using a Rovic & Leers Even Flow machine. These spray volume and air volume combinations were compared with the 12500 L/ha used by the farm and applied with a Nieuwoudt spray machine. Results indicated that variation in air volume did not influence deposition parameters on leaves and fruit. The deposition parameters were also not significantly influenced by the range of volumes evaluated. Deposition parameters achieved with the 1/2 and 1/3× spray application (in relation to the 1× TRV application) indicate promise and should be evaluated in properly managed canopies. This indicates possible effective use of lower spray volumes together with canopy adapted spraying methodology for pest and disease control. The biological efficacy of TRV based reduced volumes will therefore be evaluated in future trials.

Opsomming

Sitrus bome is vir ŉ verskeidenheid van insekpeste en swamsiektes vatbaar. Spuitaanwending van plantbeskermingsprodukte (PBP's) word algemeen gedoen deur gebruik te maak van voorafbepaalde toedieningsmetodologie sonder om die teikenpes, boord en spuitmasjien eienskappe in ag te neem. Die gevolg is die algemene gebruik van hoë spuitvolumes wat tot die vermorsing van water en PBP's, deur middel van afloop, lei. Vorige studies het getoon dat laer volumes tot hoër deposisie kwantiteit op blare en vrugte aanleiding gee in vergelyking met hoë volume aanwendings. Die hoër spuitvolumes het egter tot beter spuituniformiteit aanleiding gegee wat gelei het tot beter siekte en pesbeheer. Dit het aangedui dat laer spuitvolumes optimiseer moet word om deposisie uniformiteit en kwaliteit te verbeter. ŉ Proef is dus in ŉ volwasse Nules Clementine boord, met boomryvolume (BRV) van 3700 L/ha, gedoen. Die BRV is gebruik om die spuitvolumes in die proef te bepaal. Hierdie was 1233 L/ha (1/3 BRV), 1850 L/ha (1/2 BRV), 3700 L/ha (BRV) en 7400 L/ha (2 x BRV). Hierdie volumes is teen twee lugvolumes van 48 000 m^3/h en 36 000 m^3/h aangewend deur gebruik te maak van ŉ Rovic & Leers Even Flow masjien. Die spuit en lugvolume kombinasies is vergelyk met die plaas se standard toediening van 12500 L/ha, toegedien met ŉ Nieuwoudt masjien. Resultate het aangedui dat variasie in lugvolume nie die deposisieparameters op blare en vrugte beïnvloed het nie. Die deposisieparameters is ook nie betekenisvol deur die reeks volumes geëvalueer, beïnvloed nie. Deposisieparameters met die 1/2 and 1/3× spuitaanwending (in vergelyking met die 1× BRV toediening) toon belofte en moet in goed gesnoeide bome getoets word. Hierdie dui moontlik op effektiewe gebruik van laer spuitvolumes tesame met boomaangepaste spuitmetodologie vir die beheer van siektes in insekplae. Die biologiese effektiwiteit van BRV gebasseerde verminderde spuitvolumes moet dus in toekomstige proewe getoets word.

4.3.8 **PROGRESS REPORT: Potential biocontrol agents and host/pathogen interaction of citrus replant pathogens**
Project 1215 (RCE2-07B) by Jan van Niekerk (CRI), Prof Lizel Mostert, Dr Elodie Stempien, Gray-Lee Carelse and Soné Reens (USPP)

Summary

This study is aimed at investigating the interaction of citrus replant pathogens identified in previous studies and to identify potential biological control agents (BCA's) that can be used to manage these pathogens. In order to study the pathogen interactions, development of species-specific primers for the different pathogens was critical. To date, species specific qPCR primers were developed for *P. nicotianae*, *P. citrophthora*, *P. irregulare* and three *Neocosmospora* spp. associated with citrus replant pathogens. Having these primers and qPCR protocols will enable researchers to further study the interaction of these pathogens in citrus plants. For more sustainable management of replant pathogen, finding non-chemical alternatives is important. To this end, four potential BCA's (two bacterial and two *Trichoderma* strains) were identified from citrus roots. After extensive *in vitro* testing, it was discovered that non-volatile compounds formed by these potential BCA's successfully inhibit the different replant pathogens. Cell free culture filtrates produced by these agents will therefore be used in further studies to determine if they can also provide protection to citrus seedlings against replant pathogen infection.

Opsomming

Die doelwit van hierdie studie is om die interaksie van sitrus herplantpatogene, geïdentifiseer in vorige studies, te bestudeer en om potensiële biologiese beheeragente teen hierdie patogene, te identifiseer. Ten einde die patogeen interaksies te bestudeer, was die ontwikkeling van spesies spesifieke inleiers noodsaaklik. Tot op hede, is spesie spesifieke kPKR (qPCR) inleiers vir *P. nicotianae*, *P. citrophthora*, *P. irregulare* en drie *Neocosmospora* spp. ontwikkel. Die beskikbaarheid van hierdie inleiers en kPKR protokolle maak dit moontlik vir navorsers om die interaksie van herplant pathogene in sitrus plante te bestudeer. Meer volhoubare bestuur van herplant patogene is afhanklik van die ontdekking van nie-chemiese alternatiewe. Hiervoor is vier potensiële biologiese beheeragente (twee bakterie en twee *Trichoderma* isolate) vanuit sitruswortels geïdentifiseer. Na intensiewe *in vitro* toetsing, is dit ontdek dat nie-vlugtige verbindings, wat deur hierdie beheeragente gevorm word, die verskillende herplant patogene suksesvol inhibeer. Selvrye kultuurfiltrate wat deur hierdie agente gevorm word, sal dus in verdere studies gebruik word om te bepaal of hulle sitrussaailinge kan beskerm teen herplant patogeeninfeksie.

4.3.9 **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 Paul H. Fourie (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, with 81% of fruit having no ABS symptoms. The spray programme consisting of alternating Dipotassium phosphate applied at its registered and double rates with copper oxychloride achieved 51 and 66.4% clean fruit, respectively. Only 22.2% of the fruit in the untreated trees were free from ABS symptoms.

Opsomming

Verskillende fungisied spuitprogramme, insluitend 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-Kaap Provinsie geëvalueer. Die insluiting van boscalid en minerale olie aan die begin van 'n spuitprogram, het die beste beheer verskaf, met 81% van die vrugte met geen ABV simptome nie. Die spuitprogram bestaande uit die afwisseling van Dikalium-fosfaat, toegedien teen sy geregistreerde en dubbel dosis, met koper-oksichloried, het onderskeidelik 51 en 66.4% skoon vrugte gelewer. Slegs 22.2% van die vrugte in die onbehandelde bome was vry van ABV simptome.

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 tangerine such as the '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.

Objectives

To evaluate different spray programmes for ABS control in a susceptible 'Nova' mandarin orchard.

Materials and methods

A susceptible 22-year-old 'Nova' mandarin orchard, located in Kirkwood in the Eastern Cape, was selected as a trial site for the 2018-2019 season. Spray programmes included alternating copper and dipotassium phosphate and the inclusion of boscalid as part of a spray programme (Table 4.3.9.1). 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 2019, 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 Fisher'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
Apr 2019 – Mar 2020 To evaluate different spray programmes for ABS control in a susceptible 'Nova' mandarin orchard.	In the trial conducted all the spray programmes led to significantly more ABS free fruit and the application of boscalid at the start of the spray programme also significantly improved ABS control.

All treatments, irrespective of spray programme, had significantly higher percentage of clean exportable fruit when compared to the untreated control, which yielded 22.2% clean fruit (Table 4.3.9.1). The highest percentage (81% clean fruit) of clean fruit was achieved with the spray programme that included the application of boscalid with mineral oil at the beginning of the spray season. The standard spray programme used by the farmer (treatment 5) yielded the least amount of clean fruit (46.6%) compared to other fungicide treatments.

Table 4.3.9.1. 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 between October 2018 and March 2019.

Treatments		**Dosage (g/ml per 100L water tank mixture)**	**Percentage of fruit in each class**		
			Lesions/fruit[v]		
			0	**1-5**	**≥6**
1	Untreated control		22.2d	55.6a	22.2a
2	Boscalid+ mineral oil/mancozeb + pyraclostrobin + mineral oil/copper oxychloride/copper oxychloride + pyraclostrobin + mineral oil /copper oxychloride[w]	25g + 250ml/150g + 10ml + 250ml/200g/200g + 10ml + 250ml/200g	81.0a	17.4d	1.6c
3	Copper oxychloride/dipotassium phosphate/copper oxychloride/dipotassium phosphate/copper oxychloride/copper oxychloride[x]	200g/100ml/200g/100ml/ 200g/200g	51.0c	40.6b	8.4b
4	Copper oxychloride/dipotassium phosphate/copper oxychloride/dipotassium phosphate/copper oxychloride/copper oxychloride[y]	200g/200ml/200g/200ml/ 200g/200g	66.4b	30.6c	3.0c
5	Grower's standard spray		46.6c	47.8ab	1.6c

[v]Means 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.
[w]Spray dates were: 10 October 2018; 7 November 2018; 19 December 2018; 23 January 2019; 6 March 2019
[x]Spray dates were: 10 October 2018; 14 November 2018; 12 December 2018; 16 January 2019; 13 February 2019; 20 March 2019
[y]Spray dates were: 10 October 2018; 14 November 2018; 12 December 2018; 16 January 2019; 13 February 2019; 20 March 2019

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 made available 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. Alternative fungicides will have to be identified because of the withdrawal of mancozeb from the European Union which is South Africa's biggest export market.

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

Project 970 (Ongoing) by P. Moyo, S. Masikane and Paul H. Fourie (CRI)

Summary

There is a constant need to test novel control measures, including the evaluation of new fungicides, against citrus black spot (CBS). Several new systemic and contact fungicides, including experimental fungicides from various companies, were tested alone or in combination with registered fungicides on Valencia oranges for the control of citrus black spot. This was done according to predetermined protocols. Excellent CBS control (100% clean fruit) was achieved with several fungicides tested, but certain experimental fungicides including XDE-659 (GF 3509) and an experimental copper resulted in phytotoxicity. No phytotoxicity was observed on fruit treated with the industry standard CBS spray programmes. The substitution of mineral oil in mancozeb-strobilurin-mineral oil tank mixtures with adjuvants, NuFilm 17 and Entreé, yielded great CBS control. Testing of novel fungicides and re-evaluation of existing registered fungicides is necessary to improve CBS control.

Opsomming

Daar is ŉ konstante behoefte vir die toets van nuwe beheermaatreëls, insluitend die evaluasie van nuwe fungisiedes, teen sitrusswartvlek (SSV). Verskeie nuwe sistemiese en kontak fungisiedes, insluitend eksperimentele fungisiedes, vanaf verskeie maatskappye, is alleen of in kombinasie met geregistreerde fungisiedes op Valencia lemoene vir die beheer van sitrusswartvlek getoets. Hierdie is gedoen volgens vooraf-bepaalde protokolle. Uitstekende SSV beheer (100% skoon vrugte) is met verskeie getoetste fungisiedes verkry, maar sekere eksperimentele fungisiedes, insluitend 'XDE-659 (GF 3509)en ŉ eksperimentele koper, het tot fitotoksisiteit gelei. Geen fitotoksisiteit is waargeneem op vrugte wat met die industrie standaard SSV spuitprogramme behandel is nie. Die vervanging van minerale olie in mankoseb-strobilurien-minerale olie tenkmengsels met bymiddels, NuFilm 17 en Entreé, het tot groot SSV beheer gelei. Die toets van nuwe

fungisiedes en her-evaluasie van bestaande geregistreerde fungisiedes is nodig ten einde SSV beheer te verbeter.

Introduction

Citrus Black Spot (CBS), caused by *Phyllosticta citricarpa* (McAlpine) van der Aa, is a major concern for the South African citrus industry. *Phyllosticta citricarpa* is an A1 quarantine pathogen in the European Union and other CBS sensitive markets where there is a zero tolerance for CBS on fruit.

Citrus black spot holds the potential to reduce the South African competiveness on the global citrus market, due to its phytosanitary status and the impact thereof on trade of citrus fruit. Hence, research has focussed even more on protecting fruit from infection by the CBS pathogen. Currently, all commercial fungicide applications aimed at protecting fruit from CBS infection begin in mid-October in South Africa, based on research findings from ascospore release and trap data (Kellerman and Kotzé, 1977; Kotzé, 1981). Due to the withdrawal of certain fungicides from the market, as a result of concerns of risks posed to human health and the environment as well as resistance, there is a constant need to evaluate old and new fungicide formulations that may possess activity against CBS, and to structure spray programmes to improve control whilst adhering to permitted maximum residue levels (MRL) and limiting fungicide resistance.

A number of adjuvants are regularly used with systemic fungicide applications in South Africa, to enhance the efficacy of fungicides. However, most systemic fungicides registered for the control of CBS are used in combination with mineral oils. These oils have been shown to enhance the penetration of fungicides into the plant tissues, ultimately increasing the efficacy of the fungicides against CBS (Kellerman and Kotzé 1977). The efficacy of newly developed non-mineral oil adjuvants, as substitutes for mineral oil in standard strobilurin spray programmes, also need to be investigated for the control of *Phyllosticta citricarpa* on citrus in South Africa.

Fungicide manufacturers often develop new fungicide formulations for disease control but they also modify and upgrade old fungicide products to possess new characteristics such as rain fastness and particle size. The evaluation of new fungicide products and re-evaluation of old products for efficacy against CBS remains an integral part of staying globally competitive in the citrus market place.

Objectives

To evaluate any new potential fungicides and alternative spray programmes for the control of citrus black spot.

Materials and methods

The trial was carried out in a 1.4 ha commercial orchard (Crocodile Valley Citrus Co.) located in Nelspruit (GPS: 25° 28' 16.38” S and 31° 04’ 20.70” E). The orchard was planted in 1986 and consisted of Olinda ‘Valencia’ orange (*Citrus sinensis*) trees grafted on Rough lemon rootstock (*Citrus jambhiri*), with spacing of 8.3 m x 5.6 m. The orchard consists of sandy-loam soil.

Different fungicides were applied either alone or in tank mixtures with other fungicides. Spray applications were conducted according to predetermined intervals. The efficacy of the different fungicides in tank mixtures, as well as that of experimental spray programmes, was compared with the registered industry standard CBS treatments (Table 4.3.10.1).

Commercial fungicide applications against CBS are recommended from fruit set in South Africa and therefore, treatment applications began in mid-October 2018 and were applied using a trailer-mounted, high-volume, high-pressure (2,500-3,000 kPa) sprayer with two hand-held spray guns. Each treatment was replicated four times on single-tree plots arranged in a randomized complete-block design. Fungicide volumes varied according to the size and canopy density of the tree, but all trees were sprayed to the point of runoff.

On 14 August 2019 (a week before harvest), 100 fruit per data tree were evaluated according to a 3-point index: 0 = clean fruit with no CBS lesions; 1 = one to three CBS lesions per fruit; and 2 = four or more CBS lesions per fruit. ANOVA was carried out using XLSTAT, Version 2014.5.03 (Addinsoft, New York, USA) to determine the efficacy of each treatment on CBS severity and incidence. Tukey's least significant difference (LSD) test (P = 0.05) was used to compare means. The effect of each treatment or spray programme on the fruit rind (phytotoxicity) was evaluated simultaneously. No rating scale was used in determining phytotoxicity, however, evaluations were based on visual appearance of the fruit. A fruit was considered to be affected by the experimental fungicide, irrespective of the size of the rind area affected.

Results and discussion

Objective / Milestone	Achievement
Apr 2018 – Mar 2019 To evaluate any new potential fungicides and alternative spray programmes for the control of citrus black spot.	The experimental spray programmes all reduced CBS significantly. The best results were obtained with the standard programme of strobilurins and mancozeb. The application of Product X with oil also gave very good results but in some cases led to phytotoxicity.

The largest export markets for South African citrus have zero tolerance of CBS on fruit, and therefore, only the criteria of fruit without lesions was used in determining the efficacy of the treatments. The trial site was characterised by a high incidence of citrus black spot, during the 2018-19 season, with the untreated control trees yielding only 25.75% fruit without CBS lesions (Table 4.3.10.1).

Most experimental fungicides and spray programmes significantly reduced CBS infection compared to the untreated control (Table 4.3.10.1). The highest percentage of clean fruit (100%) was achieved with the registered CBS spray programme consisting of mancozeb followed by the application of two sprays of azoxystrobin + mancozeb + mineral spray oil and lastly mancozeb (treatment 4) as well as different experimental fungicides.

The experimental fungicide 'Product X' produced maximum percentage of clean fruit, when applied together with mineral oil (e.g. in treatments 9 and 10). Although results showed that 'Product X' has the ability to significantly reduce CBS infection, when applied timely during the fruit susceptibility period, the fungicide also resulted in phytotoxicity. Discoloured circular marks were observed on one-half of the rind of fruit treated with this fungicide (Figures 4.3.10.1 and 4.3.10.2). The substitution of mineral oil with adjuvants, NuFilm 17 and Entreé, in certain spray programmes also achieved 100% clean fruit (Table 4.3.10.1). No phytotoxicity was evident on fruit treated with treatments incorporating NuFilm 17 as well as on fruit treated with the industry standard CBS spray programmes. However, discoloured marks were observed when double the rate of Entreé was used in a spray programme (Figure 4.3.10.2). The experimental copper fungicide (treatments 22, 23, 38 and 39), also showed the ability to significantly reduce CBS infection. However, seven successive applications of the fungicide caused severe stippling of the fruit rind. Incorporating this copper into a tank mixture with azoxystrobin and mineral oil slightly reduced stippling (Figures 4.3.10.1 and 4.3.10.2).

Also noteworthy, among other treatments, is treatment 24 which also produced 100% clean fruit. In this treatment, the usual first mancozeb spray in normal CBS spray programmes was replaced with a mancozeb-strobilurin-mineral oil tank mixture and a second mancozeb-strobilurin-mineral oil tank mixture was applied before two mancozeb sprays were applied (Table 4.3.10.1).

Table 4.3.10.1. Evaluation of spray programmes for the control of Citrus black spot conducted at Crocodile Valley Citrus Co., Nelspruit, Mpumalanga during the 2018-19 season

Treatments		Dosage (g/ml per 100L water tank mixture)	Average number of fruit with CBS lesions[s]		
			0 lesions (%)	1-3 lesions (%)	≥4 lesions (%)
1	Untreated control		25.75 b	20.00a	54.25a
2	Mancozeb[t]	200g	98.00 a	1.75 b	0.25 b
3	Copper oxychloride[u]	200g	99.25 a	0.25 c	0.50 b
4	Mancozeb/mancozeb+azoxystrobin+mineral oil/ mancozeb+azoxystrobin+mineral oil /mancozeb[v]	200g/150g+20ml+250ml/ 150g+20ml+250ml/200g	100.00 a	0.00 b	0.00 b
5	Zinc oxide (x5)[w]	300ml	76.00 a	7.00 abc	17.00 b
6	Zinc oxide (x5)[w]	600ml	91.00 a	3.50 bc	5.50 b
7	Cuprous oxide + zinc oxide (x4)[x]	150g	93.25 a	4.50 b	2.25 b
8	Cuprous oxide + zinc oxide (x4)[x]	300g	99.00 a	1.00 b	0.00 b
9	'Product X' 100 SC + mineral oil (x4)[y]	120ml+250ml	100.00 a	0.00 b	0.00 b
10	'Product X' 100 SC + mineral oil (x4)[y]	150ml+250ml	100.00 a	0.00 b	0.00 b
11	Mancozeb/ 'Product X' + mineral oil/'Product X' + mineral oil/mancozeb[z]	200g/150ml+250ml/ 150ml+250ml/200g	99.25 a	0.50 b	0.25 b
12	'Product X' 100 SC + mineral oil (x4)[y]	180ml+250ml	99.00 a	0.25 b	0.75 b
13	'Product Y' + mineral oil/ mancozeb+azoxystrobin+mineral oil/ mancozeb+azoxystrobin+mineral oil /mancozeb[z]	120ml+250ml/150g+20ml+250ml/ 150g+20ml+250ml/200g	99.000 a	1.000 c	0.000 b
14	'Product Y' + mineral oil/ mancozeb+azoxystrobin+mineral oil/ mancozeb+azoxystrobin+mineral oil /mancozeb[rx]	150ml+250ml/150g+20ml+250ml/ 150g+20ml+250ml/200g	100.000 a	0.000 c	0.000 b
15	Mancozeb/mancozeb + azoxystrobin + mineral oil/ mancozeb + azoxystrobin + mineral oil/mancozeb[z]	200g/ 150g + 20ml + 250 ml/ 150g + 20ml + 250ml/ 200g	99.50 a	0.25 b	0.25 b
16	'Product Y' + mineral oil/ mancozeb+azoxystrobin+mineral oil/ mancozeb+azoxystrobin+mineral oil /mancozeb[ry]	180ml+250ml/150g+20ml+250ml/ 150g+20ml+250ml/200g	100.000 a	0.000 c	0.000 b
17	'Product X' + Aidsee C80W (x4)[w]	120ml+30ml	99.75 a	0.00 b	0.25 b

18	'Product X' + Aidsee C80W (x1)[ry]	120ml+30ml	25.250 b	23.750 a	51.000 a
19	Mancozeb/mancozeb+azoxystrobin+Wetcit/ Mancozeb+azoxystrobin+Wetcit/mancozeb[z]	200g/150g+20ml+100ml/ 150g+20ml+100ml/200g	99.750 a	0.250 c	0.000 b
20	Mancozeb/mancozeb+azoxystrobin+Wetcit/ Mancozeb+azoxystrobin+Wetcit/mancozeb[z]	200g/150g+20ml+200ml/ 150g+20ml+100ml/200g	99.000 a	0.250 c	0.750 b
21	Mancozeb/mancozeb+azoxystrobin+Wetcit/ Mancozeb+azoxystrobin+Wetcit/mancozeb[z]	200g/150g+20ml+400ml/ 150g+20ml+400ml/200g	100.000 a	0.000 c	0.000 b
22	Experimental copper (x7)[sx]	300ml	94.00 a	2.75 b	3.25 b
23	Experimental copper (x7)[sx]	600ml	98.00 a	1.25 b	0.75 b
24	Mancozeb+azoxystrobin+mineral oil/ mancozeb+azoxystrobin+mineral oil /mancozeb/mancozeb[sy]	150g+20ml+250ml/ 150g+20ml+250ml/200g/200g	100.000 a	0.000 c	0.000 b
25	Mancozeb/mancozeb + ortiva/ mancozeb + ortiva/ mancozeb[z]	200g/ 150g + 20ml/ 150g + 20ml/ 200g	97.75 a	1.50 b	0.750 b
26	Mancozeb/mancozeb + azoxystrobin + NuFilm 17/ mancozeb + azoxystrobin + NuFilm 17/ mancozeb[z]	200g/ 75g + 10ml + 15ml/ 75g + 10ml + 15ml/ 200g	98.75 a	0.50 b	0.750 b
27	Mancozeb/mancozeb + azoxystrobin + NuFilm 17/ mancozeb + azoxystrobin + NuFilm 17/ mancozeb[z]	200g/ 150g + 20ml + 15ml/ 150g + 20ml + 15ml / 200g	100.00 a	0.00 b	0.00 b
28	Mancozeb/mancozeb + azoxystrobin + Entreé/ mancozeb + azoxystrobin + Entreé/ mancozeb[sz]	200g/ 75g + 10ml + 20ml/ 75g + 10ml + 20ml/ 200g	79.75 a	1.250 b	19.00 b
29	Mancozeb/mancozeb + azoxystrobin + Entreé/ mancozeb + azoxystrobin + Entreé/ mancozeb[sz]	200g/ 150g + 20ml + 20ml/ 150g + 20ml + 20ml / 200g	100.00 a	0.00 b	0.00 b
30	Mancozeb/mancozeb + pyraclostrobin/ mancozeb + pyraclostrobin/ mancozeb[z]	200g/ 150g + 10ml/ 150g + 10ml/ 200g	100.00 a	0.00 b	0.00 b
31	Mancozeb/mancozeb + pyraclostrobin + NuFilm 17/ mancozeb + pyraclostrobin + NuFilm 17/ mancozeb[z]	200g/ 150g + 10ml + 15ml/ 150g + 10ml + 15ml / 200g	99.75 a	0.25 b	0.00 b
32	Mancozeb/mancozeb + pyraclostrobin + NuFilm 17/ mancozeb + pyraclostrobin + NuFilm 17/ mancozeb[z]	200g/ 75g + 5ml + 15ml/ 75g + 5ml + 15ml / 200g	100.00 a	0.00 b	0.00 b

33	Mancozeb/mancozeb + pyraclostrobin + mineral oil/mancozeb + pyraclostrobin + mineral oil/mancozeb[sz]	200g/ 150g + 10ml + 250ml/ 150g + 10ml + 250ml/ 200g	100.00 a	0.00 b	0.00 b
34	Mancozeb/mancozeb + pyraclostrobin + Entreé/ mancozeb + pyraclostrobin + Entreé/ mancozeb[sz]	200g/ 75g + 5ml + 20ml/ 75g + 5ml + 20ml / 200g	99.00 a	0.75 b	0.25 b
35	Mancozeb/mancozeb + pyraclostrobin + Entreé/ mancozeb + pyraclostrobin + Entreé/ mancozeb[sz]	200g/ 150g + 10ml + 20ml/ 150g + 10ml + 20ml/ 200g	99.75 a	0.00 b	0.25 b
36	Dipotassium phosphate WP (DPP)/ DPP + experimental pyraclostrobin + mineral oil/ DPP + experimental pyraclostrobin + mineral oil/ DPP[z]	35g/35g+10ml+250ml/ 35g+10ml+250ml/35g	82.50 a	1.00 b	16.50 b
37	Dipotassium phosphate WP/ DPP + experimental pyraclostrobin + mineral oil/ DPP + experimental pyraclostrobin + mineral oil/ DPP[z]	70g/70g+10ml+250ml/ 70g+10ml+250ml/70g	99.25 a	0.25 b	0.50 b
38	Experimental copper/experimental copper + azoxystrobin +mineral oil/experimental copper + azoxystrobin +mineral oil/experimental copper[tx]	300ml/ 300ml + 20ml + 250ml/ 300ml + 20ml + 250ml/ 300ml	99.00 a	0.50 b	0.50 b
39	Experimental copper / experimental copper + azoxystrobin +mineral oil/experimental copper + azoxystrobin +mineral oil/ experimental copper[tx]	600ml/ 600ml + 40ml + 250ml/ 600ml + 40ml + 250ml/ 600ml	93.75 a	1.75 b	4.50 b
40	Mancozeb/mancozeb + azoxystrobin + NuFilm 17/ mancozeb + azoxystrobin + NuFilm 17/ mancozeb[z]	200g/ 300g + 40ml + 30ml/ 300g + 40ml + 30ml/ 200g	100.00 a	0.00 b	0.00 b
41	Mancozeb/mancozeb + azoxystrobin + Entreé/ mancozeb + azoxystrobin + Entreé/ mancozeb[z]	200g/ 300g + 40ml + 40ml/ 300g + 40ml + 40ml/ 200g	99.25 a	0.75 b	0.00 b
42	Mancozeb/mancozeb + pyraclostrobin + NuFilm 17/ mancozeb + pyraclostrobin + NuFilm 17/ mancozeb[sz]	200g/ 300g + 20ml + 30ml/ 300g + 20ml + 30ml/ 200g	100.00 a	0.00 b	0.00 b
43	Mancozeb/mancozeb + pyraclostrobin + Entreé/ mancozeb + pyraclostrobin + Entreé/ mancozeb[sz]	200g/ 300g + 20ml + 40ml/ 300g + 20ml + 40ml/ 200g	100.00 a	0.00 b	0.00 b
44	Cuprous oxide + zinc oxide/ cuprous oxide + zinc oxide + experimental pyraclostrobin + mineral oil/cuprous oxide + zinc oxide + pyraclostrobin + mineral oil/ cuprous oxide + zinc oxide[ty]	75g/75g+10ml+250ml/ 75g+10ml+250ml/75g	100.00 a	0.00 b	0.00 b
45	Cuprous oxide + zinc oxide/ cuprous oxide + zinc oxide + experimental pyraclostrobin + mineral oil/	150g/150g+20ml+250ml/ 150g+20ml+250ml/150g	99.25 a	0.75 b	0.00 b

	cuprous oxide + zinc oxide + experimental pyraclostrobin + mineral oil/ cuprous oxide + zinc oxide[ty]				

[s]Means followed by the same letter in the same column do not differ significantly (P = 0.05) according to Tukey's least significant difference test.

[t]Spray dates were: 8 October 2018; 5 November 2018; 26 November 2018; 21 December 2018 and 15 January 2019.

[u]Spray dates were: 8 October 2018; 12 November 2018; 12 December 2018 and 16 January 2019.

[v]Spray dates were: 8 October 2018; 5 November 2018; 12 December 2018 and 23 January 2019.

[w]Spray dates were: 19 October 2018; 16 November 2018; 12 December 2018; 22 January 2019 and 6 February 2019.

[x]Spray dates were: 8 October 2018; 5 November 2018; 12 December 2018 and 16 January 2019.

[y]Spray dates were: 8 October 2018; 19 November 2018; 31 December 2018 and 11 February 2019.

[z]Spray dates were: 8 October 2018; 5 November 2018; 26 November 2018; 21 December 2018 and 15 January 2019.

[rx]Spray dates were: 8 October 2018; 26 November 2018; 9 January 2019; 20 February 2019.

[ry]Spray dates were: 8 October 2018; 3 December 2018; 14 January 2019; 25 February 2019.

[rz]Spray dates were: 8 October 2018.

[sx]Spray dates were: 8 October 2018; 29 October 2018; 19 November 2018; 12 December 2018; 4 January 2019; 25 January 2019 and 15 February 2019.

[sy]Spray dates were: 8 October 2018; 19 October 2018; 31 December 2018; 25 January 2019.

[sz]Spray dates were: 8 October 2018; 9 November 2018; 21 December 2018 and 1 February 2019. Treatments were not applied on 5 November or a few following days because of rain.

[tx]Spray dates were: 8 October 2018; 29 October 2018; 12 December 2018 and 23 January 2019.

[ty]Spray dates were: 8 October 2018; 12 November 2018; 21 December 2018 and 1 February 2019.

Conclusions to date

Despite the high disease pressure in the trial site, during the 2018-2019 season, most of the experimental treatments and tank mixtures were successful in controlling citrus black spot. With such encouraging results, several of these fungicides and both NuFilm 17 and Entreé should be investigated further with the ultimate goal of registering them for the control of CBS, especially if mancozeb will be banned for use on citrus in the EU during the next few months. However, further trials should determine the effect of 'Product X' and experimental copper applied at lower rates and longer intervals, respectively, on phytotoxicity and CBS control.

Technology transfer

Talks at study groups and CRI IPM and Disease Management workshops.

Future objectives and work plan

Research in the future will consist of further evaluation of the promising spray programmes as well as fungicides identified during the 2018-2019 season. There is still a lot of market pressure on several of the most effective fungicides, such as benzimidazoles and dithiocarbamates. Alternatives should be identified and developed.

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Kotzé, J. M. 1981. Epidemiology and control of citrus black spot in South Africa. Plant Dis. 65:945-950.

4.3.11 **PROGRESS REPORT: Susceptibility period of sweet orange fruit to *Phyllosticta citricarpa* in commercial orchards**

Project 1186 (2017/04 - 2020/03) by Providence Moyo, S. Masikane and Paul H. Fourie (CRI)

Summary

Citrus black spot is one of the most important fungal diseases of citrus worldwide. The disease is characterized by a long latency period in which symptoms may not appear until fruit ripening and its severity depends on a number of factors including the age of the fruit at the time of infection. It has been demonstrated that fruit becomes resistant to CBS infection with maturity, *i.e.* ontogenic resistance development; however, recent research from Brazil has indicated that fruit is susceptible to infection for longer periods than previously assumed and thus, the need for longer periods of fruit protection have been proposed. To quantitatively and more conclusively demonstrate ontogenic resistance development of citrus fruit to *P. citricarpa* infection, fruit in commercial Valencia orchards were inoculated with different concentrations (10^1, 10^3 and 10^5 conidia/mL) of *P. citricarpa* suspensions on a monthly basis or exposed to *P. citricarpa* natural infection at different times through a staggered spray programme. The general trend observed is a decrease in the incidence and severity of the disease as fruit matures.

Opsomming

Sitrus swartvlek is wêreldwyd een van die belangrikste swamsiektes van sitrus. Die siekte word deur 'n lang latente periode gekenmerk, waartydens simptome moontlik nie voor vrugrypwording sigbaar word nie, en die erns van die siekte van 'n aantal faktore afhanklik is, insluitend die ouderdom van die vrug tydens infeksie. Daar is al aangetoon dat vrugte met rypheid weerstandbiedend teen SSV-infeksie word, dit is ontogeniese weerstandsontwikkeling. Onlangse navorsing in Brasilië het egter aangetoon dat vrugte vir langer periodes vatbaar vir infeksie is as wat voorheen aangeneem is, en gevolglik is die behoefte vir langer periodes van vrugbeskerming voorgestel. Ten einde ontogeniese weerstandsontwikkeling van sitrusvrugte teen *P. citricarpa* infeksie kwantitatief en onweerlegbaar te demonstreer, is vrugte in kommersiële Valencia boorde met verskillende konsentrasies (10^1, 10^3 en 10^5 konidia/mL) van *P. citricarpa* suspensies op 'n maandelikse basis

geïnokuleer, of aan *P. citricarpa* natuurlike infeksie op verskillende tye deur ŉ trapsgewyse ("staggered") spuitprogram blootgestel. Die algemene tendens wat gedurende evaluasie van die inokulasieproef waargeneem is, is ŉ afname in die voorkoms en erns van die siekte soos wat die vrugte ryp word. Resultate vanaf die trapsgewyse spuitproewe dui daarop dat die beskerming van die vrugte ná Maart, nie tot ŉ afname in die voorkoms van die siekte onder hierdie toestande sal lei nie.

4.3.12 PROGRESS REPORT: Influence of shade nets on Alternaria brown spot and citrus black spot: comparing epidemiological model output for covered (under shade nets) and uncovered (normal/open) orchards' weather datasets

Project 1187 (2017/04 - 2020/03) by Providence Moyo, Siyethemba L. Masikane and Paul H. Fourie (CRI)

Summary

The use of shade/hail nets is increasing in South African orchards. These nets are mainly to mitigate the loss of yield due to detrimental climatic conditions including extreme temperatures, hailstorms and high winds. They also protect crops against insects and birds. The use of hail nets can, however, lead to the modification of the orchard microclimate, in particular humidity and temperature, which are crucial in the growth and development of pathogens such as *Phyllosticta citricarpa*, *Alternaria alternata* and *Botrytis cinerea*, which cause citrus black spot (CBS), Alternaria brown spot (ABS) and Botrytis, respectively. Thus, the use of shade nets can directly or indirectly affect the development of these diseases in citrus orchards. Only disease predictions from the Nadorcott orchard in Nelspruit were analysed for the 2019/2020 season. A high number of 3-hourly periods (22) with possible CBS pycnidiospore infections were recorded in the orchard under the net compared to 17 3-hourly periods outside the net, but the number of days with possible ascospore infection were the same between the two orchards. High ABS and Botrytis infections were predicted under the net compared to outside the net. Data from Patensie have not yet been received.

Opsomming

Die gebruik van skadu-/haelnette neem toe in Suid-Afrikaanse boorde. Hierdie nette is hoofsaaklik om die verlies aan opbrengs weens skadelike klimaatstoestande, insluitend uiterste temperature, haelstorms en sterk winde, te versag. Hulle beskerm ook gewasse teen insekte en voëls. Die gebruik van haelnette kan egter tot die verandering van die boord se mikroklimaat lei, veral humiditeit en temperatuur, wat noodsaaklik is in die groei en ontwikkeling van patogene soos *Phyllosticta citricarpa*, *Alternaria alternata* en *Botrytis cinerea*, wat onderskeidelik sitrusswartvlek (SSV), Alternaria bruinvlek (ABV) en Botrytis, veroorsaak. Die gebruik van skadunette kan dus, direk of indirek, die ontwikkeling van hierdie siektes in sitrusboorde affekteer. Slegs siekte-voorspellings van die Nadorcott boord in Nelspruit is vir die 2019/2020 seisoen geanaliseer. ŉ Hoë getal van 3-uurlikse periodes (22) met moontlike piknidiospoor-infeksies is in die boord onder net aangeteken, in vergelyking met 17 3-uurlikse periodes buite die net, maar die aantal dae met moontlike askospoor-infeksie was dieselfde tussen die twee boorde. Meer ABV infeksies is onder die net voorspel in vergelyking met buite die net. Data vanaf Patensie is nog nie ontvang nie.

4.3.13 PROGRESS REPORT: Management of pruning debris as part of citrus black spot control strategy

Project 1223 (2019/04 – 2021/12) by P. Moyo and P.H. Fourie (CRI)

Summary

Initially, two orchards (lemon orchard in Hoedspruit and Valencia orchard in Nelspruit) had been identified as trial sites for investigating the suitability of chopped pruning debris as a source of CBS inoculum. However, the Valencia orchard in Nelspruit could not be used as a trial site because the grower decided not to prune trees since the orchard was to be uprooted after harvest in 2020. Chopped and un-chopped pruning debris were collected from the orchard in Hoedspruit, placed in wire frames and left under the trees in the orchard to undergo decomposition. Naturally fallen leaves were also collected. The decomposing leaves were sampled at monthly intervals from December 2019 to February 2020 and sent to DAFF in Stellenbosch, for analysis for

the presence of ascospores of *Phyllosticta* using a Kotzé inoculum monitor. We are still awaiting the results of the analysis.

Opsomming

Aanvanklik is twee boorde (suurlemoenboord in Hoedspruit en Valencia boord in Nelspruit) geïdentifiseer as proefpersele vir die ondersoek na die geskiktheid van opgekapte snoeisels as bron van SSV inokulum. Die Valencia boord in Nelspruit kon egter nie as proefperseel gebruik word nie omdat die produsent besluit het om nie die bome te snoei nie, aangesien die boord ná oes in 2020 uitgehaal sou word. Opgekapte en nie-opgekapte snoeisels is van die boord in Hoedspruit versamel, in draadrame geplaas, en onder die bome in die boord gelos om te ontbind. Natuurlik afgevalde blare is ook versamel. Die ontbinde blare is in maandelikse intervalle vanaf Desember 2019 tot Februarie 2020 versamel en na *DAFF* in Stellenbosch gestuur, vir analise vir die teenwoordigheid van askospore van *Phyllosticta*, deur gebruik te maak van 'n Kotzé inokulum monitor. Ons wag nog vir die resultate van die analise.

4.3.14 **PROGRESS REPORT: Further validation and improvements of CRI-PhytRisk**

Project RCE-2-12 (1238) (Apr 2019 – Mar 2022) by Providence Moyo and Paul H. Fourie (CRI)

Summary

CRI-PhytRisk is a CBS-risk management platform which allows improved decision support to citrus growers, in CBS areas, to improve fungicide spray timing as well as indicate CBS-risk on fruit destined for export based on the past season's weather conditions and CBS infection predictions. To enable forecasting of the CBS risks of each orchard and therefore help growers make informed decisions on market destinations, it is important that CRI-PhytRisk be linked with PhytClean (Integrated Information Management System for the South African citrus industry). A software development company (Paltrack) has been asked to start with the programming phase in which PUC and orchard data of each grower (PhytRisk user) is ported from eCert. CRI-PhytRisk will have a data input page where growers can input their spray records so that the daily CBS risk is overlaid with information showing whether the orchard was protected by CBS sprays or not. Further developments include the successful integration of the Botrytis forecast model into CRI-PhytRisk.

Opsomming

CRI-PhytRisk is 'n SSV-risiko bestuurplatform wat verbeterde besluitneming ondersteuning aan sitrusprodusente in SSV areas verleen, om fungisiedspuit tydsberekening te verbeter, asook om SSV risiko op vrugte wat vir uitvoer bestem is, aan te dui, gebaseer op die vorige seisoen se weerstoestande en SSV infeksie voorspellings. Ten einde voorspelling van die SSV risiko's van elke boord moontlik te maak, en sodoende produsente te help om ingeligte besluite oor markbestemmings te maak, is dit belangrik dat CRI-PhytRisk aan PhytClean (Geïntegreerde Inligting Bestuursisteem vir die Suid-Afrikaanse sitrus-industrie) gekoppel moet wees. 'n Sagteware ontwikkeling maatskappy (Paltrack) is gevra om met die programmeringsfase te begin waarin PUC en boorddata van elke produsent (PhytRisk gebruiker) vanaf die elektroniese sertifiseringsdatabasis oorgedra word. CRI-PhytRisk sal 'n data invoerblad hê waar produsente hul spuitrekords kan invoer, sodat die daaglikse SSV risiko oorlê word met inligting wat aandui of die boord met SSV spuite beskerm was of nie. Verdere ontwikkelings sluit die suksesvolle programmering van die Botrytis voorspellingsmodel in CRI-PhytRisk in; hierdie model word tans met boord data gevalideer.

4.3.15 **PROGRESS REPORT: Comparison of the rind phytochemistry and wax composition of CBS resistant and susceptible citrus cultivars**

Project 1242 (2019/20) Melida Mabogoane, Puseletso Tswaai, Wilma Augustyn (TUT), Obiro Wokadala (ARC), and Wilma du Plooy (CRI)

Summary

Extraction and characterisation of the phenolic compounds from the rind of 'Eureka' lemons, 'Bitter Seville' sour orange, 'Late Valencia' sweet orange, 'Nagami' kumquats and 'Tahiti' lime throughout all stages of

development, were done. Fruit samples from the five different citrus types have been collected from May 2017 until the end of the 2019 season. An efficient method for the extraction of total phenolic compounds was developed, with five solvent mixtures screened to determine the most suitable solvent for the extraction of soluble phenolic compounds. The highest yields of phenolic compounds were extracted using methanol: acetone: water (7:7:1 v/v/v). These extracts are being used for total phenolic content (TPC) determinations, using HPLC and UPLC analysis. Total phenolic contents were also determined in triplicate by the Folin-Ciocalteu method, and the TPC calculated and referenced to a gallic acid standard curve. The volatile oil of citrus orange rinds was obtained by hydro distillation (HD) and solvent extraction and samples were analysed by GC–MS. To achieve better separation and identification of as many components as possible, two extraction methods were tested. Several constituents such as ∝-pinene, ∝-terpene, limonene were typical in all citrus oil, with limonene identified as the major compound. Some of the identified compounds possess important bioactivities. The results showed a gradual decrease of TPC with fruit maturity, being highest at the immature fruit stage and decreasing as the fruit matures. A solvent extraction method was developed and a total of 89 polar compounds were identified, with n-alkaloid acids, alkanes, alkenes, esters, ethers and sugars, but the isolates were dominated by the presence of homologous series of long chain-aliphatic compounds. The chromatographic separation was problematic due to the large number of compounds identified from the samples. In order to address this, a CG programme with increased run time was developed to ensure sufficient chromatographic separation. Future extraction of volatile compounds will be conducted using HD as this method has already resulted in 41 compounds being identified.

Opsomming

Die ekstraering en karakterisering van die fenoliese verbindings in die skil van ‘Eureka’ suurlemoene, ‘Bitter Seville’, ‘Laat Valencia’ lemoene, ‘Nagami’ kumkwarte en ‘Tahiti’ lemmetjies deur alle groeistadiums was gedoen. Vrugmonsters was geneem van al vyf sitrustipes vanaf Mei 2017 tot die einde van die 2019 seisoen. ŉ Effektiewe ekstraksietegniek vir die totale fenoliese inhoud was ontwikkel, met vyf verskillende oplosmiddelmengsels wat getoets was om die beste een vir die ekstraering van oplosbare fenoliese verbindings (OFV) te bepaal. Die beste opbrengs van OFV was behaal met ŉ methanol:asetoon:water mengsel (7:7:1 v/v/v). Hierdie ekstrakte word gebruik vir OFV bepalings deur middel van HPLC en UPLC tegnieke. Totale fenoliese inhoudsbepalings is ook in triplikaat met die Folin-Ciocalteu metode gedoen, met die fenoliese inhoud bereken en verwys na ŉ galleïensuur standaardkurwe. Die vlugtige olies in die sitrusskille is deur hidrodistillasie (HD) en oplosmiddelekstrakie bekom, en geanaliseer deur GC-MS. Om verbeterde skeiding en identifikasie van soveel verbindings as moontlik te kry, is twee verskillende ekstraksiemetodes gebruik. Verskeie bydraende verbindings soos ∝-pineen, ∝-terpeen en limoneen is tipies van alle sitrusolies, met limoneen die hoofkomponent. Sommige van die geïsoleerde verbindings toon bioaktiwiteit. Die resultate toon ook ‘n geleidelike afname in OFV met vrugrypheid, waar die PFV die hoogste is in onvolwasse vrugte en afneem na mate die vrute ryp word. ŉ Oplosmiddelekstraksie was ontwikkel en ŉ totaal van 89 polêre verbindings geïdentifiseer, met n-alkaloïede sure, alkane, alkene, esters en suikers, maar die isolate was oorheers deur die teeenwoordigheid van lank-ketting alifatiese verbindings. Die chromatografiese skeiding was problematies weens die groot aantal verbindings wat geïdentifiseer moes word. Ten einde die probleem aan te spreek, was ŉ GC program met ŉ langer looptyd ontwikkel sodat genoegsame chromatografiese skeiding plaasvind. In die toekoms sal die ekstraksie van vlugtige stowwe gedoen word met HD aangesien hierdie metode reeds die identifikasie van 41 verbindings tot gevolg gehad het.

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

Project RCE-2-14 (1244) by Providence Moyo, Siyethemba L. Masikane and Paul H. Fourie (CRI)

Summary

Citrus Black Spot (CBS) is the most important fungal disease of citrus in South Africa, because of its quarantine status in major export countries. Epidemiology of CBS is not fully understood, since there is currently no method to distinguish between ascospores of the CBS pathogen and those of the non-pathogenic *P. capitalensis*. Furthermore, actual infection has not been measured yet, although generic infection models are available. The first pycndiospore inoculation trial conducted in Nelspruit was recently evaluated. No symptoms

have been observed yet on the inoculated leaves of the second trial and therefore, it was decided to incubate the trees for a little longer. There has been a breakthrough with the production of *P. citricarpa* ascospores in culture but these were produced only in very low numbers. Ascospore production in culture is continuing to try to get enough ascospores for inoculation trials and infection studies. The *P. capitalensis* isolate did not grow when cultured and therefore, the optimisation of the qPCR protocol has not been done yet. We are still trying to source a viable culture of *P. capitalensis* to begin with qPCRs.

Opsomming

Sitrusswartvlek (SSV) is die belangrikste swamsiekte van sitrus in Suid-Afrika, weens sy kwarantynstatus in belangrike uitvoerlande. Epidemiologie van SSV word nie ten volle verstaan nie, aangesien daar tans geen metode is om tussen askospore van die SSV patogeen en dié van die nie-patogeniese *P. capitalensis* te onderskei nie. Verder is werklike infeksie nog nie gemeet nie, hoewel generiese infeksiemodelle beskikbaar is. Die eerste piknidiospoor inokulasieproef wat in Nelspruit uitgevoer is, is onlangs geëvalueer. Geen simptome is nog op die geïnokuleerde blare van die tweede proef waargeneem nie en daar is dus besluit om die bome vir nog ŉ rukkie te inkubeer. Daar was ŉ deurbraak met die produksie van *P. citricarpa* askospore in kultuur, maar hierdie is slegs in baie lae getalle geproduseer. Askospoor produksie in kultuur gaan steeds voort ten einde te probeer om genoeg askospore vir inokulasieproewe te verkry, asook vir infeksiestudies. Die *P. capitalensis* isolaat het nie in kultuur gegroei nie en gevolglik is die optimalisering van die qPCR protokol nog nie gedoen nie. Ons poog steeds om ŉ lewensvatbare kultuur van *P. capitalensis* te kry ten einde met qPCRs te kan begin.

4.4 PROGRAMME: POSTHARVEST DISEASES

Programme coordinator: W du Plooy (CRI)

4.4.1 Programme summary

Packhouses were faced with many uncertainties regarding the future of postharvest chemicals available for use in export programmes in 2019. Project 123, the service project in this programme, looked at new and possible alternative products, testing them for efficacy and compatibility with currently used actives. Most of these were still focussed on sanitation, and included an extraordinary number of solutions based on copper as an antimicrobial. None of these copper-based products were effective. Similarly, two phenolic-based products did not offer any efficacy. Two products based on bacteria and their enzymes, had moderate, but variable results. In contrast, a GRAS chemical and a preservative used in other food industries, gave excellent results. A UV-C treatment technology was tested, with good results against the sour rot pathogen, *Galactomyces citri-aurantii*. 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. A new administrative procedure for accepting products for testing will be implemented in 2020.

The use of OPP caused residue problems on fruit stacked on pallet bases treated with the active (Project 1165, PHI 8/19). A major issue is the deposition of SOPP on fruit that was never treated with any chemical actives, but were shipped in containers that may have been exposed to SOPP. This problem affects not only organic fruit, but also fruit where the maximum number of residues are exceeded. The decision to use OPP was taken due to pressure to remove methylbromide as a permissible product in wood treatment. Through the collaboration of the industry and PPECB, it has been determined that the pallets are the most likely source of the OPP contamination. This was done through visiting three different container depots, with 10 storage containers at each, in the Western Cape Province, as well as six cold storage facilities, where swabs were collected and tested for possible OPP detection. A total of 151 swabs were collected from different areas and 150 of these showed zero detection of OPP, and one only had 0,09 ppm OPP detected. This led to the conclusion that the contamination is not coming from the cold storage facility where fruits are stored, nor the containers that are used during shipping. The chemicals that are used to wash the containers also showed zero detection of OPP. However, from the results of 15 samples of wood shavings sent for analysis, it was clear that the problem is the pallets. 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. From a total of 279 isolates, 89 species were identified using PCR techniques, with 120 species still in process. Final confirmation of the 89 species were with sequencing, employing ITS primers 1 and 4. To date, none of the isolates pose any phytosanitary risk.

In project 1198, fungicide management of brown rot, caused by *Phytophthora* spp is investigated. Currently, it relies on preharvest strategies only, with no postharvest remedies registered for the management of this disease. The curative and protective efficacy of azoxystrobin (1125 µg/ml), fludioxonil (598 µg/ml) and potassium phosphite (1500 µg/ml) as aqueous dip treatments for the postharvest management of *Phytophthora* brown rot on different citrus types (lemons, oranges and mandarins) were evaluated. Additionally, azoxystrobin (2500 µg/ml for all three fruit types) and fludioxonil (2300 µg/ml for lemons and 4600 µg/ml for oranges and mandarins) amended wax was evaluated for the prevention of spreading of brown rot (nesting) within cartons during transit. Results indicated that the three tested fungicides have good curative action, reducing brown rot incidence significantly when the fungicide was applied 12 h after inoculation. The protective ability of all three fungicides was better the longer the fungicides remained on the fruit before inoculation. The data obtained from this study can add additional value to the already registered postharvest azoxystrobin and fludioxonil fungicides and preharvest registered potassium phosphite.

A serious potential threat to the South African citrus industry and citrus postharvest treatments, is the loss of a European Union maximum residue level (MRL) for imazalil. Although this is not a reality yet, Project 1250 (PHI-4 27/19) investigates the five registered actives allowed in the European Union. Azoxystrobin, fludioxonil, thiabendazole, pyrimethanil and ortho phenyl phenol were tested for efficacy against *Penicillium digitatum*, and measured against imazalil as the standard. A total of 15 different trials were run throughout the season. Soft citrus, oranges and lemons were used in each of the trials, with all trials done in triplicate and a full repeat. All the trials are done at 22 - 24°C, which is the ambient temperature in the laboratory, the incoming water pH 5,5 – 6, and with an exposure time of 1 minute, with the different variables selected to suit the available conditions and water quality. The trials were run in the custom-built drenchers (140 L capacity). Apart from the curative ability of individual actives against *Penicillium digitatum*, they were also evaluated for sporulation inhibition. In terms of disease control, AZO and PYR did well (70 – 80%), with FLU being more variable (60 – 80%), but still giving reasonable control, while OPP did not do well on any of the three citrus types tested (<30%). To this end OPP was eventually omitted from the trials. The chemicals tested were not able to give 100% sporulation control, with OPP failing altogether. Fludioxonil, did give some variable sporulation control (30 - 50%), and while PYR and AZO were also variable, they were more successful (40 – 80%).

As an extension of the curative and anti-sporulation study, Project 1251 aimed to investigate four fungicides (azoxystrobin, fludioxonil, pyrimethanil and OPP) for their potential to be used as alternatives or replacements for imazalil in wax application. In the stand-alone fungicide treatments, azoxystrobin (AZO) had the best protective control with 98% of control when averaging all fruit types. Percentage control was 83% for fludioxonil (FLU) and 63% for pyrimethanil (PYR). From these results, pyrimethanil did not perform well when compared to the registered concentration of imazalil (IMZ) (94%). In terms of sporulation inhibition, AZO in wax resulted in sporulation inhibition of 54% over all fruits, whereas FLU and pyrimethanil had 83% and 63% control respectively. For combination fungicide treatments AZO+FLU performed the best with 98% protective control. Protective control obtained with FLU+PYR was 92%, and for the PYR+AZO combination, 87%. Considering sporulation inhibition, AZO+FLU delivered the best results with 93% sporulation inhibition. Sporulation inhibition with FLU+PYR was 72%, and 60% with a combination of PYR+AZO. An additional product, Evolve, was also tested in wax, evaluating its protective control. The results indicated that it did not differ significantly from IMZ treatments on all fruit types and a pooled average of 98.5% protective control was obtained over all fruit types. It also gave good anti-sporulation results, inhibiting spore development up to 88%. Although it is a registered option in South Africa, OPP gave poor results, with the pooled average over the three fruit types only 31%, and sporulation inhibition at only 16%, which is why it was dropped from the study early on. Azoxystrobin performed best with a high protective control action at the registered concentration on citrus, while sporulation was largely inhibited at the registered concentration of this fungicide. As far as combination fungicide treatments are concerned, AZO+FLU performed the best for both protective control as well as sporulation inhibition.

Programopsomming

Pakhuise moes in 2019 baie onsekerhede rondom die toekoms van na-oes chemie beskikbaar vir uitvoerprogramme, die hoof bied. Projek 123, die diens projek in die program, het na nuwe en moontlike alternatiewe produkte gekyk, met evaluasie vir effektiwiteit en verenigbaarheid met huidige chemie. Meeste van die produkte is steeds gefokus op sanitasie en het ‘n groot aantal was koper-gebaseerde antimikrobiese formulasies. Nie een van hierdie koper-gebaseerde formulasies was effektief nie. Soortgelyk was twee produkte gebaseer op fenoliese verbindings, ook oneffektief. Twee produkte waarvan die werking gebaseer is op bakterië en hulle ensieme, het wisselende resultate gelewer. In teenstelling het ‘n GRAS produk, asook ‘n preserveermiddel wat in ander dele van die voedselindustrie gebruik word, uitstekende resultate gelewer. Tegnologie vir UV-C behandeling het ook goeie resultate gelewer teen die suurvrot patogeen, *Galactomyces citri-aurantii*. Die assesering van saniteermiddels sal voortgaan, aangesien die toenemende druk op na-oes chemie beteken sanitasie in na-oes siektebestuur word krities belangrik. ŉ Nuwe administratiewe prosedure vir die inname van toetsprodukte word in 2020 geimplimenteer.

Die gebruik van ortofenielfenol (OPP) op hout vir palletbasisse is problematies vir die vrugte wat daarop gestapel word (Projek 1165, PHI 8/19). Die hoofprobleem is vrugte wat nog nooit met die aktief behandel was nie, maar wat residue daarvan toon op die invoermarkte. Herdie raak nie net organiese vrugte nie, maar ook vrugte met ‘n maksimum aantal residue, wat dan tipies oorskry word. Die besluit om OPP te gebruik was teweeggebring deur die onttrekking van metielbromied as ‘n houtbehandeling. Deur die samewerking van die industrie en PPECB was bepaal dat die palette die mees waarskynlike bron van die residue is. Hierdie gevolgtrekking was bereik nadat drie verskillende behoueringsdepots met 10 behoueringseenhede by elk, asook ses kouestoorfasiliteite besoek was en daar uit 151 oppervlakmonsters slegs een was met 0,09 ppm OPP daarop. Verder het die monsters wat geneem was van die skoonmaakmiddels wat gebruik word vir die was van die behoueringseenhede, ook negatief getoets. Daarteenoor het die 15 monsters van pallethoutskaafsels positief getoes vir OPP, wat beteken dit is die bron van die probleem. Die toenemende voorkoms van fungi op die palletbassisse en die moontlike houtdegradering laat twee bekommernisse: (1) potensieële strukturele verswakking en (2) moontlike fitosanitêre risikos. Uit ‘n totaal van 279 isolate is 89 spesies to dusver geidentifiseer deur middel van polimerasekettingreaksie tegnieke, met nog 120 spesies in proses. Die finale bevestiging van identifikasie was gedoen met volgordebepaling, waar ITS voorvoeg 1 en 4 ingespan was. Tot hede toe is geen fitosanitêre risiko geïdentifiseer nie.

In projek 1198 is die fungisiedbestuur van bruinvrot in Suid-Afrika ondersoek. Tans bestaan dit slegs uit voor-oes strategieë, met niks vir die na-oes bestuur van hierdie siekte geregistreer nie. De uitwissende en beskermende effektiwiteit van azoxystrobin (1125 µg/ml), fludioxonil (598 µg/ml) en kaliumfosfiet (1500 ug/ml) as waterige doopbehandelings vir die na-oes bestuur van *Phytophthora* bruinvrot op verskillende sitrustipes (suurlemoene, lemoene en mandaryne) was ge-evalueer. Addisioneel, is azoxystrobin (2500 µg/ml vir al drie vrugtipes) en fludioxonil (2300 µg/ml vir suurlemoene en 4600 µg/ml vir lemoene en mandaryne) gewysigde waks, vir die voorkoming van die verspreiding van bruinvrot kruiskontaminasie, binne kartonne gedurende vervoer, geëvalueer. Resultate het aangetoon dat die drie getoetste fungisiedes goeie uitwissende aksie gehad het, wat die voorkoms van bruinvrot betekenisvol verminder het wanneer die fungisied 12 ure ná inokulasie toegedien is. Verer is bevind dat die beskermende vermoë van al drie fungisiedes was beter hoe langer die fungisiedes op die vrug gebly het vóór inokulasie. Die data wat vanuit hierdie studie verkry is, kan addisionele waarde tot die reeds geregistreerde na-oes azoxystrobin en fludioxonil fungisiedes en voor-oes geregistreerde kaliumfosfiet toevoeg.

ŉ Ernstige bedreiging vir die Suid-Afrikaanse sitrus industrie en na-oesbehandelings, is die verlies van ŉ maksimum residuvlak toelating (MRL) vir imazalil. Alhoewel dit nog nie realitieit is nie, is Projek 1250 (PHI 27/19) vroegtydig in die studie van die vyf geregistreerde aktiewes wat wel in Europa toegelaat word. Azoxystrobien (AZO), fludioksonil (FLU), thiabendasool (TBZ), pirimethaniel (PYR) en OPP was getoets vir hulle effektiwiteit teen *Penicillium digitatum*, en gemeet teen imazalil as standaard. ‘n Totaal van15 proewe was gedoen deur die seisoen. Sagte sitrus, suurlemoen en lemoene was ingelsuit vir elke proef, met alle proewe was in triplikaat, en ŉ volledig herhaling deur die loop van die seisoen. Al die proewe is gedoen by 22 - 24°C wat die laboratorium se temperatuur is, ŉ inkomende water pH van 5,5 – 6 en ŉ blootstellingstyd van 1 minuut. Die veranderlikes is dus so gekies om die beskikbare omgewingsfaktore in ag te neem. Buiten vir die genesende vermoeë van die individuele aktiewes teen *Penicillium digitatum*, was hulle ook geëvalueer vir hul vermoeë tot sporulasie inhibisie. In terme van siektebeheer het AZO en PYR goed gedoen (70 – 80%), met meer varieërende resultate vir FLU (60 – 80%), maar steeds aanvaarbaar; OPP het egter nie dieselfde mate van beheer gegee nie (<30%). Vir hierdie rede is dit uiteindelik weggelaat uit die studie. Nie een van die aktiewes het 100% sporulasiebeheer gegee nie, met OPP wat weereens heeltemal gefaal het. Fludioksonil het varieënde sporulasiebeheer gegee (30 - 50%), terwyl PYR en AZO, alhoewel varieërend, beter gevaar het met 40 – 80% beheer. Die moontlike sinergistiese werking van kombinasies met die aktiewes word in 2020 ondersoek.

In ʼn verlenging van Projek 1250 was ʼn studie gedoen op die vermoeë van AZO, FLU, PYR en OPP om as beskermende alternatiewe vir imazalil in waks gebruik te word (Projek 1251). In die beskermende aktiwiteitsproewe met alleenstaande aktiewes het AZO die beste gevaar met gemiddeld 98% oor al die vrugtipes. Die persentasie beheer vir FLU was 83% en vir PYR, 63%. Uit hierdie resultate is dit duidelik dat PYR nie so goed gevaar het as die IMZ kontrole nie (94%). In terme van sporulasiebeheer, het AZO 54% beheer getoon, met FLU 83% en PYR 63% beheer. Wat betref kombinasiebehandelings, het AZO+FLU die beste gevaar met 98% beskermende beheer, 92% vir FLU+PYR en 87% vir AZO+PYR. Ten opsigte van sporulasie inhibisie, het AZO+FLU die beste gevaar (93%), gevolg deur FLU+PYR met 72% en AZO+PYR met 60%. ʼn Addisionele produk, Evolve, was ook in waks getoets vir moontlike beskermende behaar. Die resultate het aangedui dat dit nie noemenswaardig van IMZ verskil nie, met 98,5% beheer gemiddeld oor al die sitrustipes. Wat sporulasie inhibisie betref, het dit tot 88% inhibisie getoon. Alhoewel dit ʼn geregistreerde opsie is in Suid Afrika, was OPP uiteindelik weggelaat weens die feit dat dit slegs 31% beskerming kon gee, en 16% sporulasie inhibisie. Azoxytrobien het uitgestaan met die beste beskermende en sporulasie beheer teen die geregistreede dosisse. In terme van die kombinasiebehandelings, het AZO+FLU die beste gevaar in beide aspekte van beheer.

4.4.2 PROGRESS REPORT: Provision of an industry service whereby new packhouse treatments are comparatively evaluated, fungicide resistance is monitored and standardised recommendations are provided

Project 123 (Ongoing) by Wilma du Plooy, Lindokuhle Mamba and Jan van Niekerk (CRI)

Summary

Several products were tested and evaluated against industry standards. The most successful of these products were two GRAS compounds, namely cinnamaldehyde, a natural plant extract, as well as a food preservative, natamycin. Both of these, unfortunately, have to address regulatory hurdles before commercialisation in the citrus industry. The results obtained, however, do encourage further development of the actives. An unusual number of copper-based products were received for evaluation as water sanitisers. None of them was successful in diminishing any spore loads in the trials. No new peroxyacetic acid formulations were received, but a new technology using UV-C did open up a new opportunity when it proved successful against sour rot. No ring tests were conducted, but the tests for resistance monitoring in collaboration with the Diagnostic Centre were successful. The work done in 2019 was presented at the Packhouse Workshops during January and February 2020.

Opsomming

Verskeie produkte was getoets en ge-evalueer vir prestasie teen bestaande industrie standaarde. Die mees suksesvolle produkte was twee GRAS verbindings, naamlik ʼn gekommersialiseerde plantekstrak, sinnamaldehied, asook ʼn voedselpreseerveermiddel, natamisien. Ongelukkig, op die oomblik, het beide hierdie aktiewes regulatoriese beperkinge op kommersialisering in die sitrusbedryf. Dit is egter belowend dat dit goed vaar en dus verdere ontwikkeling sal aanmoedig. ʼn Besondere aantal produkte met kopergebaseerde formulasies het in 2019 die lig gesien. Geeneen daarvan was suksesvol in die na-oessituasie nie. Daar was geen nuwe peroksieperasynsuur formulasies getoets nie, maar daar is die geleentheid om nuwe tegnologie in die pakhuis te sien ontplooi, waar baie suksesvolle loodsproewe met UV-C lig teen suurvrot gedoen is. Geen ringtoetse was gedoen nie, maar die weerstandtoetse in samewerking met die Diagnostiese Sentrum het goed verloop. Die 2019 seisoen se werk was by die Pakhuis Werkswinkels in Januarie en Februarie 2020 aangebied.

Introduction

This ongoing project offers an industry service to evaluate potential new postharvest disease control products or options, as well as to conduct *ad hoc* experiments. Products are mostly submitted from private companies on a voluntary basis, or projects/products are selected by the researchers involved. Given limited time and resources, requests are screened based on industry priorities.

Objectives

1. Testing new potential products as fungicides, as well as evaluate possible synergistic reactions between chemicals, with specific focus on sour rot.
2. Evaluate available chemistries for use in the heated flooder – the effect of temperature, pH and exposure time, as well as combinations thereof to be evaluated.
3. Introduce and implement the application of GRAS chemicals and sanitizers into the citrus postharvest industry.
4. Analytical lab focus – ring test with the aim to reduce variability.
5. Assessment of fungicide resistance in citrus packhouse.
6. Technology transfer – primarily at the workshops and through collaboration with extension.

Materials and methods

1. *Ad hoc* tests are performed on products that may be of postharvest use to the citrus industry. Fresh, untreated fruit is collected from a reputable commercial packhouse, sanitised and stored at ≈23 °C for two days before the trial commences. The fruit is removed from cold storage and allowed to reach ambient temperature before use in the trial.
2. Each treatment has five replicates with 10-12 fruit in each repeat, except where residue samples are collected, in which case the appropriate repeat has six extra prepared for residue determination.
3. For all trials a sensitive strain of *Penicillium digitatum* (PD) and/or *Galactomyces citri-aurantii* (GCA) are used.
4. A 10^6 spore suspension of PD and GCA are prepared using the standard in-house laboratory technique.
5. Inoculations are done 6 hours prior to treatment.
6. Fruit are treated according to the label instruction of each of the various remedies that are being tested.
7. Lesions are evaluated 4-6 days after inoculation, once >80% of the untreated controls show positive lesion development.

Results

Objective / Milestone	Achievement
1. New potential products will be tested as sanitation agents and/or fungicides.	A total of 11 products and a single technology were tested. The results are available in the full reports attached herewith.
2. Introduce and implement the application of GRAS chemicals into the citrus postharvest industry	Several trials were conducted. Both natamycin and cinnamaldehyde have promise as alternative postharvest remedies, but have not yet met regulatory compliance.
3. Assist CRI DC with packhouse resistance testing	Swabs are either collected by extensionists visiting packhouses, or sent to the DC. Most do not indicate shifts in sensitivity, however, there were detections. The implicated packhouses were consulted for remedial action.
4. Analytical lab focus – ring test with the aim to reduce variability	No ring tests were conducted in 2019, due to an overwhelming number of research trials that were run during the season. The tests will be run in 2020, including local, as well as overseas laboratories.

Alternative products

In terms of GRAS chemicals, natamycin and cinnamaldehyde were tested. The results are available in appendices A and B.

Packhouse sanitation

No new products were successful as water sanitation options, with several having been offered and tested. An interesting sanitation technology has been investigated, with excellent results, whereby inoculated fruit were exposed to UV-C light at a short range and >90% disease control achieved.

Resistance monitoring

Swabs from actively working packhouses are tested regularly throughout the season. This service will be continued and expanded in 2020. A few incidences of sensitivity shifts in pathogen resistance were detected and the packhouses concerned consulted about the issue. Suitable measures to curb this problem were suggested in cooperation with the extensionists.

Technology transfer

Presentation and talks at postharvest workshops, and presentations at conferences where results from CRI-funded research were presented.

Further objectives (milestones) and work plan

1. New potential products will be tested as sanitation agents and/or fungicides; this specifically includes 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 A

TRIAL: Sanitation of water used in aqueous application in citrus packhouses
On behalf of: Natamycin, Jeanine Hordijk (Citrashine)
BY: Dr Wilma du Plooy, Lindokuhle Mamba and Thabang Mgwenya

Objectives

A formulation that claims to be based on natamycin; a standard food preservative in fresh-cut produce, dairy products and fruit juice, was received. It is believed that it will be effective against *Penicillium digitatum* (PD) and *Galacatomyces citri-aurantii* (GCA) in the packhouse. Since *Penicillium* is the most prevalent of the two organisms, as well as the more readily controlled of the two, it was decided to use only PD as test organism.

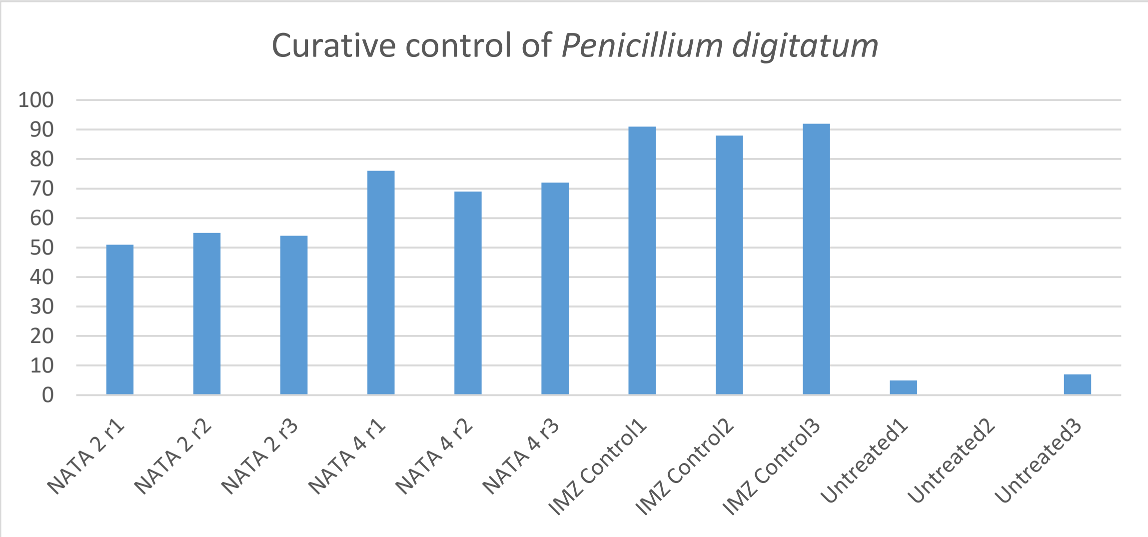
Crop: Valencia
Origin: Ngodwana District
Trial date: 20 August 2019
Trial site: CRI, 2 Baker Street, Nelspruit, 1201
Report date: 25 September 2019

Materials and methods

1. Freshly picked, mature Valencia fruit were collected from a commercial packhouse, rinsed in chlorine (150 ppm total chlorine, pH 6 for 90 seconds), and allowed to air dry.
2. The fruit was stored at ~8°C for three days before the trial commenced. The day before the trial commenced the fruit was moved into ambient temperature (22°C) in order for fruit temperature to also reach ambient, and to allow any possible condensation to evaporate.
3. Dip solutions were prepared in 5 L buckets, using tap water corrected to temperature immediately before the fruit was dipped, with a temperature of ~30°C. The temperature at which water enters the laboratory is about 20°C, and adjustments were made to closely resemble typical temperatures found in a postharvest packhouse.
4. Each treatment had 3 replicates with 10 fruit in each repeat.
5. A *Penicillium* spore suspension was prepared from a virulent, purified culture harvested from fruit, at a 10^6 spore concentration.
6. Control treatments were a standard Imazalil treatment and clean water.
7. The fruit were inoculated by wounding each 4 times on the top shoulder circumference, at equidistant intervals.
8. The wounding instrument is dipped in the spore suspension for every wound made.
9. After wounding the fruit, they were submerged into the treatment solutions for 1 minute, removed from the solution and air dried in a wind tunnel.
10. Treated fruit were placed on a nectarine liner to separate fruit during incubation. The liners were placed in an open top carton, slipped into a transparent polyethylene bag and closed.
11. Four small holes are punched into the bags to facilitate gaseous exchange and prevent build-up of CO_2 and ethylene.
12. The treatments are incubated until >80% decay is visible on the water controls.

Results and discussion

In comparison to Imazalil as the industry standard, natamycin gave good disease control against *Penicillium digitatum*, in this pilot trial (Graph 1). This was an improved result from a previous trial run in 2016.



Graph 1: Comparison of Natamycin to Imazalil as a curative postharvest disease control option

Conclusion

The product has exellent potential, but may still be facing regulatory hurdles. At this point no comment can be made regarding the implementation of the active as a packhouse treatment.

DISCLAIMER
This report contains information and results from a confidential trial to determine any combination of product efficacy, compatibility and phytotoxicity. By conducting these trials the CRI is assisting the citrus industry in finding postharvest sanitation and disease control options. This does not imply any product endorsement by the CRI. Any successful interaction still requires that the necessary accreditations be acquired (Act 5 or Act 36).

APPENDIX B

TRIAL: Sanitation of water used in aqueous application in citrus packhouses
On behalf of: Cinnamaldehyde for Jeanine Hordijk (Citrashine)
BY: Dr Wilma du Plooy, Jeanine Hordijk, Hannelie Kellerman, Lindokuhle Mamba and Thabang Mgwenya

Objectives

A formulation that claims to be based on a cinnamaldehyde extract (CA) was received. It is believed that it will be effective against *Penicillium digitatum* (PD) and *Galacatomyces citri-aurantii* (GCA) in the packhouse. Since *Penicillium* is the most prevalent of the two organisms, as well as the more readily controlled of the two, it was decided to use only PD as a test organism. The synergetic effect of CA with azoxystrobin (AZO) and thiabendazole (TBZ) was also investigated.

Crop: Valencia
Origin: Nelspruit area
Trial date: 25 July 2019
Trial site: CRI, 2 Baker Street, Nelspruit, 1201
Report date: 26 August 2019

Materials and methods

1. Freshly picked, mature Valencia fruit were collected from a commercial packhouse, rinsed in chlorine (150 ppm total chlorine, pH 6 for 90 seconds), and allowed to air dry.
2. The fruit was stored at ~8°C for three days before the trial commenced. The day before the trial commenced the fruit was moved into ambient temperature (22°C) in order for fruit temperature to also reach ambient, and to allow any possible condensation to evaporate.
3. Dip solutions were prepared in a dip tank, using tap water corrected to temperature immediately before the fruit was dipped, with a temperature of ~30°C. The temperature at which water enters the laboratory is about 20°C, and adjustments were made to closely resemble typical temperatures found in a postharvest packhouse.
4. Each treatment had 3 replicates with 12 fruit in each repeat.
5. A *Penicillium* spore suspension was prepared from a virulent, purified culture harvested from fruit, and a 10^6 spore concentration.
6. Control treatments were a standard Imazalil treatment and clean water.
7. The fruit were inoculated by wounding each four times on the top shoulder circumference, at equidistant intervals.
8. The wounding instrument is dipped in the spore suspension for every wound made.
9. After wounding the fruit, they were submerged into the treatment solutions for 1 minute, removed from the solution and air dried in a wind tunnel.
10. Fruit that needed to be waxed were treated after drying by applying 300 µL to each piece of fruit, and then vigorously spreading it on the surface with a gloved hand. The gloves are prewetted with 300 µL wax. The amount applied this way relates to an application rate of 1,2 L/tonne. The wax used in this trial was a carnauba based, natural wax (18% solids).
11. Treated fruit were placed on a nectarine liner to separate fruit during incubation. The liners are placed in an open top carton, slipped into a transparent polyethylene bag and closed.
12. Four small holes were punched into the bags to facilitate gaseous exchange and prevent build-up of CO_2 and ethylene.
13. The treatments are incubated until >80% decay is visible on the water controls.

Results and discussion

The curative action of cinnamaldehyde against *Penicillium digitatum* under pressured artificial inoculation is summarised in Figure 1.

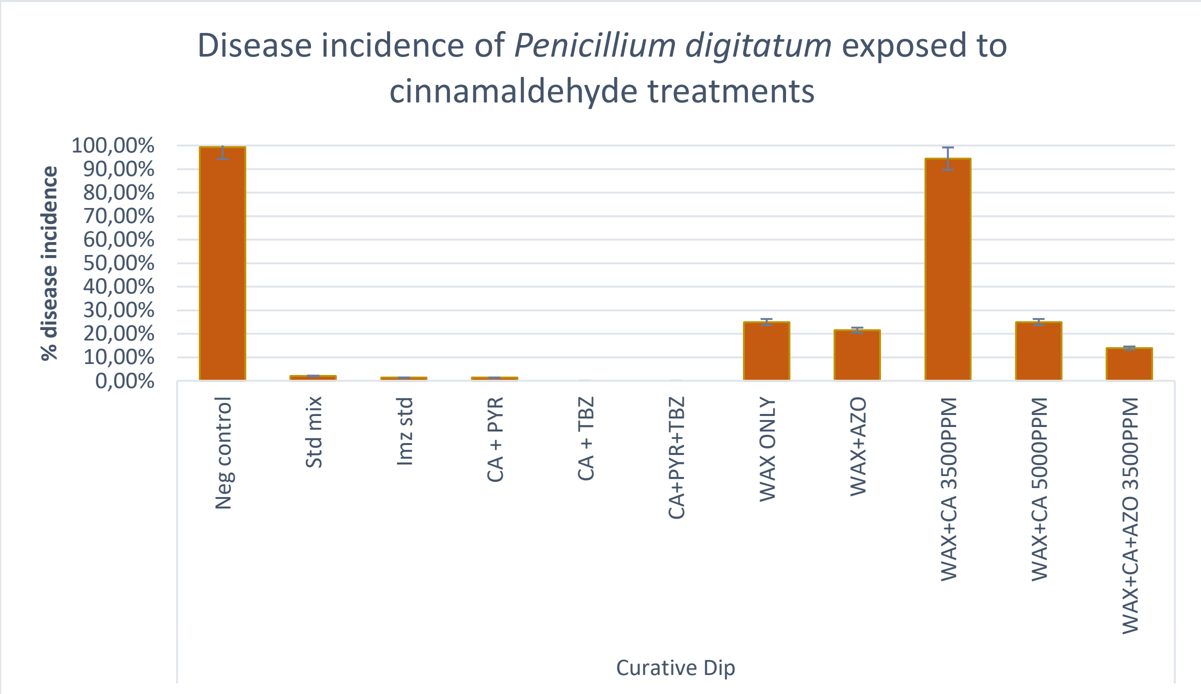


Figure 1: Curative action of cinnamaldehyde and other postharvest chemicals, against *Penicillium digitatum*, with the graph indicating disease incidence.

The curative action of CA in a standard mix was not statistcally different from the control, Imazalil. It has a small, positive interaction with pyrimethanil and thiabendazole, which makes the use of such mixes seem unnecessary. This should be investigated more, to confirm this observation. Wax has a negative effect on the curative action of CA, and it is hypothesized CA actually binds to the wax to an extent, which is why the higher concentration (5000 ppm) does have a significant control action in comparison to the lower concentration (3500 ppm), as it is probably past the saturation point of CA in the wax. The curative action seen with WAX + CA (3500 ppm) + AZO is most likely due to the AZO, based on the result from the WAX + CA at 3500 ppm. Cinnamaldehyde occurs naturally as predominantly the trans-(E) isomer, and gives the characteristic cinnamon flavour and odour, but none of that was detected in any of the fruit tested.

Conclusion

Cinnamaldehyde has good curative action when not used with wax, and should be considered for development in aqueous application products on the line. As an organic compound of natural origin, this holds potential as a replacement for synthetic chemicals.

DISCLAIMER
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APPENDIX C

TRIAL: Sanitation of water used in aqueous application in citrus packhouses
On behalf of: Prosol, Citrosol, PureAgri and Hygrotec
BY: Dr Wilma du Plooy, Lindokuhle Mamba and Thabang Mgwenya

Objectives

During the 2019 season, there was a marked increase in products containing copper and other metals, all claiming levels within the acceptable micronutrient allowance. Six such products were tested: Prosol, OptiBlue, PureAgri (2500 and 100), and Hygrotec (Sol3).

Crop: Valencia
Origin: Hoedspruit, Nelspruit, and Ngodwana Districts
Trial date: July - August 2019
Trial site: CRI, 2 Baker Street, Nelspruit, 1201
Report date: 25 September 2019

Materials and methods

1. Freshly picked, mature Valencia fruit were collected from commercial packhouses in weeks 28, 29, 30 and 31. Rinsed in chlorine (150 ppm total chlorine, pH 6 for 90 seconds), and allowed to air dry.
2. The fruit was stored at ~8°C for three days before the trial commenced. The day before the trial commenced the fruit was moved into ambient temperature (22°C) in order for fruit temperature to also reach ambient, and to allow any possible condensation to evaporate.
3. pH adjustments were made to get to a pH of 6.5.
4. Solutions were prepared with tap water at the correct temperature immediately before the fruit was dipped, with a temperature of ~30°C. The temperature at which water enters the laboratory is about 20°C, and adjustments were made to closely resemble typical temperatures found in a postharvest packhouse.
5. Each treatment had 3 replicates with 10 fruit in each repeat.
6. A *Penicillium* spore suspension was prepared from a virulent, purified culture harvested from fruit, and a 10^6 spore concentration.
7. Buckets with 5 L of the copper solution were seeded with 50 ml *Penicillium* spores to get to a final concentration of ~10^4.
8. Control treatments were a standard 150 ppm chlorine solution (FREXUS Chlorine) and clean water to which spore suspensions were added.
9. The solutions were left for 3 minutes to react with the spores.
10. Immediately before treatment, fruit were injured using a metal plate with 9 x 7mm spikes that were rolled across the cheek section of the fruit, on two sides.
11. Injured fruit were placed in the sanitiser solution with the spores, and left in the solution for 1 minute while slightly agitating it.
12. Treated fruit were removed and dried before placing the 10 fruit from individual replicates in a nectarine liner to separate fruit during incubation. The liners are placed in an open top carton, slipped into a transparent polyethylene bag and closed.
13. Four small holes were punched into the bags to facilitate gaseous exchange and prevent build-up of CO_2 and ethylene.
14. The treatments were incubated until >80% decay was visible on the water controls.

Results and discussion

The products did not have enough efficacy to replace chlorine as a sanitiser in aqueous application points on the packline (Table 1). An observation made with all these products was the poor wettability they seemed to have on the fruit. This means that there is equally low wound penetration and possiblly also low wetting of the fungal spores. Since *Galactomyces* develops as a sub-cuticular organism this organism was not included in the tests. The products are apparently based on solutions with cocktails of micro elements, and may not be suitable for postharvest application.

Table 1: Schedule of treatments used to compare the % disease control achieved with copper-based products in comparison to standard calcium hypochlorite as a water sanitiser for use in aqueous application

Treatment	% Disease Control
Water	0
$Ca(ClO)_2$ (Frexus)	81,1
Optiblue	33,3
Hygrotech Sol3	3,3
Prosol	2,8
PureSan2500	13,9
PureSan100	8,3

Conclusion

None of the products tested were effective as water sanitisers. The claimed efficacy of the various solutions may be directed more toward situations where prolonged exposure may precipitate a lethal action by the actives in the products, i.e. soil application, foliar application.

DISCLAIMER
This report contains information and results from a confidential trial to determine any combination of product efficacy, compatibility and phytotoxicity. By conducting these trials the CRI is assisting the citrus industry in finding postharvest sanitation and disease control options. This does not imply any product endorsement by the CRI. Any successful interaction still requires that the necessary accreditations be acquired (Act 5 or Act 36).

APPENDIX D

TRIAL: Sanitation of water used in aqueous application in citrus packhouses
On behalf of: CRI Extension - Catherine Savage
BY: Wilma du Plooy, Lindokuhle Mamba, and Thabang Mgwenya

Objectives

Two formulations of a product to be considered as a water sanitiser were received: MAP 4% and MAP 10%. The products are based on natural metabolic elements from plants (phenolics), with claimed antimicrobial properties. It is believed that they will be effective against *Penicillium digitatum* (PD) and *Galacatomyces citri-aurantii* (GCA) in the packhouse. Since *Penicillium* is the prevalent of the two organisms, as well as the more readily controlled of the two, it was decided to use only PD as test organism.

Crop: Valencia
Origin: Hoedspruit, Nelspruit, and Ngodwana Districts
Trial date: July - August 2019
Trial site: CRI, 2 Baker Street, Nelspruit, 1201
Report date: 25 September 2019

Materials and methods

1. Freshly picked, mature Valencia fruit were collected from commercial packhouses in weeks 28, 29, 30 and 31, rinsed in chlorine (150 ppm total chlorine, pH 6 for 90 seconds), and allowed to air dry.
2. The fruit was stored at ~8°C for three days before the trial commenced. The day before the trial commenced the fruit was moved into ambient temperature (22°C) in order for fruit temperature to also reach ambient, and to allow any possible condensation to evaporate.
3. pH adjustments were made to get to a pH of 6.5.
4. Solutions were prepared with tap water at the correct temperature before the fruit was dipped, with a temperature of ~30°C. The temperature at which water enters the laboratory is about 20°C, and adjustments were made to closely resemble typical temperatures found in a postharvest packhouse.
5. Each treatment had three replicates with 10 fruit in each repeat.
6. A *Penicillium* spore suspension was prepared from a virulent, purified culture harvested from fruit, and a 10^6 spore concentration.
7. Buckets with 50 L of each of the different MAP solutions were seeded with 50 ml *Penicillium* spores to get to a final concentration of ~10^4.
8. Control treatments were a standard 150 ppm chlorine solution (Frexus) and clean water to which spore suspensions were added.
9. The solutions were left for 3 minutes to react with the spores.
10. Immediately before treatment, fruit were injured using a metal plate with 9 x 7mm spikes that were rolled across the cheek section of the fruit, on two sides.
11. Injured fruit were placed in the sanitiser solution with the spores, and left in the solution for 1 minute while slightly agitating it.
12. Treated fruit were removed and dried before placing the 10 fruit from individual replicates in a nectarine liner to separate fruit during incubation. The liners are placed in an open top carton, slipped into a transparent polyethylene bag and closed.
13. Four small holes were punched into the bags to facilitate gaseous exchange and to prevent build-up of CO_2 and ethylene.
14. The treatments were incubated until >80% decay was visible on the water controls.

Results and discussion

Table 1: Schedule of treatments used to evaluate the % control achieved with two MAP formulations against calcium hypochlorite as a water sanitiser for use in aqueous application

Treatment	% Control
Water	0
$Ca(ClO)_2$ (Frexus)	87,7
MAP 4%	33,3
MAP 10%	3,3

The products did not have enough efficacy to replace chlorine as a sanitiser in aqueous application points on the packline. It is known that plant phenolics have varying eficacy against microbials, the success of such products are dependent on the specific application as well as the matrix within which the actives are contained. Nothing is known about the products tested, other than the claimed actives and efficacy, which could not be proven.

Conclusion

None of the products tested were effective as water sanitisers.

APPENDIX E

TRIAL: **Sanitation of water used in aqueous application in citrus packhouses**
On behalf of: NanoSilver
BY: Wilma du Plooy and Lindokuhle Mamba

Objectives

A formulation that claims to be based on micronized silver was received. It is believed that it will be effective against *Penicillium digitatum* (PD) and *Galacatomyces citri-aurantii* (GCA) in the packhouse. Since *Penicillium* is the prevalent of the two organisms, as well as the more readily controlled of the two, it was decided to use only PD as test organism.

Crop: Valencia
Origin: Ngodwana District
Trial date: 20 August 2019
Trial site: CRI, 2 Baker Street, Nelspruit, 1201
Report date: 25 September 2019

Materials and methods

1. Freshly picked, mature Valencia fruit were collected from a commercial packhouse, rinsed in chlorine (150 ppm total chlorine, pH 6 for 90 seconds), and allowed to air dry.
2. The fruit was stored at ~8°C for three days before the trial commenced. The day before the trial commenced the fruit was moved into ambient temperature (22°C) in order for fruit temperature to also reach ambient, and to allow any possible condensation to evaporate.
3. pH adjustments were made to get to a pH of 6.5.
4. Solutions were prepared with tap water at the correct temperature immediately before the fruit was dipped, with a temperature of ~30°C. The temperature at which water enters the laboratory is about 20°C, and adjustments were made to closely resemble typical temperatures found in a postharvest packhouse.
5. Each treatment had 3 replicates with 10 fruit in each repeat.
6. A *Penicillium* spore suspension was prepared from a virulent, purified culture harvested from fruit, and a 10^6 spore concentration.
7. Buckets with 50 L of the NanoSilver solution were seeded with 50 ml *Penicillium* spores to get to a final concentration of ~10^4.
8. Control treatments were a standard 150 ppm chlorine solution (FREXUS Chlorine) and clean water to which spore suspensions were added.
9. The solutions were left for 3 minutes to react with the spores.
10. Immediately before treatment, fruit were injured using a metal plate with 9 x 7mm spikes that were rolled across the cheek section of the fruit, on two sides.
11. Injured fruit were placed in the sanitiser solution with the spores, and left in the solution for 1 minute while slightly agitating it.
12. Treated fruit were removed and dried before placing the 10 fruit from individual replicates in a nectarine liner to separate fruit during incubation. The liners are placed in an open top carton, slipped into a transparent polyethylene bag and closed.
13. Four small holes were punched into the bags to facilitate gaseous exchange and prevent build-up of CO_2 and ethylene.
14. The treatments were incubated until >80% decay is visible on the water controls.

Results and discussion

Table 1: Table to compare control achieved by NanoSilver in comparison to standard chlorine as a water sanitiser for use in aqueous applications.

Treatment	% Control
Water	0
Chlorine (Frexus)	83,3
NanoSilver 1%	52,8

These products cannot be considered as a replacement for chlorine, due to a lower efficacy, as well as concern about silver residue on the fruit after treatment. This concern was not addressed and nothing more is known about the product tested.

Conclusion

The product tested was not effective as a water sanitiser.

DISCLAIMER
This report contains information and results from a confidential trial to determine any combination of product efficacy, compatibility and phytotoxicity. By conducting these trials the CRI is assisting the citrus industry in finding postharvest sanitation and disease control options. This does not imply any product endorsement by the CRI. Any successful interaction still requires that the necessary accreditations be acquired (Act 5 or Act 36).

APPENDIX F

TRIAL: Sanitation of water used in aqueous application in citrus packhouses
On behalf of: Reinardt Fourie, BioRetics
BY: Dr Wilma du Plooy, Lindokuhle Mamba and Thabang Mqwenya

Objectives

A unit containing technology based on UV-C was received. It is believed that it will be effective against *Penicillium digitatum* (PD) and *Galacatomyces citri-aurantii* (Gca) in the packhouse. With the novel approach and possible benefits, *Galactomyces citri-aurantii* was used in the trial. This decision was validated by *in vitro* tests conducted at Bioretics.

Crop: Valencia
Origin: Nelspruit Area
Trial date: 27 August 2019
Trial site: CRI, 2 Baker Street, Nelspruit, 1201
Report date: 25 September 2019

Materials and methods

1. Freshly picked, mature Valencia fruit were collected from a commercial packhouse, rinsed in chlorine (150 ppm total chlorine, pH 6 for 90 seconds), and allowed to air dry.
2. The fruit was stored at ~8°C for three days before the trial commenced. The day before the trial commenced the fruit was moved into ambient temperature (22°C) in order for fruit temperature to also reach ambient, and to allow any possible condensation to evaporate.
3. Each treatment had 3 replicates with 12 fruit in each repeat.
4. A *Galactomyces* spore suspension was prepared from a virulent, purified culture harvested from fruit, and a 10^6 spore concentration.
5. Basins with 50 L intended for the control solutions were seeded with 50 ml *Galactomyces citri-aurantii* spores to get to a final concentration of ~10^4.
6. The control solution was left for 3 minutes to react with the spores.
7. The fruit to be irradiated was dipped in a 10^6 spore suspension, left to dry completely (1 hour) and thereafter exposed to the UV light.
8. Immediately before treatment, fruit were injured using a metal plate with 9 x 7mm spikes that was rolled across the cheek section of the fruit, on two sides.
9. Injured fruit were placed in the sanitiser solution with the spores, and left in the solution for 1 minute while slightly agitating it.
10. Fruit exposed to the UV light were manually turned after 1 minute, as the trial unit does not have a rotating mechanism.
11. Treated fruit were removed and dried before placing the 12 fruit from individual replicates in a nectarine liner to separate fruit during incubation. The liners were placed in an open top carton, slipped into a transparent polyethylene bag and closed.
12. Four small holes were punched into the bags to facilitate gaseous exchange and prevent build-up of CO_2 and ethylene.
13. The treatments were incubated until >80% decay was visible on the water controls.

Results and discussion

The technology is not intended as a replacment for chlorine at the moment, since this is a first attempt at studying the potential in a packhouse environment. The results, however, indicate that this may well be worth pursuing. The untreated water did not have any control action, but is rather indicative of the difficulty to get 100% decay with *Galactomyces citri-aurantii* through artificial inoculation.

Table 1: Percentage disease control achieved by applying UV-C in comparison to standard chlorine as a water sanitiser for use in aqueous application

Treatment	% Control
Water	8,3
Chlorine (Frexus)	77,8
UV-C	88,9

Conclusion

This technology needs to be investigated further.

DISCLAIMER
This report contains information and results from a confidential trial to determine any combination of product efficacy, compatibility and phytotoxicity. By conducting these trials the CRI is assisting the citrus industry in finding postharvest sanitation and disease control options. This does not imply any product endorsement by the CRI. Any successful interaction still requires that the necessary accreditations be acquired (Act 5 or Act 36).

APPENDIX G

TRIAL: Sanitation of water used in aqueous application in citrus packhouses

On behalf of: Reinardt Fourie, BioRetics

BY: Dr Wilma du Plooy, Lindokuhle Mamba and Thabang Mqwenya

Objectives

This is a follow-up trial on work done earlier, and in which good results were obtained on Valencia fruit. In this repeat, lemons were used to give an indication if the efficacy can be repeated across different citrus types for *Galacatomyces citri-aurantii* (Gca) in the packhouse. The initial decision for the use of *Galactomyces citri-aurantii* has been validated by *in vitro* tests conducted at the laboratories of Bioretics themselves (Figure 1).

Crop: Lemons
Origin: Nelspruit Area
Trial date: 5 September 2019
Trial site: CRI, 2 Baker Street, Nelspruit, 1201
Report date: 17 November 2019

Materials and methods

1. Freshly picked, mature lemon fruit were collected from a commercial packhouse, rinsed in chlorine (150 ppm total chlorine, pH 6 for 90 seconds), and allowed to air dry.
2. The fruit was stored at ≈8°C for three days before the trial commenced. The day before the trial commenced the fruit was moved into ambient temperature (22°C) in order for fruit temperature to also reach ambient, and to allow any possible condensation to evaporate.
3. Each treatment had three replicates with 12 fruit in each repeat.
4. A *Galactomyces* spore suspension was prepared from a virulent, purified culture harvested from fruit, and a 10^6 spore concentration.
5. Basins with 50 L intended for the control solutions were seeded with 50 ml *Galactomyces* spores to get to a final concentration of ~10^4.
6. The control solution was left for 3 minutes to react with the spores.
7. The fruit to be irradiated was dipped in a spore 10^6 spore suspension, left to dry completely (1 hour) and then thereafter exposed to the UV light.
8. Immediately before treatment, fruit were injured using a metal plate with 9 x 7mm spikes that was rolled across the cheek section of the fruit, on two sides thereof.
9. Injured fruit were placed in the sanitiser solution with the spores, and left in the solution for 1 minute while slightly agitating it.
10. Fruit exposed to the UV light were manually turned after 1 minute, as the trial unit does not have a rotating mechanism.
11. Treated fruit were removed and dried before placing the 12 fruit from individual replicates in a nectarine liner to separate fruit during incubation. The liners were placed in an open top carton, slipped into a transparent polyethylene bag and closed.
12. Four small holes were punched into the bags to facilitate gaseous exchange and prevent build-up of CO_2 and ethylene.
13. The treatments were incubated until >80% decay was visible on the water controls.

Results and discussion

The technology is not intended as a replacement for chlorine at the moment, but as exploratory work to evaluate the potential thereof in a packhouse environment. From the *in vitro* work done by Bioretics (Figure 1), a clear dose-response to UV-C can be seen.

The results of this trial (Figure 2), as with the first on Valencia fruit, indicate that the technology is worth considering for further studies. The untreated water did not have any control action, but is rather indicative of the difficulty to get 100% decay with *Galactomyces citri-aurantii* through artificial inoculation.

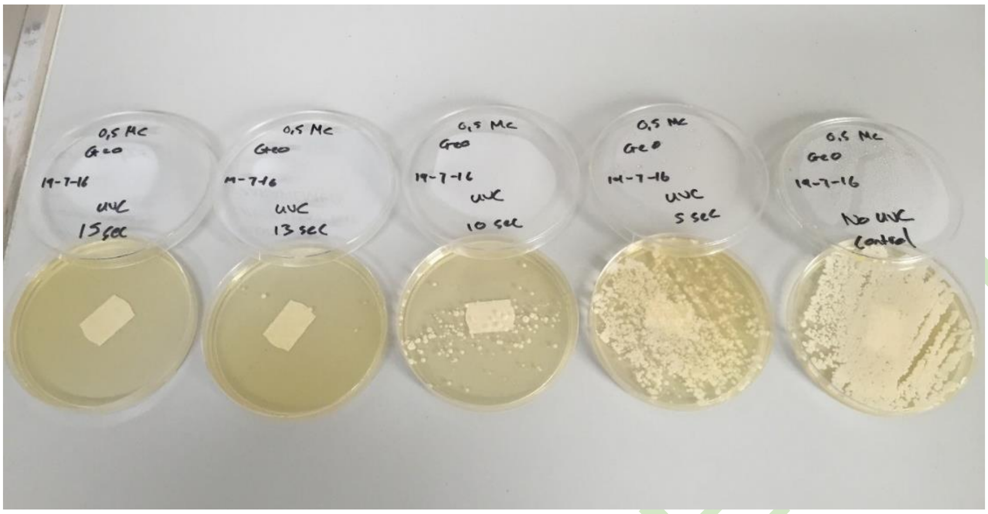

Figure 1. Dose response of Galactomyces *citrii-aurantii* exposed to UV-C for different times.

It is important to keep in mind that the small laboratory unit and perfect conditions for the trial do bias the outcome towards the technology, and that a commercialised unit will have to meet a number of safety aspects to be successful on a commercial packhouse scale. Regardless, the chlorine control was significantly less successful that the UV-C application in this trial.

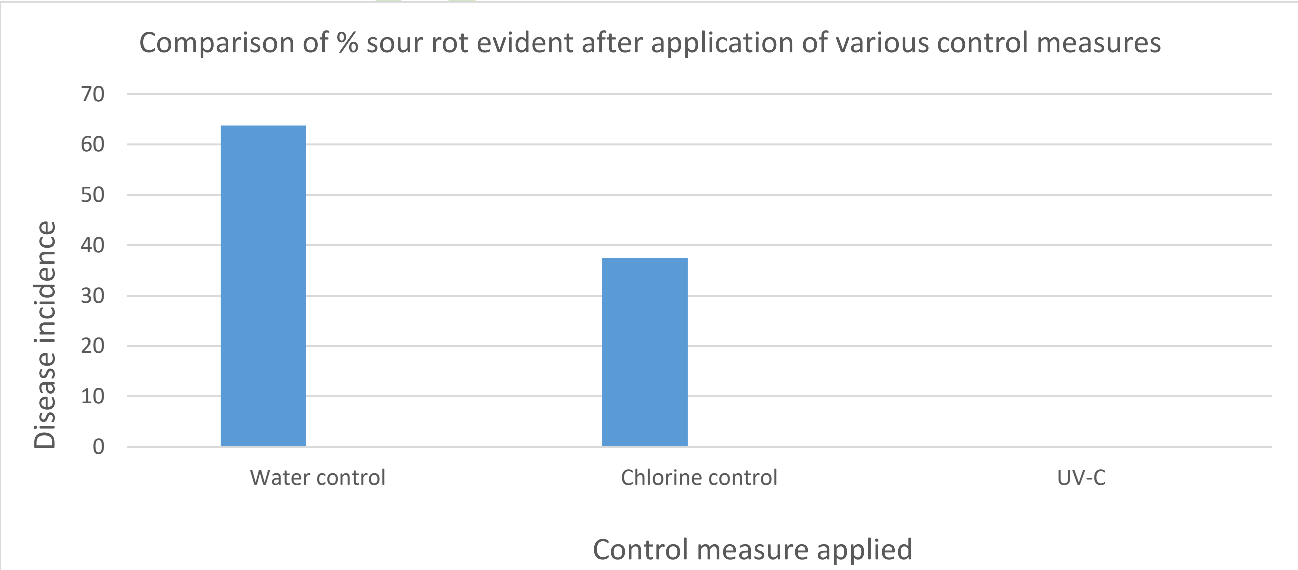

Figure 2. A comparison of the control achieved with water vs chlorine vs UV-C application

Conclusion

This technology needs to be investigated further.

DISCLAIMER

This report contains information and results from a confidential trial to determine any combination of product efficacy, compatibility and phytotoxicity. By conducting these trials the CRI is assisting the citrus industry in finding postharvest sanitation and disease control options. This does not imply any product endorsement by the CRI. Any successful interaction still requires that the necessary accreditations be acquired (Act 5 or Act 36).

4.4.3 **PROGRESS REPORT: Evaluation of new postharvest fungicides for the control of*Phytophthora* brown rot**

Project PHI4-07 (1198) (2018/9 - 2021/20) by Jan van Niekerk (CRI-USPP), Lize van der Merwe and Cheryl Lennox (USPP)

Summary

Brown rot is a postharvest disease of citrus caused by *Phytophthora* spp. Fungicide management of brown rot in South Africa currently consists only of preharvest strategies and nothing is registered for postharvest management of this disease. The objectives of this study were to evaluate the curative and protective efficacy of azoxystrobin (1125 µg/ml), fludioxonil (598 µg/ml) and potassium phosphite (1500 ug/ml) as aqueous dip treatments for the postharvest management of *Phytophthora* brown rot on different citrus types (lemons, oranges and mandarins). Additionally, azoxystrobin (2500 µg/ml for all three fruit types) and fludioxonil (2300 µg/ml for lemons and 4600 µg/ml for oranges and mandarins) amended wax was evaluated for the prevention of spreading of brown rot (nesting) within cartons during transit. Results indicated that the three tested fungicides have good curative action, reducing brown rot incidence significantly when the fungicide was applied 12 hrs after inoculation. Applications done 24 hrs after inoculation also provided some curative action but not as effective as earlier applications. Azoxystrobin and potassium phosphite furthermore provided very good protection against infection if inoculations were done up to 48 hrs after application on all three fruit types but fludioxonil did not fare as well. Interestingly, the protective ability of all three fungicides was better the longer the fungicides remained on the fruit before inoculation. Trials aimed at prevention of nesting during transit indicated that only azoxystrobin amended wax significantly reduced brown rot from spreading to healthy fruit when in contact, compared to the control. The data obtained from this study can add additional value to the already registered postharvest azoxystrobin and fludioxonil fungicides and preharvest registered potassium phosphite.

Opsomming

Bruinvrot is ‘n na-oes siekte van sitrus, veroorsaak deur *Phytophthora* spp. Fungisiedbestuur van bruinvrot in Suid-Afrika bestaan tans slegs uit voor-oes strategieë, en niks is vir die na-oes bestuur van hierdie siekte geregistreer nie. Die doelwit van hierdie studie was om die uitwissende en beskermende effektiwiteit van azoxystrobin (1125 µg/ml), fludioxonil (598 µg/ml) en kaliumfosfiet (1500 ug/ml) as waterige doopbehandelings vir die na-oes bestuur van *Phytophthora* bruinvrot op verskillende sitrustipes (suurlemoene, lemoene en mandaryne) te evalueer. Addisioneel, is azoxystrobin (2500 µg/ml vir al drie vrugtipes) en fludioxonil (2300 µg/ml vir suurlemoene en 4600 µg/ml vir lemoene en mandaryne) gewysigde waks, vir die voorkoming van die verspreiding van bruinvrot kruiskontaminasie, binne kartonne gedurende vervoer, geëvalueer. Resultate het aangetoon dat die drie getoetste fungisiedes goeie uitwissende aksie gehad het, wat die voorkoms van bruinvrot betekenisvol verminder het wanneer die fungisied 12 ure ná inokulasie toegedien is. Toedienings wat 24 ure ná inokulasie gedoen is, het ook ‘n mate van uitwissende aksie verskaf, maar nie so effektief soos vroeër toedienings nie. Azoxystrobin en kaliumfosfiet het verder baie goeie beskerming teen infeksie op al drie vrugtipes verskaf wanneer inokulasies tot 48 ure ná toediening gedoen is, maar fludioxonil het nie so goed gevaar nie. Interessantheidshalwe, die beskermende vermoë van al drie fungisiedes was beter hoe langer die fungisiedes op die vrug gebly het vóór inokulasie. Proewe wat die voorkoming van kruiskontaminasie gedurende vervoer ten doel gehad het, het aangedui dat azoxystrobin gewysigde waks, betekenisvol die verspreiding van bruinvrot na gesonde vrugte wanneer in kontak gekom het, verminder het, in vergelyking met die kontrole. Die data wat vanuit hierdie studie verkry is, kan addisionele waarde tot die reeds geregistreerde na-oes azoxystrobin en fludioxonil fungisiedes en voor-oes geregistreerde kaliumfosfiet toevoeg.

4.4.4 **PROGRESS REPORT: Fungal degradation of wooden pallet bases used in containerised export of citrus fruit**

Project 1165 (2017/18) Muriel Rikhotso, Elaine Basson, and Wilma du Plooy (CRI)

Summary

The project made good progress in all aspects. Samples of wood colonised through fungal contaminants, were collected from timber yards where pallets are manufactured, as well as from packhouses where pallets were visibly affected. From a total of 279 isolates, 89 isolates were identified using PCR techniques, with 120 isolates already in process of being identified to species level. Final confirmation of the 89 isolates' identity was achieved with sequencing, employing ITS primers 1 and 4. To date, none of the species identified pose any phytosanitary risk, as they all represent ubiquitous species.

The second leg of the study was focussed on identifying the source of OPP contamination of fruit during transit. This required visits to cold storage facilities and container depots, which were only possible with the support and assistance of PPECB. A total of 151 swabs were collected from three container depots and six cold storage facilities. No OPP was detected in any of the containers, while in the cold stores only one swab detected 0.09 mg/kg OPP. The results for the chemicals used to wash the containers also showed zero detection of OPP. From the results of the pallets it is clear that the source of the problem is the pallets on which fruit boxes are stacked during shipping. In the third leg of the study, therefore, the most cost effective and environmentally friendly way to treat the pallets was investigated.

In this third leg, in a simultaneous study, the same contaminated wood from the first part, as well as severely infected slats from local timber yards, were used. To achieve repeatability in the trials, slats of freshly cut wood were co-infected by exposing it to affected slats. This part of the study is slow and time-consuming, since co-infection takes two weeks to establish at 28°C and >95% relative humidity. These co-infected slats were the base on which wood treatments were tested for efficacy. Sterilised, and then treated pine blocks were exposed to these cultivated colonies for at least another two weeks before a result was determined. Although a total of eight products were tested, only one eventually was truly effective, with viable application methodology. A further three products are still being tested in the 2020 season.

Opsomming

Die projek het goeie vordering gemaak in alle aspekte. Fungus-gekontamineerde en gekoloniseerde houtmonsters, was vanaf saagmeulens versamel, sowel as vanaf pakhuise met sigbaar geaffekteerde pallette. Uit 'n totaal van 279 isolate, is reeds 89 isolate tot op spesies vlak geïdentifiseer met PKR tegnieke, terwyl 'n verdere 120 isolate reeds in proses van identifikasie is. Die 89 isolate was finaal geïdentifiseer met volgordebepalings, waar ITS inleiers 1 en 4 gebruik was. Tot op hede toe is geen spesies wat 'n fitosanitêre risiko inhou geïdentifiseer nie.

Die tweede been van die studie het gefokus op die bepaling van die oorsprong van die OPP kontaminasie van vrugte tydens vervoer. Dit het die besoek aan kouestoor fasiliteite en behoueringsdepots behels. Hierdie was slegs moontlik met die ondersteuning en samewerking van PPECB. 'n Totaal van 151 depper monstersis geneem by drie verskillende behoueringsdepots en ses kouestoor fasiliteite. Geen OPP was in die behoueringseenhede opgespoor nie, met slegs een van die deppers vanaf die kouestore wat 'n 0,09 mg/kg OPP residu op gehad het. Die resultate vir die chemikalieë wat gebruik word in die skoonmaak van die behoueringseenhede, het ook negatief vir OPP getoets. Uit die resultate vir die houtmonsters is dit egter duidelik dat die pallette die bron van die OPP besmetting op die vrugte wat daarop gestapel word, is. Die derde deel van die studie is dus bemoeid met die toetsing van alternatiewe produkte wat vir die bahandeling van die hout geskik sal wees.

Vir die derde been, in 'n gelyktydige studie, word dieselfde gekontamineede hout, sowel as baie ernstige gekontamineerede hout vanf die saagmeulens, gebruik. Ten einde herhaalbare resultate te verseker, was vars gesnyde balke geïnfekteer deur dit bloot te stel aan die besmette hout. Hierdie is 'n tydsame deel van die studie, aangesien mede-infeksie twee weke vat teen 28°C en >95% relatiewe humiditeit, om te vestig. Hierdie mede-geïnfekteerde balke word dan die basis waarop die produkte getoets word. Gesteriliseerde boutblokke word op die balke vasgemaak nadat hulle behandel was, en dan verg dit nog twee weke voordat 'n resultaat waarneembaar is. Alhoewel 'n totaal van agt produte getoets was, was slegs een werklik effektief en met haalbare aanwendingsmetodologie. 'n Verdere drie produkte word in die 2020 seisoen getoets.

4.4.5 **PROGRESS REPORT: Optimising available alternative postharvest remedies as replacement for imazalil use on citrus exported to Europe**

Project 1250 (2019/20 – 2020/21) Wilma du Plooy, Lindokuhle Mamba, Thabang Mgwenya, and Paul Fourie (CRI)

Summary

Five actives registered in South African and also allowed in the European Union (azoxystrobin (AZO), fludioxonil (FLU), thiabendazole (TBZ), pyrimethanil (PYR) and ortho phenyl phenol (OPP)) were tested for efficacy against *Penicillium digitatum* (PD), and measured against imazalil as the standard. The parameters of equipment to be used in the trials, were optimised before the actual trials commenced. Although the initial prospect was to evaluate each product at three different temperatures, with three different pH's, and three exposure times for each, this proved to be impossible given the time constraints, with 15 different trials run throughout the season. Soft citrus, oranges and lemons were used in all the trials, with all trials done in triplicate and a full repeat done throughout the season. All the trials are therefore done at three temperatures (25, 35 and 45°C), pH that of the incoming water, and an exposure time of 1 minute, with the variables selected to suit the available conditions and water quality.

The trials were run in the custom built drenchers with 140 L capacity. All the trials were incubated at 22 – 24 °C, which is the ambient temperature in the laboratory. The study focused on the curative ability of individual actives against PD, by applying them in a fungicide bath, and also evaluating sporulation inhibition. In terms of disease control, AZO and PYR did well, with FLU being somewhat variable, but still giving some control, while OPP did not do well on any of the three citrus types tested. The chemicals tested were not able to give 100% sporulation control, with OPP failing altogether. To this end OPP was eventually omitted from the trials. Fludioxonil, did give some variable sporulation control (30 - 50%), and while PYR and AZO were also variable, they were more successful (30 – 80%).

The results from 2019 were presented in a summarised presentation at the 2020 workshops. The 2020 studies will focus on finding more effective combinations of the currently registered actives. An attempt will also be made to combine some of these actives with GRAS chemicals.

Opsomming

Vyf aktiewes met Suid Afrikaanse registrasie en wat in die Europese Unie toegelaat word (azoxistrobien (AZO), fludioxoniel (FLU), tiabendasool (TBZ), pirimethaniel (PYR) en ortho-fenielfenol (OPP)) was getoets vir effektiwiteit teen *Penicillium digitatum* (PD), en gemeet teen imazalil as standaard. Die parameters van die toerusting vir die proewe was geoptimiseer voordat die proewe begin het. Alhoewel die aanvanklike voorstel was om drie temperature, met drie verskillende pH's en drie blootstellingstye vir elke chemie te ondersoek, het dit uiteindelik, met tydsbeperkinge in die seisoen, geblyk onmoontlik te wees. Sagte sitrus, lemoene en suurlemoen was in al die proewe gebruik, Die proewe was gedoen in die in-huis geboude stortbaddens met 140 L kapasiteit. Die temperature in al die proewe was 25, 35 en 45°C, die pH dit van die inkomende water, en 1 minuut blootstellingstyd. Die vrugte is geïnkubeer teen 22 – 24 °C, wat die omgewingstemperatuur in die laboratorium is.

Die studie fokus op die genesende vermoë van die individuele aktiewes teen PD wanneer dit in die fungisiede bad aangewend word, asook op die sporulasie inhibisievermoë van elk. In terme van siektebeheer het AZO en PYR goed gevaar, met FLU wat varieërende resultate gelewer het, maar steeds effektief was, terwyl OPP gefaal het. Die aktiewes wat getoets was het nie 100% sporulasiebeheerr kon gee nie, met OPP wat weereeens gefaal het. Vir hierdie redes was OPP uiteindelik uitgelaat uit die werk. Fludioxoniel het varieërende sporulasiebeheer gegee (30 - 50%), terwyl resultate met PYR en AZO ook varieënd was, maar meer suksesvol (30 – 80%).

Die resultate van die 2019 werk was opgesom in die 2020 werkswinkelaanbiedinge. Die studies in 2020 is gefokus op die ondersoek van moontlike effektiewe kombinasies van die huidige geregisteerde aktiewes. Daar sal ook gepoog word om van die huidige aktiewes te kombineer met GRAS chemikalieë.

4.4.6 **PROGRESS REPORT: The use of alternative fungicides in wax to control citrus green mould caused by *Penicillium digitatum***

Project 1251 (2019/20 – 2020/21) Charles Stevens and Cheryl Lennox (SU), Wilma du Plooy and Paul Fourie (CRI)

Summary

Three fungicides (azoxystrobin (AZO), fludioxonil (FLU) and pyrimethanil (PYR)) with the potential to be used as alternatives or replacements for imazalil (IMZ) in wax application, were tested at their registered dosages to determine their protective control action as well as sporulation inhibition potential. Lemons, soft citrus and oranges were used in all the trials. In the stand-alone fungicide treatments AZO had the best protective control with 98% of control when averaging control on all fruit types. Percentage control was 83% for FLU and 63% for PYR. From these results, pyrimethanil did not perform well when compared to the registered concentration of IMZ (94%), AZO and FLU. In terms of sporulation inhibition, AZO had the best sporulation inhibition with 54% sporulation inhibition over all fruits, whereas FLU and PYR had 83% and 63% sporulation respectively.

For combination fungicide treatments AZO+ FLU performed the best with 98% protective control. Protective control obtained with FLU+ PYR was 92%, and for the PYR+AZO combination, 87%. Considering sporulation inhibition, AZO+FLU delivered the best results with 93% sporulation inhibition. Sporulation inhibition with FLU+PYR was 72%, and 60% with a combination of PYR+AZO. With the two additional products tested, protective control of Evolve was very good, and it did not differ significantly from IMZ treatments on all fruit types. A pooled average of 98.5% protective control was obtained over all fruit types. Although it is a registered option in South Africa, ortho phenyl phenate (OPP) did not deliver good results, with the pooled average over the three fruit types only 31%. Evolve did also prove to have good anti-sporulant ability, inhibiting spore development up to 88%. In comparison OPP gave poor results, with sporulation inhibition at only 16%. A wax treatment was added to prove that protective control and anti-sporulant ability comes from actives used and not wax itself. The results from 2019 were presented in a summarised presentation at the 2020 workshops. In 2020 a baseline study will be done on the actives.

Opsomming

Drie fungisiede met moontlike potensiaal om imazalil (IMZ) te vervang in verpakkingswaks, naamlik [(azoxistrobien (AZO), fludioxoniel (FLU) en pirimethaniel (PYR)], was teen hulle geregistreerde aanwendingsdosisse getoets vir beskermende sowel as sporulasie inhibisievermoë. Suurlemoene, sagte sitrus en lemoene was in al die proewe gebruik. In die proewe met een enkel aktief het AZO die beste gemiddelde beskermingsresultate gegee (98%), gemiddeld oor al die sitrustipes. Die gemiddelde persentasie beheer behaal met FLU was 83%, en 63% vir PYR. Uit hierdie werk vaar PYR dus nie goed in vergelyking met die IMZ standaard (94%), AZO of FLU nie. In terme van sporulasie inhibisie het AZO die beste resultate gelewer, met 54% sporulasie imhibiie oor al die vrugte, met FLU en PYR 83% en 63% sporulasie inhibisie onderskeidelik.

In die kombinasie proewe het AZO+FLU die beste gevaar met 98% beskermende beheer. Beskermende beheer met FLU+PYR was 92% en vir PYR+AZO 87%. Wanneer gekyk word na sporulasie inhibisie, het FLU+PYR 72% inhibisie gegee, met 60% behaal met ‘n PYR+AZO kombinasie. Met die twee addisionele produkte wat getoets was, het Evolve baie goed gevaar en nie noemenswaardig van IMZ verskil in terme van beheer nie. Die gesamentlike resultaat oor al die sitrustipes was 98,5% beskermende beheer. Daarteenoor het orto fenielfenol (OPP), alhoewel dit geregister is in Suid Afrika, swak gevaar, met ‘n gemiddelde beskermingsbeheer van 31%. Evolve het ook goeie sporulasiebeheer gegee (88% inhibisie). In vergelyking hiermee, het OPP slegs 16% sporulasiebeheer gegee. ‘n Waksbehandeling was ingesluit om te bewys dat die beskerming nie deur die waks gegee word nie, maar wel deur die aktiewes soos getoets. Die resultate van die 2019 werk was opgesom in die 2020 werkswinkelaanbiedinge. In 2020 sal ‘n basislynstudie op die aktiewes gedoen word.

4.5 **CRI Diagnostic Centre** (Elaine Basson, Charmaine Olivier, Aubrey Metane, Samuel Ndlovu, Nozipho Shabangu and Jan van Niekerk)

Analysis	Citrus nurseries	Commercial samples	Other crops	Research samples
Nematode: Roots	83	1393	0	249
Nematode: Soil	0	456	28	247
Phytophthora	7140[1]	1007	211	587
Water spore trap	223	0	4	0
Black spot identification (PCR)	0	305	0	187
Black spot benzimidazole resistance	0	48	0	0
Citrus greening (PCR)	0	31	0	0
Post Harvest Sensitivity	0	110	0	3
Fruit & Foliar identification	0	78	11	69
Soil dilution plating	0	22	9	18
VIRUS/VIROID PCR	0	0	0	0
SUB-TOTALS	**7446**	**3450**	**263**	**1360**

[1] Total samples received from citrus nurseries – includes quarterly samples, re-tests and non-certified nurseries

Citrus Certified Nurseries

It is compulsory for all citrus nurseries participating in the Citrus Improvement Scheme to send samples for *Phytophthora* analysis on a quarterly basis. The irrigation water must also be tested for *Phytophthora* by making use of the spore trap method. In total, 6216[2] nursery samples were received by the Diagnostic Centre for *Phytophthora* analyses. Of these samples, 3.28% 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, and Western Cape. Most of the samples received from citrus growers were analysed for *Phytophthora nicotianae* and the citrus nematode, *T. semipenetrans*. Fifty-five percent of the 1393 samples analysed for citrus nematode had counts above the threshold value of 1000 females per 10 g of roots, and nematicide treatments were recommended. Sixty-one percent of the 1007 samples analysed for *Phytophthora* tested positive.

Other crops

Nematode counts were done on soil or root samples of Avocado, Banana, Indigenous trees, Macadamia, Mango, Pecan, Sorghum, Stevia and Tomato. Nematodes found present on these crops included: *Pratylenchus, Rotylenchulus, Scutellonema,* and *Xiphinema. Phytophthora* and *Pythium* analyses were done on Avocado, Banana, Blueberries, Indigenous trees, Macadamia, Mango, Pecan, Peony, Sorghum, Stevia and Tomato. The Diagnostic Centre analysed 104 soil samples from avocado and macadamia nurseries for the presence of *Phytophthora cinnamomi*.

Research samples

Nematode and *Phytophthora* analyses were done on 1083 samples from experimental trials and extension samples. The Diagnostic Centre assisted in trials to identify possible citrus black spot lesions using PCR protocols on 187 fruit samples.

Footnote:

[2] Sample number and the percentage positive are only for certified nurseries and only for the quarterly samples received.

CRI Diagnostiese Sentrum (Elaine Basson, Charmaine Olivier, Aubrey Metane, Samuel Ndlovu, Nozipho Shabangu en Jan van Niekerk)

Ontleding	**Sitrus kwekerye**	**Kommersiële monsters**	**Ander gewasse**	**Navorsings-monsters**
Aalwurms: Wortels	83	1393	0	249
Aalwurms: Grond	0	456	28	247
Phytophthora	7140[1]	1007	211	587
Water spoorlokval	223	0	4	0
Swartvlek (PKR)	0	305	0	187
Swartvlek benzimidazole bestandheid	0	48	0	0
Sitrusvergroeningsiekte (PKR)	0	31	0	0
Na-oes bestandheid (Imazalil)	0	110	0	3
Vrug- en blaar identifikasie	0	78	11	69
Grondverdunningsplate	0	22	9	18
Virus / Viroïedes PCR	0	0	0	0
TOTAAL	**7446**	**3450**	**263**	**1360**

[1] Totale hoeveelheid monsters ontvang van gesertifiseerde kwekerye – sluit in kwartaal monsters, hertoets monsters en nie-gesertifiseerde kwekerye

Sitrus Gesertifiseerde Kwekerye

Dit is verpligtend vir al die sitruskwekerye wat aan die Sitrus Verbeteringskema deelneem om kwartaalikse monsters vir *Phytophthora* te laat ontleed. Die besproeiingswater moet ook deur middel van die spoorlokval metode vir *Phytophthora* getoets word. In totaal 6216[2] monsters is deur die diagnostiese sentrum vir *Phytophthora* ontleding ontvang, waarvan 3.28% 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. semipenetrans*.

Kommersiële monsters

Monsters is uit die volgende sitrusverbouingsareas ontvang: Oos-Kaap, KwaZulu-Natal, Limpopo, Mpumalanga, Noord-Kaap, Noord-Wes, en Wes-Kaap. Die meeste van die monsters wat van sitrusprodusente ontvang is, is vir *Phytophthora nicotianae* en die sitrusaalwurm, *Tylenchulus semipenetrans*, ontleed. Vyf-en-vyftig persent van die 1393 aalwurmmonsters wat ontleed is, het tellings hoër as die drempelwaarde van 1000 wyfies per 10g wortels gehad. Aalwurmdoderbehandelings is in daardie gevalle aanbeveel. Een-en-sestig persent van die 1007 monsters wat vir *Phytophthora* ontleed is het positief getoets.

Ander Gewasse

Aalwurmtellings is op grond- of wortelmonsters van Avokado, Piesang, Inheemse bome, Makadamia, Mango, Pekan, Sorghum, Stevia en Tamaties. Aalwurms teenwoordig gevind op hierdie gewasse sluit in: *Pratylenchus, Rotylenchulus, Scutellonema,* and *Xiphinema.* Avokado, Piesang, Bloubessies, Inheemse bome, Makadamia, Mango, Pekan, Pioenrose, Sorghum, Stevia en Tamaties monsters is vir *Phytophthora* en *Pythium* ontleed. Die diagnostiese sentrum het 104 monsters vanaf avokado en makadamia kwekerye ontvang om vir *Phytophthora cinnamomi* te ontleed.

Navorsingsmonsters

Aalwurm en *Phytophthora* ontledings is op 1083 monsters afkomstig uit navorsingsprojekte gedoen. Die Diagnostiese Sentrum het ook hulp verleen aan navorsingsprojekte in die identifikasie van moontlike sitrus swartvlek letsels deur middle van PKR op 187 vrug monsters.

Voetnota:
[2] Monster hoeveelheid en die persentasie positief is net vir gesertifiseerde kwekerye en slegs vir die kwartaal monsters ontvang.

5 PORTFOLIO: CITRICULTURE

5.1 PORTFOLIO SUMMARY

By Paul Cronje (Portfolio Manager: Citriculture, CRI)

Consistent improvement in yield and fruit quality as well as reduction of postharvest losses remain key in the Citriculture portfolio. Research programmes and projects are structured to address grower identified research priorities. The first focus area is to continually supply information on new cultivars and rootstocks as their suitability to all the various climatic regions are evaluated. The expansion and improvement of our cultivar options is an important aspect that underpins our sustained competiveness. A neglected part of citriculture is being addressed in a study to improve seed number in rootstock cultivars and has already shown significant differences between cultivars indicating diverse management actions need to be developed. During the past season a better understanding of nutritional balance in seedless cultivars and the relationship to fruit set was developed. The use of plant growth regulators such as gibberellic acid, to improve fruit set of seedless Valencia trees remains the most effective option, even though it is also not optimal. Foliar-applied nutrients to increase fruit set in two seedless cultivars indicated that responsiveness of these cultivars to these treatments is not well understood in terms of fruit set. The efficacy of aerial application of foliar nutrients was found to be increased by Masterlock® which aided in distribution in the canopy. Stemming from various projects and efforts of researchers and technical specialists a new nutritional guideline was completed and will be made available in early 2021. New strategies and technologies were tested in the ever increasingly complex citrus cold chain. This research has enabled the constant improving in temperature management, especially in the case of cold protocol markets. Due to the increased exposure of SA citrus fruit to temperatures below 2°C, an increase in chilling injury is experienced. A research project structured to evaluate all aspects which could determine fruit susceptibly to chilling injury as well as actions which could prevent symptom development, is ongoing.

PORTEFEULJE OPSOMMING

Deurlopende verhoging in opberging en verbetering van vrugkwaliteit tesame met ŉ vermindering in na-oes verliese bly sleutel aspekte van die Sitrusverbouing portefeulje. Navorsing programme en projekte word dus gestruktureer om die produsent geïdentifiseerde behoefte aan te spreek. Die eerste fokus is op ŉ deurlopend insamel en verskaffing van evaluasie inligting oor nuwe cultivar en onderstamme in terme van hulle aanpasbaarheid in die verskeie klimaatzones waar sitrus geproduseer word. Die uitbreiding en verbetering van die kultivar en onderstam opsies is nodig as onderbou vir die bedryf se kompeterende inslag voort te sit. Daar word tans aandag gegee aan ‘n afgeskeepte area in sitrusnavoring in ŉ studie waarin die faktore wat saadproduksie van onderstambomme kan verhoog ondersoek word. Alreeds was daar vordering wat dui om meer cultivar spesifieke bestuur aksies wat benodig word. Gedurende die afgelope seisoene in verder insigte in die voedingsbehoeftes en balans van saadlose culitvars gekry. Daar is egter gevind dat die plantgroei reguleerder, Gibberliensuur, die beste opsie is om vrugset te verseker in saadlose Valencias, al is dit ook nie optimaal nie. Die effektiwiteit om bemesting deur lugbespuitings toe te dien is ondersoek en daar is ŉ definitiewe positiewe impak gevind deur die gebruik van Masterlock® om die bespuiting beter te versprei in die boomdak. As ŉ uitvloeiing van verskei navorsing projekte in insette na tegniese persone is die nuwe plantvoeding handleiding voltooi en sal begin 2021 aan die bedryf beskikbaar gestel word. Nuwe tegnologie en strategie was getoets om temperatuur beheer te verbeer in ons komplekse koeiketting, veral ten opsigte van kouesterlisasie markte. As gevolg van die verhoogde volume uitvoere teen 2°C en laer word al meer koueskade gerapporteer in die markte. Die navorsings projek is so gestruktureerd om van alle voor en naoes se impak te bepaal in terme van sensitiwiteit en verhindering van simptoom ontwikkeling.

5.2 PROGRAMME: RIND CONDITION AND COLD CHAIN

Programme coordinator: Paul Cronje (CRI)

5.2.1 Programme summary

Challenges within the citrus cold chain have increased, not only due to the higher export volumes but also due to implementation of the FMS to the EU. Therefore, new approaches to packing, handling and shipping of our fruit are necessary to remain cost effective, comply with phytosanitary regulations and maintain fruit quality. Non-conformance of consignments exported in containers to cold sterilisation markets in the Far East are of a concern. In a novel approach the advantage of constant landside visibility by using GPS temperature sensors was evaluated and shows that monitoring the flow of our consignments could add value. Alternative stowing options to improve temperature management were evaluated in commercial shipments and showed that the Otflow floor cover decreases cooling performance, however, some novel air re-direction options could improve uniformity. Due to phytosanitary pests, a large portion of fruit will have to be exported to countries demanding a cold disinfestation protocol and this is likely to reduce the quality of fruit due to the development of chilling injury (CI) symptoms in the fruit rind. During the 2019 season a long-term project to determine the contribution of rootstock, cultivars, production area and harvest window was initiated. From the first season's results, clear differences were seen due to cultivar/type as well as rootstocks. Furthermore the impact of shade nets on chilling injury susceptibility was non-significant. Currently the most consistently effective management action to reduce the incidence of chilling injury remains adequate wax application.

Opsomming

Die druk op die sitrus koueketting het nie net alleenlik toegeneem as gevolg van die groeiende uitvoer volumes nie maar ook a.g.v. die implementering van die FMS vir die EU mark. Daar is dus ŉ behoefte na nuwe benaderings tot verpakking, hantering en verskeping van die sitrusvrugte wat koste-effektief is, inval met fitosanitere maatreëls en ook verseker vrugkwaliteit word nie benadeel nie. Een so ŉ aspek wat probleme bied is buite protokol spesifikasies tydens uitlaai van vragte vir die kouesterilisasie protokol na China en Koreaanse markte. In ŉ projek word gepoog om te bepaal of selfoon gebaseerde dataloggers die sigbaarheid van temperature in die vragte kan verhoog en so waarde toevoeg en vroeg probleme te kan aanspreek. In uitbreiding van die projek op verskeping in verkoeldehouers is die alternatiewe uitlaai van palette in houers getoets ten opsigte van tempertuur profiel en beheer. Dit is gevind dat die Otflow vloerbedekking nie effektiewelik verkoeling verbeter nie. Daar is egter gevind dat nuwe eksperimentele behandelings wel lugvloei beïnvloed en suksesvol was om verhoogde uniformiteit te bewerkstellig. As gevolg van die fitosanitere maatreëls word ŉ groot persentasie van die vrugte teen lae temperature uitgevoer wat tot koueskade kan lei. Gedurende die 2019 seisoen is ŉ projek begin om na faktore se bydrae tot vrug-sensitiwiteit te ondersoek. Die faktore sluit in skadunette, onderstam, kultivar, produksie area asook klimaat te ondersoek. Voorlopige data toon dat cultivar en onderstam wel ŉ invloed op koueskade sensitiwiteit uitoefen maar dat skadunette nie die skilkwaliteit negatief beïnvloed nie. Tans bly effektiewe en genoegsame aanwending van waks op vrugte die beste bestuursaksie om die voorkoms van koueskade te beperk.

5.2.2 PROGRESS REPORT: Ambient loading of citrus fruit for cold sterilization markets

Project 1125 (2018 – 2021) by T M Berry and Paul Cronje (CRI)

Summary

Projections indicate that future citrus production will increase significantly, resulting in increased stress on fruit precooling facilities. Additionally, international phytosanitary requirements are expected to become progressively more stringent, further increasing the cooling demand in precooling facilities (e.g. FMS). Ambient loading is a cold chain approach whereby palletised fruit bypass a precooling facility and are loaded warm into the reefer container, after which they are cooled within the container. This approach is a highly valued solution towards reducing stress on facilities, costs and waiting times. The aim of this study was thus to better apply ambient loading strategies. Specifically, the study examined the use of alternative container loading approaches that improve cold airflow distribution in the container. Results demonstrated once again that the

Otflow floor cover decreases cooling performance in containers for both ambient and cold treatment shipments. The study further showed that the use of an extended void plug and/or T-floor cover improved airflow distribution and cooling performance in ambient loaded containers. Numerical simulations further indicated that a combination of these two approaches will have an accumulative effect on cooling performance. Future studies should examine the effect on cooling of this loading approach at higher resolutions of data collection (i.e. more loggers).

Opsomming

Voorspellings dui aan dat toekomstige sitrus produksie drasties sal verhoog wat verhoogde stres op vrugte voorverkoelingsfasiliteite sal plaas. Daar word ook verwag dat internasionale fitosanitêre vereistes nog strenger sal word, wat verder druk op die voorverkoelingsfasiliteite sal plaas. Warm-laaing is 'n koueketting benadering waar vrugte 'n voorverkoelingsfasiliteit systap en warm gelaai word in die vraghouer, waarna hulle verkoel word in die vraghouer. Hierdie metode is 'n waardevolle oplossing om stres op fasiliteite, kostes en wagtye te verminder. Die doel van hierdie studie was dus om omgewingsladingstrategieë beter toe te pas. Die studie het spesifiek die gebruik van alternatiewe benaderings vir laai houers ondersoek wat die verspreiding van koue lugvloei in die houer verbeter. Resultate het weereens aangedui dat die Otflow vloerbedekking die verkoeling verminder het in houers vir beide warm-laai en kouebehandelde verskeping. Die studie het aangedui dat die gebruik van 'n verlengde void plug en/of T-vloerbedekking lugvloei distribusie en verkoeling verbeter het in warm-gelaaide houers. Numeriese simulasies het verder aangedui dat 'n kombinasie van hierdie twee benaderings 'n akkumulatiewe effek op verkoeling sal hê. Toekomstige studies moet die effek van verkoeling op die laaibenadering by hoër resolusies van data-insameling (d.w.s. meer loggers) ondersoek.

Stated objectives

The aim of this project is to determine the viability and improve on the large-scale adoption of ambient loading in the FMS without quality loss.

The specific objectives for 2019 were as follows:

1. In view of the FMS, determine the commercial implementation of ambient loading in terms of cooling rate, logistical efficiency and fruit quality.
2. Determine the impact of carton types and loading approaches on cooling rate during ambient loading conditions.
3. Validate the FMS shipment regimes.

Computational modelling of Refrigerated Containers

Simulation setup
Computational fluid dynamics (CFD) simulations were used to simulate airflow processes in a refrigerated container. Modelling methods and setup can be found in Berry et al., (2019) and Getahun et al., (2017). Although validated, the modelling approach is currently still being optimised and improved. This CFD approach was thus only used to evaluate various loading approaches with respect to the resulting airflow distributions in the pallet stacks.

Loading approaches
Figure 5.2.2.1 indicates the 12 loading approaches that were investigated. Each of these approaches attempts to manipulate the airflow distribution in the container. The specific goal is to evenly distribute airflow along the bottom of the pallet stacks, so that it flows vertically through the pallets and then returns to the refrigeration unit via the top plenum of the container. Horizontal airflow movement in the pallets is thus highly undesirable, as air is rapidly saturated with heat at these flow rates.

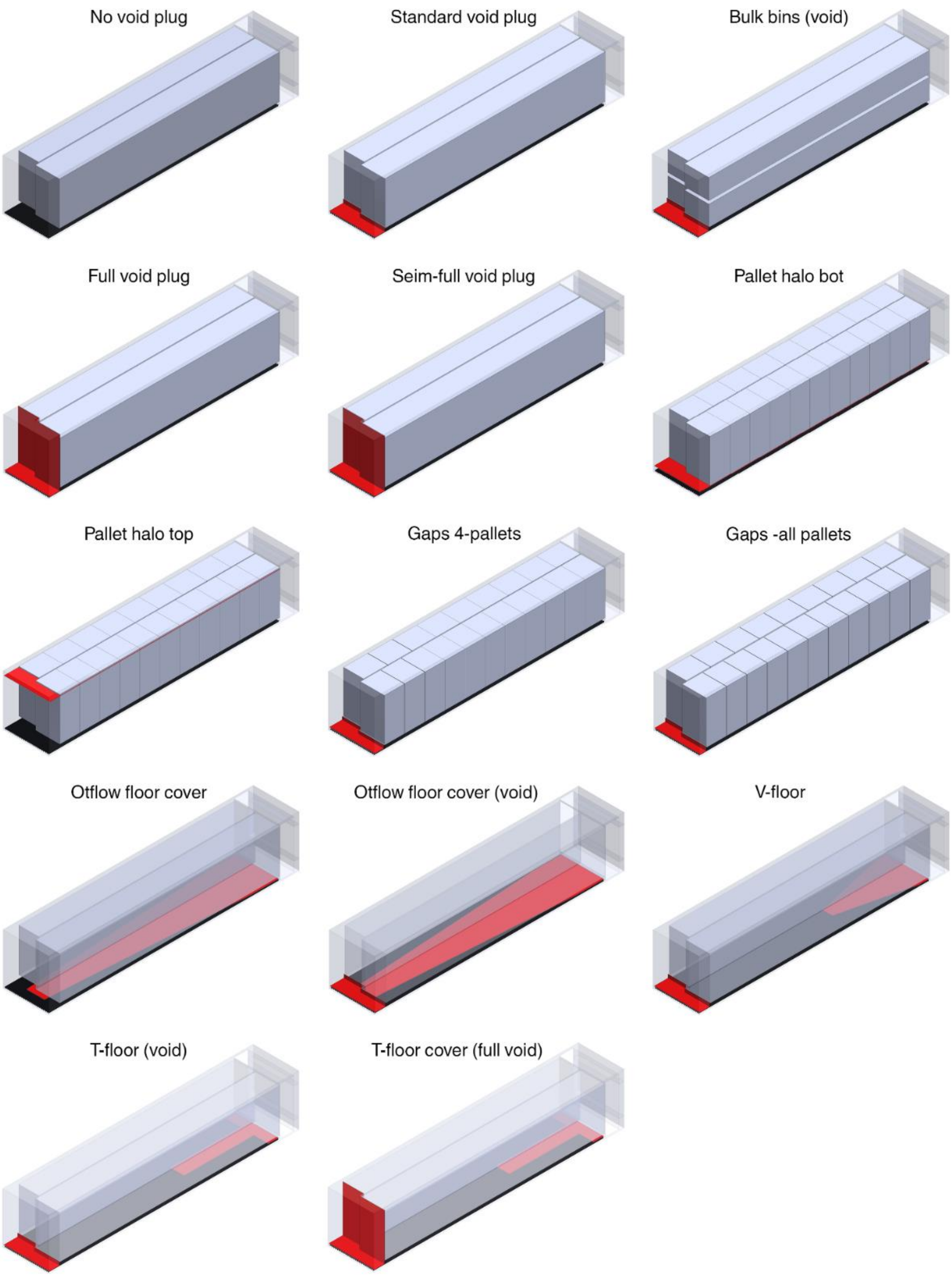

Figure 5.2.2.1. Illustration of the various container loading aids that were evaluated using CFD. Red colours indicate loading aid devices. The T-bar floor is indicated in black near the bottom of the container. The door end of the container is on the left.

Simulation results

Figure 5.2.2.3 shows the vertical airflow rates in the eight octants of the pallet stacks volume within a refrigerated container. In these cases, higher velocities are desirable, as they indicate improved airflow throughput within the pallet. Lower air velocities indicate airflow was either traveling horizontally (i.e. into an adjacent pallet), or the position was in an airflow “dead zone”. The scope of this evaluation approach is of course limited and improvements are already being made towards better methods to more holistically (and simply) quantify a container’s performance. However, for each container, the airflow properties and distributions were examined in detail (CFD postprocessing; Figure 5.2.2.2).

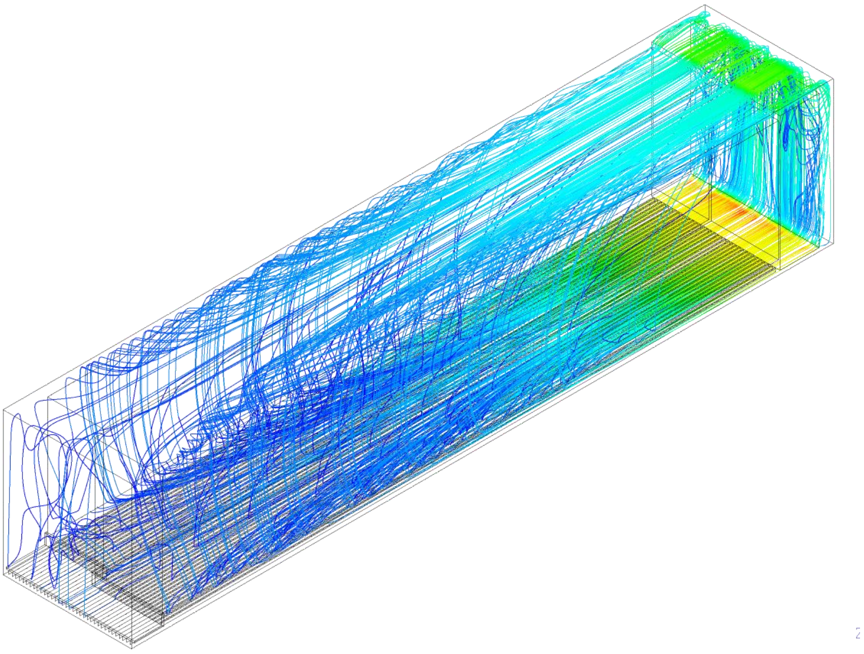

Figure 5.2.2.2. CFD simulation showing airflow rate streamlines throughout the reefer container.

The results in Figure 5.2.2.3 show a relatively close approximation to the performance observed during detailed analysis, which provides a strong validation for this methodology. The most critical regions in this graph are the back regions of the container, namely the bot_P4 (red) and top_P4 (grey). These regions are furthest from the refrigeration unit and have thus often lost momentum and gained heat. Additionally, the back regions are close to the door, which has less insulation than the walls and frequently allows some warm air to escape into the container through the joins of the door. The back pallet regions are thus a priority to achieve higher vertical air velocities.

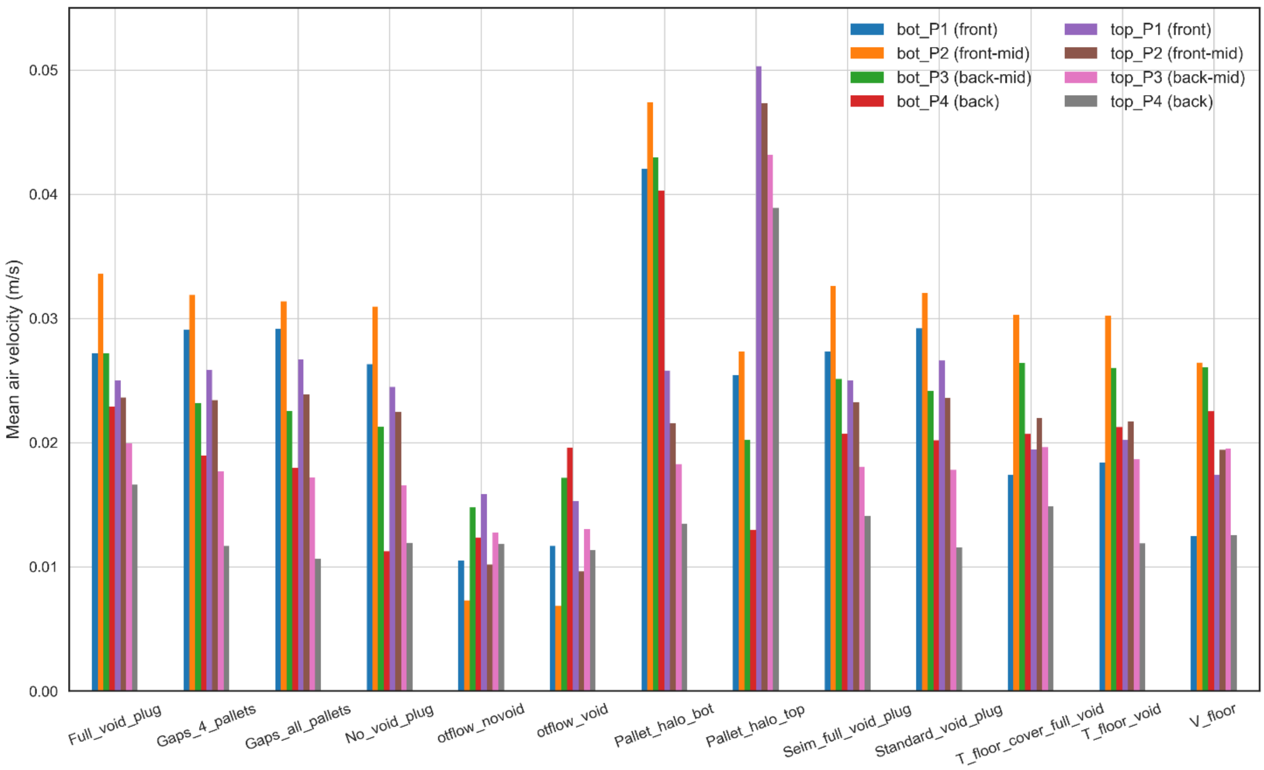

Figure 5.2.2.3. Vertical airflow in pallets for the examined container loading approaches.

Standard setups

For the standard container (with a void plug), the main challenges transpire near the top back regions, which appear as low vertical airflow rates. The effectiveness of placing gaps between the pallets was explored, but was ineffective. It was theorised that gaps could eliminate horizontal air flow between the pallets near the back container. However, the gaps simply increased airflow short circuiting and were thus largely ineffective compared to the control.

Otflow

The Otflow floor cover was the poorest performing container aid, as it facilitated substantially less vertical airflow than any of the other treatments, including the control container. The low air velocities can be attributed to the large T-bar floor coverage of the Otflow sheet (with or without a void plug), which impeded the fan system and reduced the overall flow rates. Similar findings are demonstrated in experimental ambient loading studies from 2018 and were also confirmed in experimental cold treatment studies (2019, results below). An important take-away lesson here, is that no container loading aid should be implemented into a container if it will substantially increase the airflow resistance of the refrigeration system. Out of interest, a smaller V-shaped floor cover, with a similar design to the Otflow was examined. However, although its performance was substantially better than the Otflow, the V-floor also did not improve on the Standard design.

Pallet halos

A more viable solution was the pallet halos, which are envisioned as cardboard washers around the pallet bases or at the top of the pallets. The objective here is to force all airflow through the pallet and eliminate all airflow short-circuiting. Regrettably, the pallet halo top and bottom devices resulted in vertical air velocities that were lower than the control containers for the bottom and top regions (back of the container), respectively. Another notable challenge is the expected difficulties in installing the device, which is expected to be difficult to implement at large shipping scales.

Extended void plugs

The extended void plugs were designed to eliminate horizontal airflow into or out of the back sides of the exposed pallet stacks. The flow of air being delivered from the bottom of the pallet is thus determined by the Coandă effect (Reba, 1966), whereby the air ‘sticks’ to the void plug and doesn’t drift toward horizontal flow. The results for both the full and semi-void plug showed promising results in simulation and it is expected that the semi-void plug system will be more practical to setup than a full void plug.

T-floor

One of the design trade-offs of refrigerated containers is that the refrigerated unit is at the far end of the container. Airflow rates are thus more concentrated at this end versus the door end. The T-floor was thus designed to alleviate this challenge by distributing more of the air towards the door end of the container, without drastically increasing airflow resistance in the system.

The overall results were promising, particularly when combined with an extended void plug. A combination of these two aids may thus offer a solution to the heterogeneous cooling conditions in the container, as well as reducing the incidence of “warm spots” which are sometimes observed during shipping.

Experimental ambient loading investigations

Experimental setup

Refrigerated container, cartons and fruit

Commercial cooling experiments were replicated twice using two identical state-of-the-art 40-foot, high-cube refrigerated containers (outer dimensions L × W × H = 12.2 × 2.4 × 2.9 m). Every container was stuffed with

20 pallets, each of which was stacked with 80 individual cartons (8 layers of 10 cartons). Each A15C telescopic carton was place-packed with Valencia citrus fruit and had an average carton mass of about 15.5 kg. Cornering strips and pallet straps were further applied to ensure the pallet stacks remained stable during transport.

Full scale refrigerated container experiments

Citrus fruit were harvested (near Sundays River Valley, SA) and taken directly to a packhouse where the fruit received standard commercial treatments. Fruit were then packaged, palletised and transported by road (1 hour) to a nearby cold store, where the stacks were loaded into a refrigerated container and placed onto a container vessel. The vessel sailed from the Port Elizabeth to the Port of Rotterdam (Netherlands) over a 28-day period. The refrigerated container's temperature set-point was programmed to 2°C (Moore et al., 2016) and vents for fresh air exchange were set to 4.2 L s-1 as per South African citrus export regulations (PPECB, 2006).

Three container load-out approaches were examined as illustrated in Figure 5.2.2.4. Treatments thus included a (i) standard container setup, with a void plug. The void plug was manufactured from corrugated fibreboard and covered both the exposed T-bar floor as well as the horizontal openings of the exposed pallet bases. The void plug thus stopped airflow delivered via the T-bar floor from bypassing the pallet stacks (GDV, 2014). (ii) An extended void plug setup, whereby the vertical wall of the void plug was extended to cover the whole side wall of the last pallet stacks. (iii) Finally, the T-floor cover in combination with a normal void plug was examined.

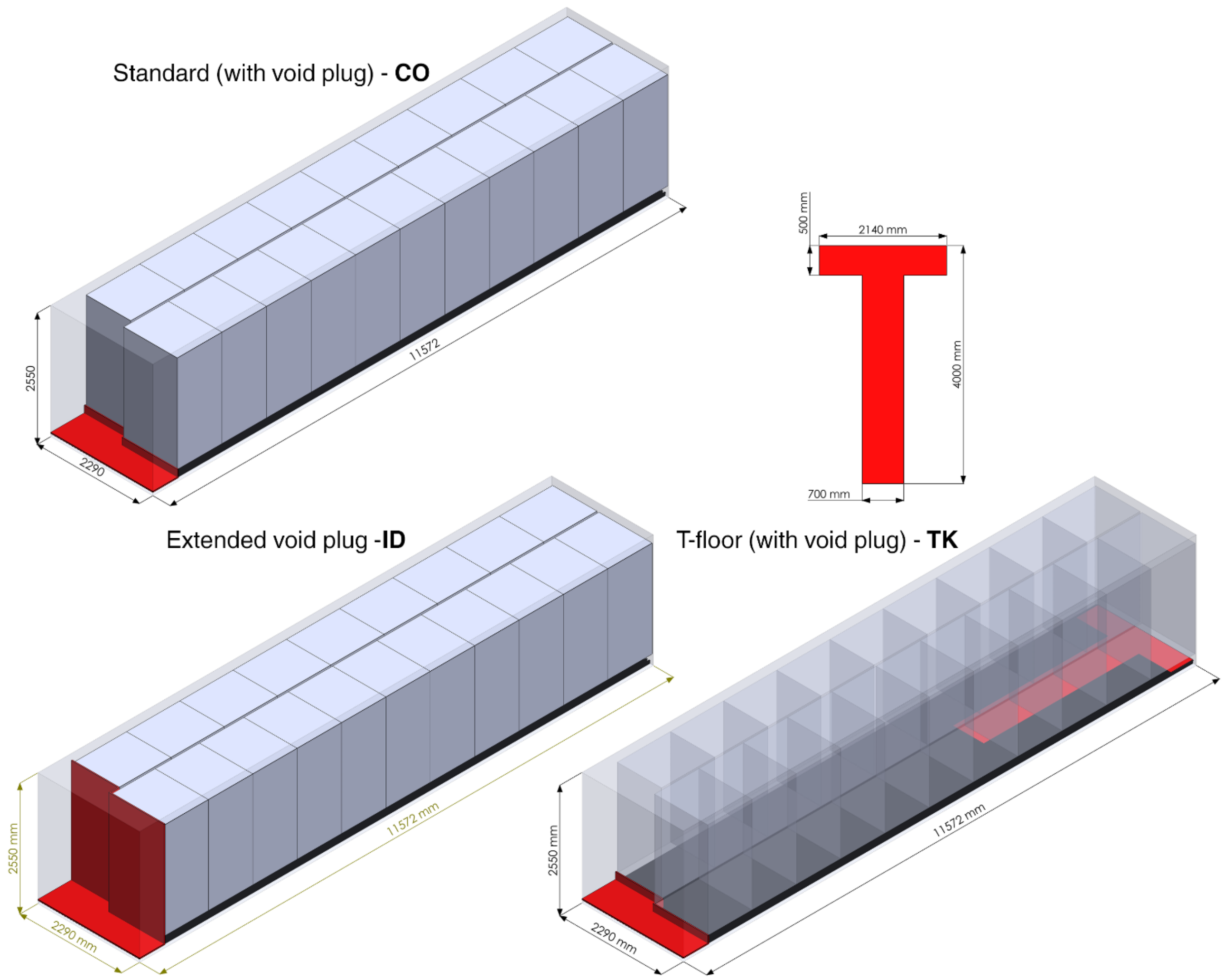

Figure 5.2.2.4. The three experimental container setups that were examined.

Temperature monitoring and data analysis

Before container stuffing, six pallet stacks (as indicated in Figure 5.2.2.5: R03, R06, R09, L01, L05, L10) were probed with temperature loggers (accuracy of ±0.5°C, data logging every 10 min). For each pallet, three cartons were probed, namely at the top, middle and bottom layers. Loggers were inserted in the centre of the fruit, which were then placed at the centre of the cartons. The spatial cooling distribution of fruit cooling within the container could thus be monitored throughout the voyage.

Figure 5.2.2.5. Layout of temperature loggers in each refrigeration unit.

Ambient loading results

Figures 5.2.2.15 and 5.2.2.16 illustrate cooling distributions throughout the refrigerated container using contour plots. These plots highlight regions in the container with poor cooling performance. Results show promising results from the extended void plug and the T-floor cover. As expected, high variations in cooling were observed between the container replicates. The first control container (CO1) showed multiple warm spots in the container compared to CO2, which cooled relatively evenly. This again illustrates the challenge of the current loading approach whereby containers with standard loadouts have a probability of developing warm spots.

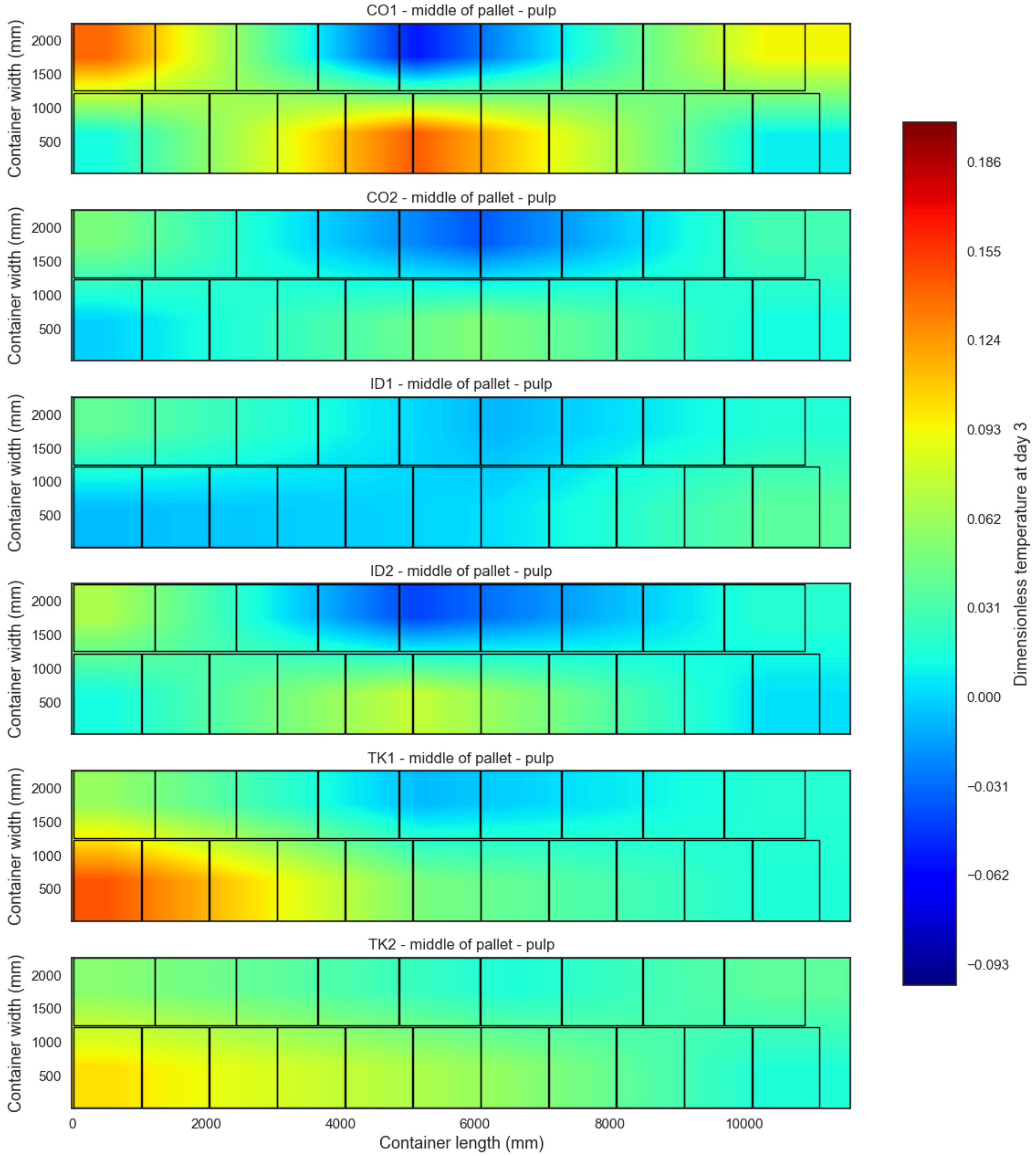

Figure 5.2.2.6. Dimensionless temperature after 3 days of cooling. Note, dimensionless temperature represents initial temperature as 1 and final (set-point) temperature as 0.

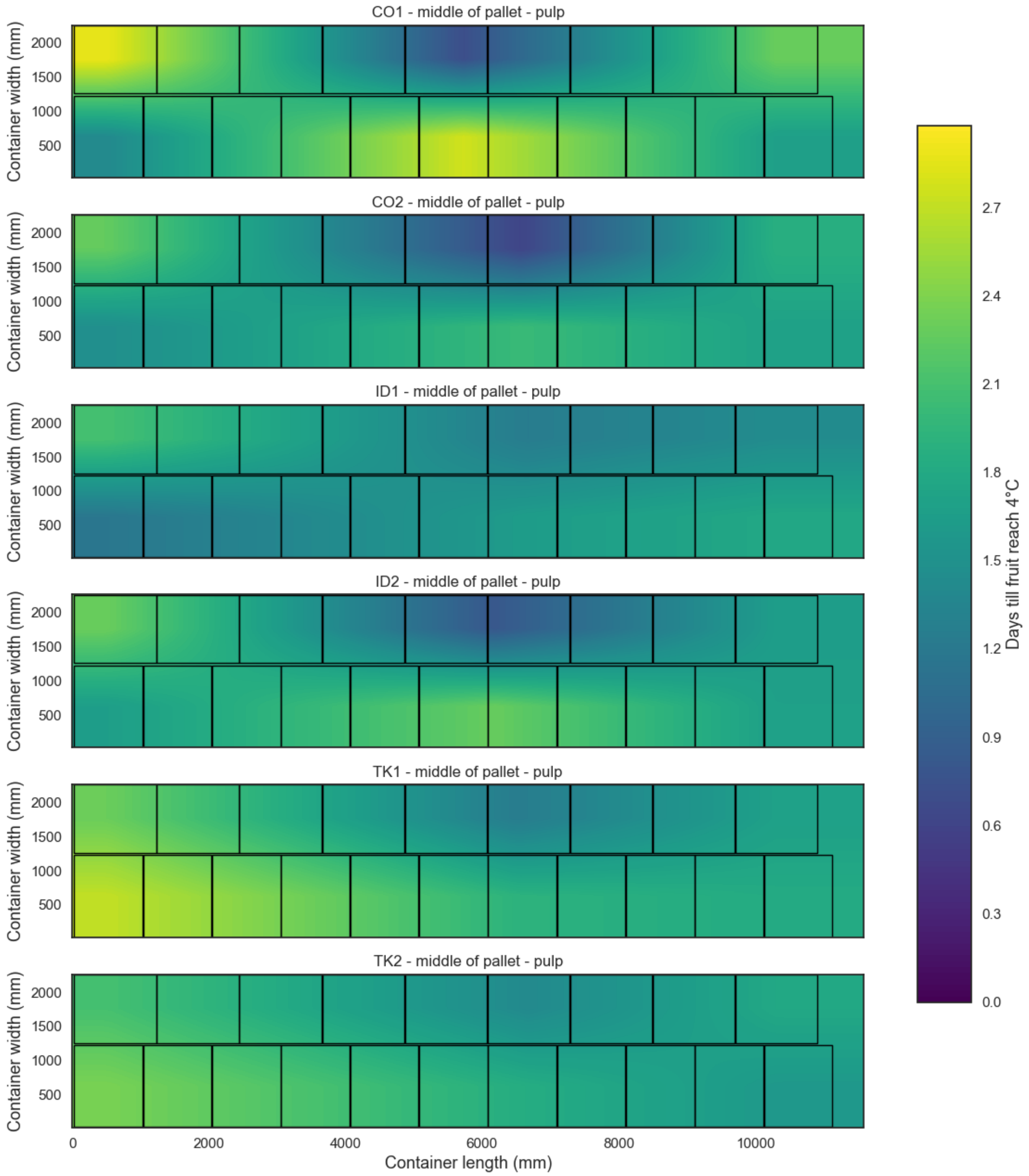

Figure 5.2.2.7. Contour plot illustrating duration (days) of cooling taken for fruit to reach to 4°C.

The extended void plug (ID1 and 2) showed promising results by facilitating comparatively rapid and uniformly cooling throughout the containers. It should be noted, however, that the replication numbers would need to be increased to fully evaluate this approach. These results help to validate the hypothesis of applying the Coanda effect by way of the extended void plug. The results for the individual logger locations in Figures 5.2.2.17, 18, 19 and 20, further indicate the top and bottom regions of the pallet stacks also cooled effectively.

Similarly, the containers using the T-floor cover also cooled relatively uniformly. However, cooling rates were slightly higher near the refrigerated unit, where the cover obstructed the floor, but were still within desirable rates. Of note, is that the pallet stacks near the door end of the container showed negligible signs of having warm spots. Although one temperature logger at the back of the container (Figure 5.2.2.11) did record a slight decrease in cooling rate. The results thus indicate that more of the cold air was distributed to the door-end of the container.

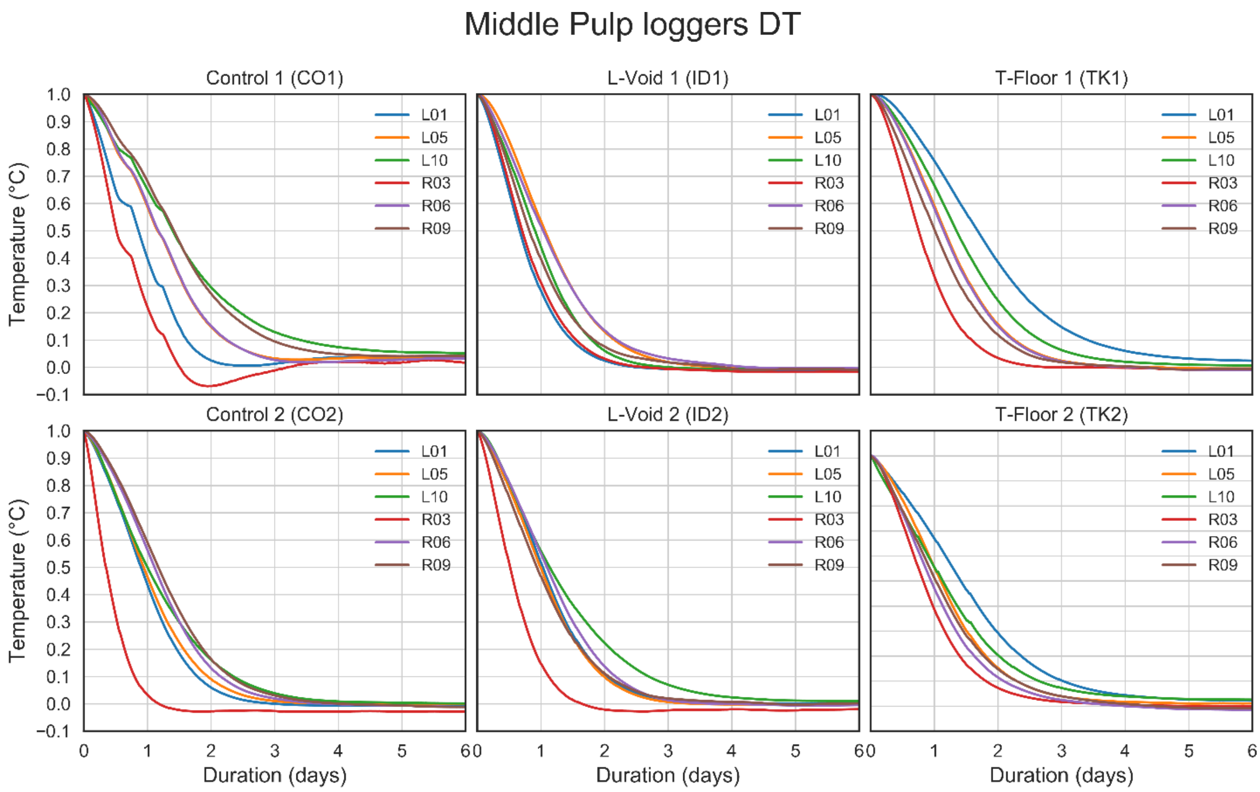

Figure 5.2.2.8. Dimensionless temperature curves for middle pulp temperature loggers.

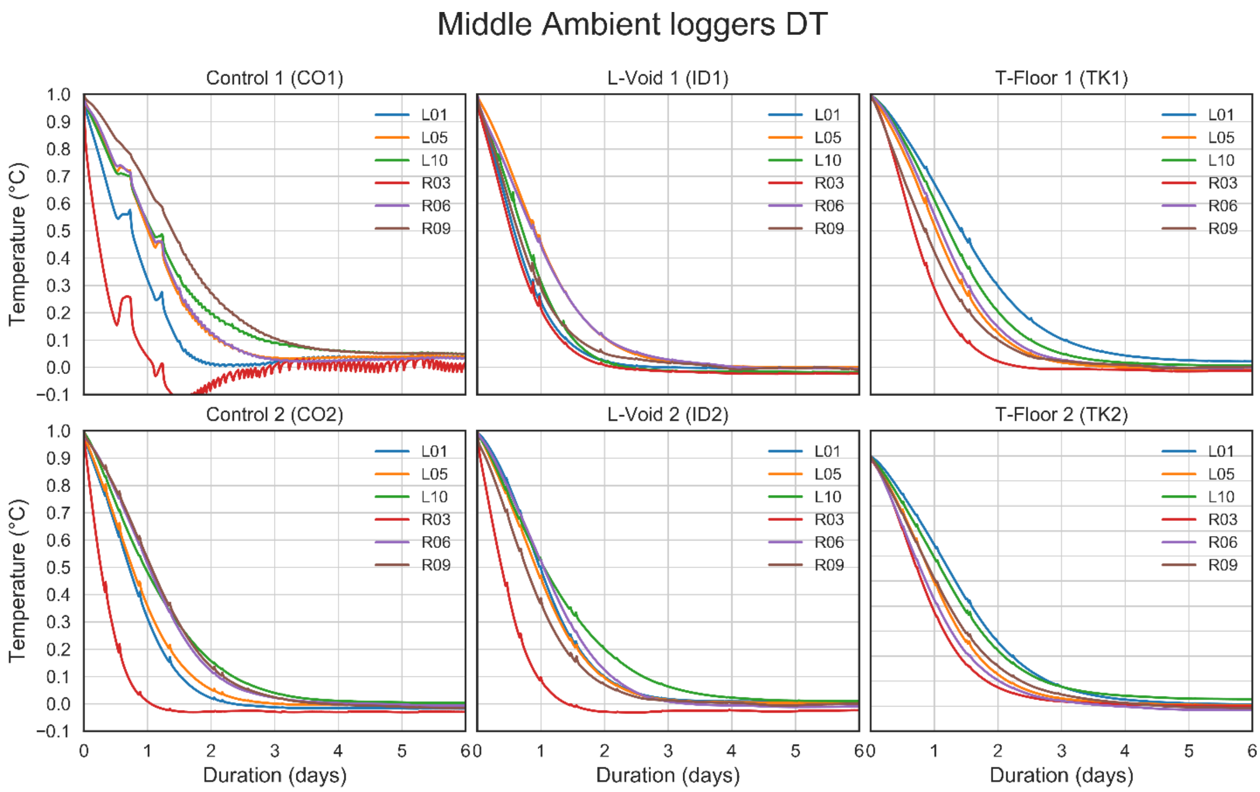

Figure 5.2.2.9. Dimensionless temperature curves for middle ambient temperature loggers.

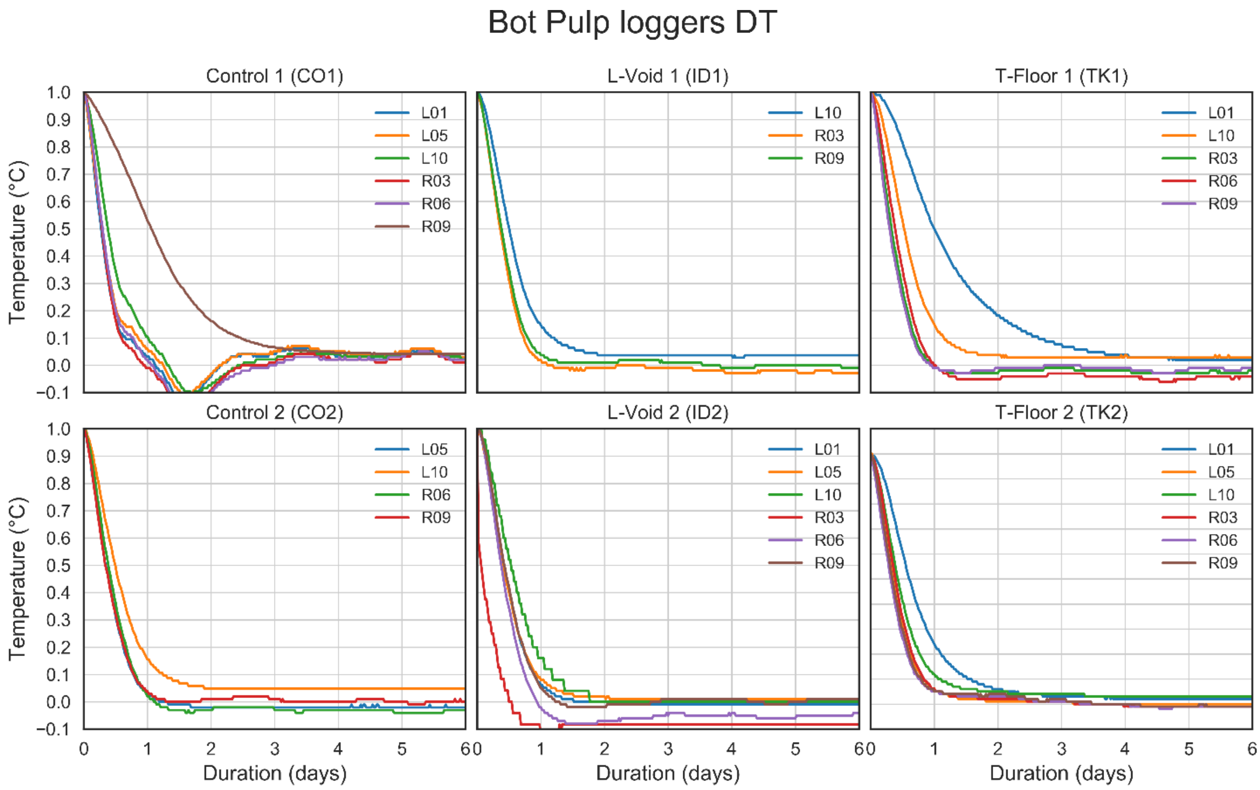

Figure 5.2.2.10. Dimensionless temperature curves for Bottom pulp temperature loggers.

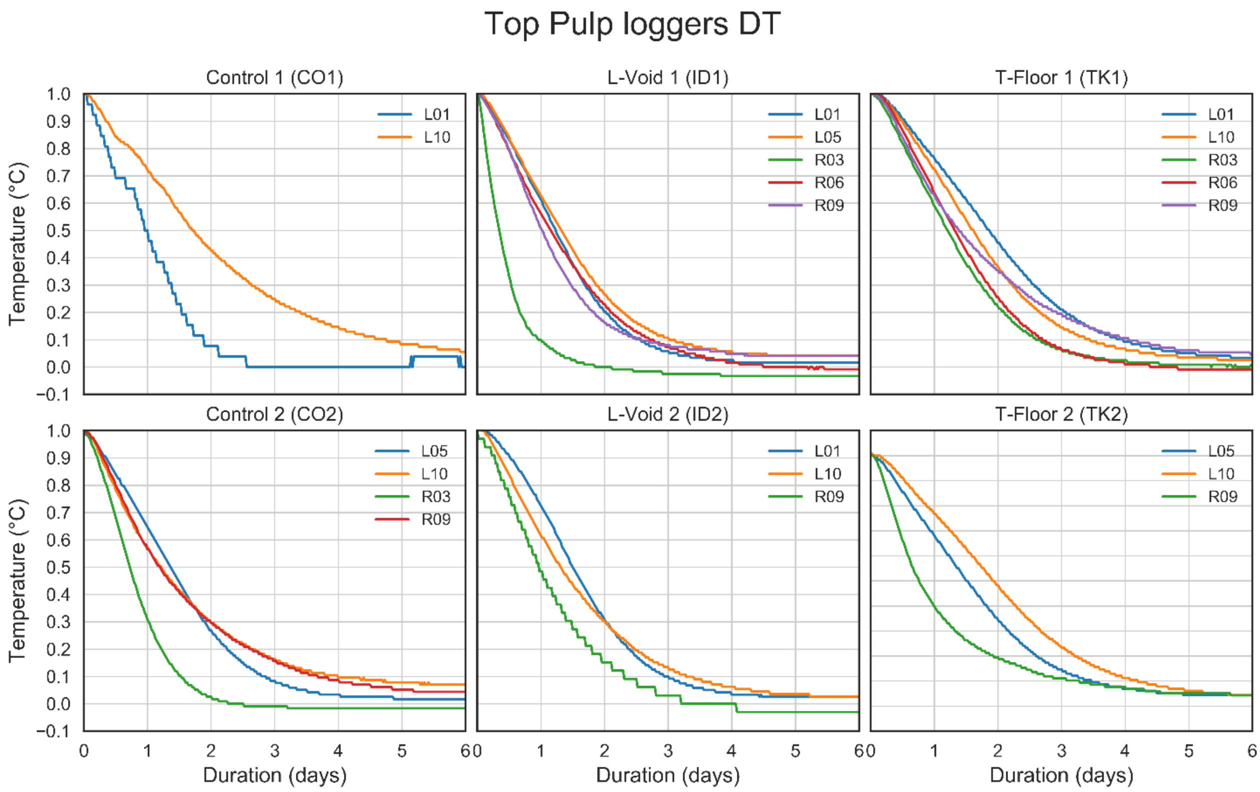

Figure 5.2.2.11. Dimensionless temperature curves for Top pulp temperature loggers.

Results from the two loading aids showed promising results with respect to improving airflow distribution in the container and consequently reducing the incidence of warm spots. Future work should thus further examine these approaches at larger scales (more containers) and at high resolution (more temperature loggers).

Numerical simulations should also be included in these evaluations to better examine how the aids/devices are actually influencing the airflow distribution and what other factors in the container could be important.

Experimental setup – Otflow in Cold treatment containers (China)

Experimental Setup

The Otflow floor covers were tested in three containers, by three exporters, under normal commercial shipping conditions for the Chinese market. The results were then compared to containers that were loaded on the same vessel and packed with the same fruit and packaging type. Multiple control containers were used in this study, since temperature readings always differ slightly between even similarly packed and loaded containers (Table 5.2.2.1). The additional comparisons thus provide some insight into whether observed differences were a result of the expected error or from the loading approaches.

Table 5.2.2.1. Number of containers examined in the study.

Exporter	Otflow containers	Control containers
A	1	4
B	1	2
C	1	0

Results

On paper, the principles behind the Otflow design show promise. However, its successful application seems to be hindered by several practical factors in the South African citrus industry. For example, simulations indicate the Otflow would be more effective in a container with a more even distribution of pallets, as was tested in the initial development study (Lukasse et al., 2017). In contrast, SA citrus is loaded with a relatively large opening near the door, which allows airflow to bypass pallet stacks. This factor was accounted for (in this study) by plugging the exposed openings along with the pallet bases nearest the door. The approach did appear to improve cooling performance compared to the standard Otflow setup (which performed poorly).

Regretfully, the Otflow provided no apparent benefit to cooling performance in any of the examined containers and one case contributed to full cold treatment failure (China; Figure 5.2.2.12). Additionally, installation of the floor covers was generally disruptive to the loading process and either hindered forklift operations (wheel slippage) or was in turn damaged/torn by the forklift's wheels. The Otflow floor cover is thus not recommended for use in the South African citrus industry to cold protocol markets or during ambient loading.

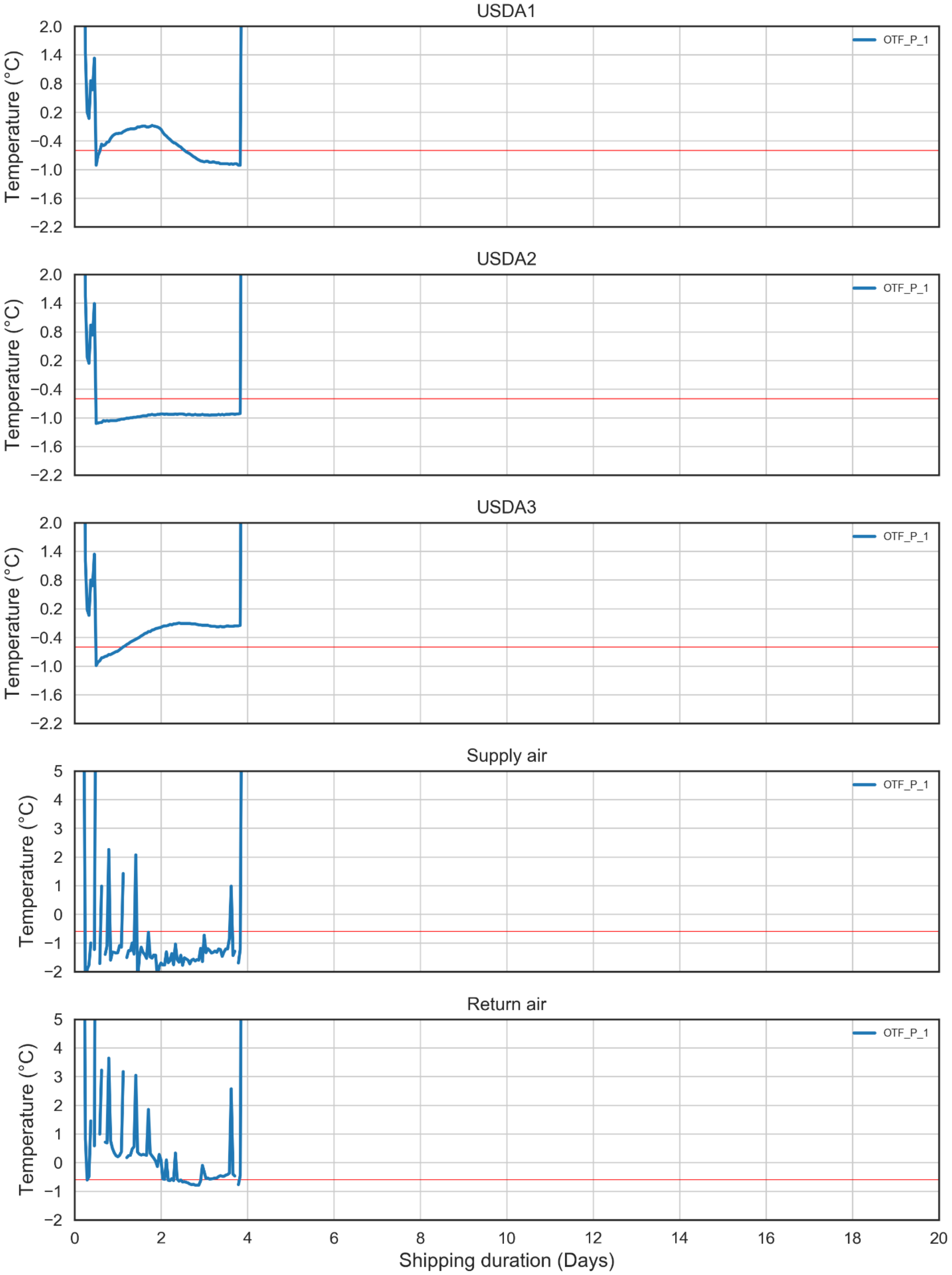

Figure 5.2.2.12. Temperature profiles for containers loaded with Exporter C.

Publications

A publication has been accepted in Biosystem Engineering to be publised 2021. Title: "Cooling of Ambient-Loaded Citrus in Refrigerated Containers: What Impact Do Packaging and Loading Temperature play?"

Conclusion

This study is exploring novel packaging solutions to more effectively apply phytosanitary cold treatments to citrus fruit during shipping. The industry has a long and relatively complex history with respect to the design of its current packaging systems. An important lesson identified in this project is that these factors need to be understood and applied to future designs, or the mistakes of the past will be repeated.

The studies show substantial promise in the new container loading approaches (T-floor and extended void-plug). The next step is to evaluate these loading aid devices at larger scales. This can be achieved by using the units in several hundred containers and then making use of the new cellular logger data system for FMS shipping.

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5.2.3 **PROGRESS REPORT: An investigation into aspects affecting chilling injury in Citrus fruit**

Project 1247 PHI-4-12 (2019/20 - 2021) by PJR Cronje, JJ North (CRI), Bahlebi Kibreab Eiasu and Phillipine Moabelo (University of Fort Hare)

Summary

Citrus fruit is one of the most important export commodities for the South African economy, with a significant increase in export volumes forecasted. Due to phytosanitary pests, a large portion of this fruit will have to be exported to countries demanding a cold disinfestation protocol of various severities. However, these low temperature regimes ≤2°C are known to reduce the quality of fruit due to the development of chilling injury (CI) symptoms in the fruit rind. Citrus fruit may develop necrosis and pitting in the flavedo tissue when stored at temperatures ≤4°C, thereby reducing the market value of the fruit. In order to reduce the susceptibility and severity of CI that affects sustainable export to all markets, this project is focused on identifying pre-harvest factors that contribute to CI susceptibility and develop postharvest options to reduce the severity. The aim of this project is firstly to develop detailed recommendations as to the impact of: cultivar x area interactions, shade netting, fruit maturity, genetic contribution as well as the influence of rootstock choice on CI susceptibility. Secondly, to reduce the severity of CI by incorporating postharvest management and technologies. For all trials (unless stated otherwise), fruit was harvested in the commercial optimum window and transported either to HortSci/SU or CRI/Nelspruit for cold storage at -0.6°C for 32 days. The following data were collected: maturity indexing and flavedo sampling on 10 fruit prior to cold storage, colour, size, TSS, TA, juice %, sampled flavedo for pigment and carbohydrate analysis, rind characteristics, thickness and dry weight. All fruit were evaluated for CI incidence after a 7-day shelf life period. 2020 will be the last season of data collection, after which a final report will be written in 2021.

Opsomming

Sitrusvrugte as een van die belangrikste uitvoerprodukte vir die Suid-Afrikaanse toon beduidende toename in uitvoervolumes. Maar as gevolg van fitosanitêre plae, word 'n groot volume vrugte uitgevoer na lande wat 'n koue-sterilisasieprotokolle vereis. Hierdie lae-temperatuurregimes, ≤ 4 °C, benadeel vrugkwaliteit as gevolg van die ontwikkeling van koueskade in die skil. Sitrusvrugte ontwikkel koueskade gesien as nekrotiese en gepokte merke wanneer dit by temperatuur ≤ 4 °C gestoor word, wat die markwaarde van die vrugte verlaag. Ten einde die voorkoms van koueskade wat die volhoubare uitvoer benadeel te verminder, fokus hierdie projek eerstens op die identifisering en kwantifisering van voor-oes faktore wat bydra tot die sensitiwiteit en tweedens die ontwikkeling van opsies om die graad van koueskade te verminder. Die projek het ten doel om gedetailleerde aanbevelings te ontwikkel rakende die impak van interaksie tussen kultivar en produksie area, vrugrypheid, die genetiese bydrae sowel as die invloed van die onderstok keuse op koueskade sensitiwiteit. Verder meer word gepoog om die graad van koueskade te verminder deur na-oes behandelings soos waks en chemikalie in te sluit proewe. Vir alle proewe (tensy anders vermeld) word vrugte in die kommersiële optimum plukvenster per area ge-oes en word dit óf na HortSci/US of CRC-Nelspruit vervoer vir koelopberging teen -0.6 ° C vir 32 dae. Die volgende data is versamel: rypheid-indeksering en flavedo monsters vir pigment onleding; skilkleur, vruggrootte, TSS, TA, Sap%, asook pigment- en koolhidraatanalise van die flavedo. Die skileienskappe word gekwantifiseer as skil dikte en droë gewig en alle vrugte word beoordeel vir die voorkoms van koueskade na 7 dae rakleeftyd. Gedurende 2020 vind die laaste seisoen van data-insameling plaas, waarna 'n finale verslag in 2021 beskikbaar sal wees.

1: <u>Determine the influence of production area on the susceptibility of fruit to CI</u>
The six commercially significant cultivars grown in the major production areas of South Africa were evaluated i.e. cultivar x area interactions. Five orchards per cultivar in each major production area were identified; Western Cape, Eastern Cape, Mpumalanga and Limpopo. The following commercially important cultivars were selected;

- Soft Citrus: Nova (Western and Eastern Cape only) and Nadorcott mandarin
- Grapefruit: Star Ruby and Marsh (Limpopo/Mpumalanga only)
- Valencia: Turkey and Midknight

The harvesting, cold storage and data collection of all cultivars in the various production areas was completed in the 2019 season. The 2020 harvesting and cold storage treatments are currently underway. After the 2 seasons, the climate data (temperature, rainfall and RH) from each production area and harvest date will be used to quantify any significant difference or changes of the pre-harvest climatic conditions on the susceptibility of CI.

2: <u>Ascertain the extent to which fruit maturity at harvest influences the susceptibility of fruit to develop Chilling Injury</u>
Nova and Midknight fruit were harvested from orchards in Citrusdal at 2 week intervals over a period of 14 weeks. The 2019 preliminary results indicate that immature fruit is more susceptible to CI than fruit harvested at commercial maturity. As fruit becomes over mature sensitivity to CI increases once again. This was more severe in Nova, than in Midknight Valencia.
This will be repeated on Midknight Valencia in the current season (2020). Unfortunately Nova harvests were unable to resume during stage 5 Covid-19 lockdown.

3: <u>Determine the impact of rootstocks on CI susceptibility</u>
Current rootstock research blocks established in 1997 and 2002 were identified; Letaba Estates (Letsitele), and Golden Frontier (Komati/Hectorspruit) respectively. Delta, Midknight and Star Ruby fruit grown on a total of 35 rootstock types are used for evaluation.
Fruit sampling, cold storage and data collection was completed in the 2019 season. There was low CI observed in ‘Star Ruby’, however, some significant differences were observed between fruit of Delta and Midknight fruit grafted onto various rootstocks. This trial will be repeated in the current season and sampling is underway. After 2 seasons of data, the influence of rootstocks on fruit quality and susceptibility to CI will become more evident.

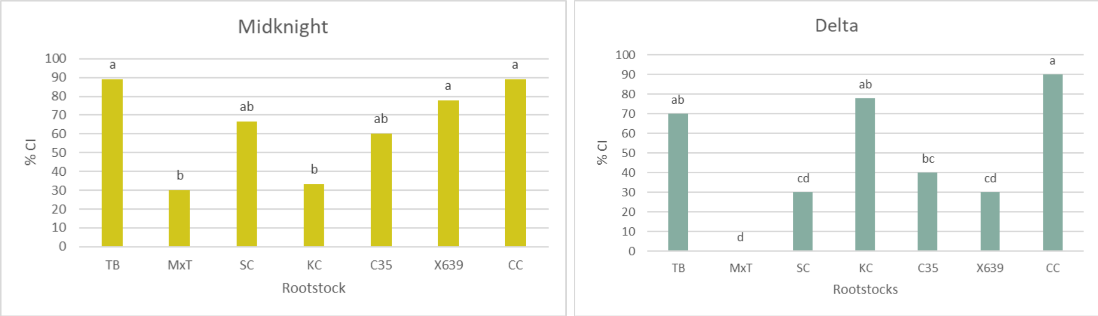

Figure 5.2.3.1. Influence of different rootstocks on % Chilling injury in Midknight and Delta Valencia from Letsitele and Hectorspruit ($p \leq 0.05$).

4: <u>Determine the genetic contribution to CI susceptibility</u>
Fruit from a genetic pool as wide as possible, which included fruit from rootstocks, were obtained from the CFB throughout the season. At full maturity fruit were sampled and transported to HortSci/SU for cold storage treatment (-0.6°C for 32 days), whereafter it was evaluated for CI incidence. The aim is to try to group cultivars in susceptibility groups, and thereafter to determine whether the ancestral admixture contributes to the susceptibility to CI.
In the 2019 season, 88 different cultivars were included. In addition to these, permission from numerous cultivar development companies has been sought to include some commercially important protected cultivars in the 2020 season. Sampling and cold treatments are currently underway for the current season. In addition to CI susceptibility, maturity indexing and flavedo sampling will be conducted for pigment analysis.

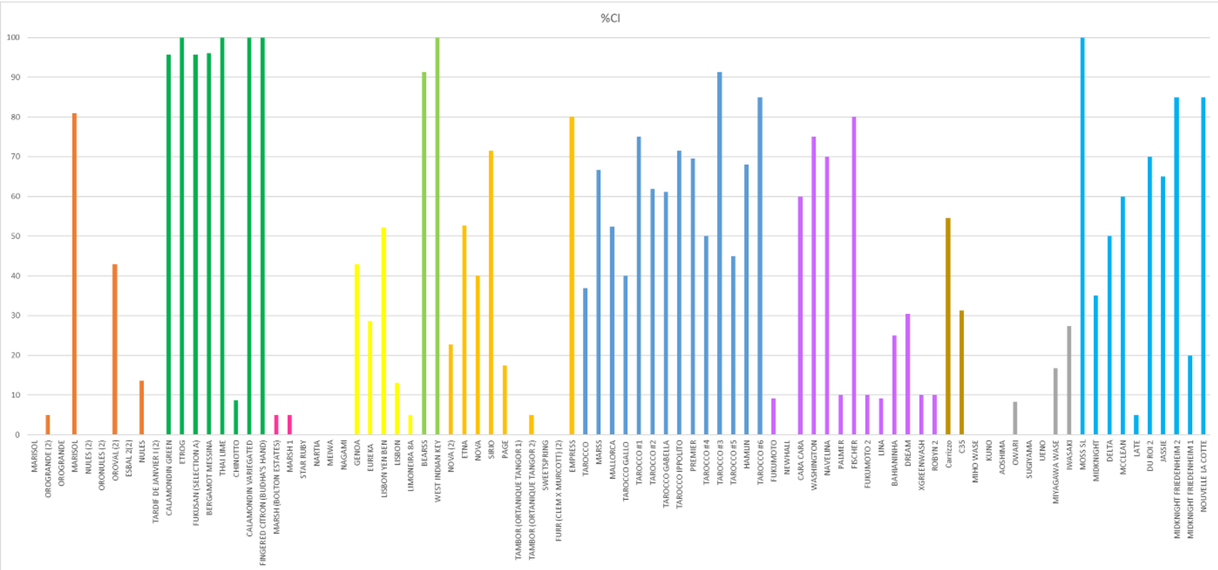

Figure 5.2.3.2. Graphic representation of the % chilling injury found in the 88 different citrus cultivars after 32 days at -0.6°C.

5: <u>Impact of different cold treatment protocols on CI incidence</u>
To determine the impact of different cold sterilisation protocols on CI incidence and severity (Time X Temperature), Nova and Midknight fruit were stored at various cold storage treatments and durations; -0.6°C, 2°C and 7°C for 12, 22, 32 and 42 days. Fruit were evaluated for CI after cold storage, flavedo sampling at each interval was done for pigment and carbohydrate analysis. The fruit respiration rates at each interval and for 7 days after shelf life at ambient temperature, was measured. This trial is being repeated in the current season.

6: <u>Investigate the post-harvest use of wax types and solids as a curative treatment on reducing CI incidence</u>
To determine whether a wax can protect fruit against developing CI when shipped at low temperatures, both natural and synthetic waxes with varying solid contents were evaluated to try to identify the most suitable wax type and solid content for ‘Nova’ mandarin and ‘Midknight’ Valencia.

In the 2019 season, 7 different wax treatments were applied in commercial packhouses to ‘Nova’ (Sitrusrand packhouse, Kirkwood) and ‘Midknight’ (Groenkloof packhouse, Citrusdal): 14% (synthetic, polyethylene), 14% (natural, carnauba), 18% (synthetic), 18% (natural), 18% (synthetic + TBZ), 20% (natural), 20% (synthetic), 2x20% + TBZ, control (untreated). This was repeated in the 2020 season, with the ‘Nova’ wax trial already applied with fruit currently in cold storage for 32 days.

7. <u>Determine the impact of shade netting in the Sundays River Valley on the external and internal fruit quality of Navel, Mandarin and Eureka lemon.</u>
In order to establish the possible impact of shade netting on chilling susceptibility and internal quality a study was conducted over two seasons in Addo, Eastern Cape, South Africa (2018-2019) in commercial blocks of shade netted cover and open orchards. The monthly recordings were taken between May and September. Citrus external quality aspects such as fruit size, rind colour, turgidity, rind thickness, dry matter percentage, fruit weight loss were measured in addition to the juice %, Brix, Total acid. Climatic changes were recorded in the structure and compared with open orchards. The results indicated that shade net improves the fruit size compared to the open. In ‘Cambria’ Navel the net also improved the rind fruit colour whereas other cultivars’ colour was not affected. Results also indicated that fruits from the net do have a softer rind, making them possibly more susceptible to bruising. No chilling injury was detected on waxed fruit.

Experimental layout

The samples were collected at four farms around Addo, Eastern cape, South Africa. Eureka lemons and ‘Washington’ Navels were collected at Allendale farm, ‘Cambria’ Navels were collected at Nuwelande and Olifantsbos farm and lastly ‘Valley gold’ mandarin were collected at Dunbrody farm. Samples were stored at Addo cold store (SRCC packhouse) for a period of 30 days. Evaluations of external quality (chilling injury) were conducted 7 days after 30 days of cold storage treatment at SRCC (Sunday River Citrus Company) in Kirkwood. Internal qualities were conducted at CRI in Port Elizabeth in the first season. Both external and internal qualities in the second season were conducted at University of Fort Hare, department of Horticulture laboratory.

Conclusions

Shade net improves the size of citrus fruit, which is advantageous for exports. Preliminary results indicate that maturity indexing was not influenced by the shade net. Shade net reduced the incidence of sunburn and wind blemishes, although citrus rind was softer under net making it more susceptible to post harvest injury. The use of 4°C and waxed samples for the export market is highly recommended. The extension of the research trials and additional parameters will be conducted and evaluated in the third season (2020) after the Covid-19 pandemic settles.

5.2.4 PROGRESS REPORT: Integration of pallet bases and carton designs to improve ventilation of citrus exports

Project 1237 (2019 – 2020) by T M Berry and Paul Cronje (CRI)

Summary

In recent years, the citrus industry has become increasingly more reliant on cooling to meet phytosanitary requirements. Cold treatment markets for example, require extensive precooling at cold store facilities. Furthermore, European markets now require fruit to be shipped at temperatures below 2°C. For these markets, fruit are often loaded at ambient temperatures so that they can bypass cold store facilities. Ambient loading is a promising approach towards reducing stress on facilities, costs and waiting times. However, cooling rates and uniformity are often inadequate and could be improved if the package ventilation facilitated better airflow distribution within the refrigerated containers and during precooling. The cooling process could thus be better controlled, allowing the industry to reduce incidence of chilling injury, while also achieving desirable cooling rates. The aim of this study is to develop and design integrated packaging systems for citrus. These packaging systems should ensure that all packaging components (pallet, cartons, securing sheets, etc.) function

effectively together and facilitate ventilation alignment. This project has reviewed current packaging systems being utilised in the industry and will now evaluate them with respect to cooling performance. Using these designs as a benchmark, the study will then develop novel integrated packaging systems towards a more optimised citrus cold chain. To date, much of the ground work for the study has been completed and work is to be directed towards exploring novel designs.

Opsomming

In die afgelope paar jaar het die sitrusbedryf meer afhanklik geword van verkoeling om aan fitosanitêre vereistes te voldoen. Koelbehandelingsmarkte benodig byvoorbeeld uitgebreide voorverkoeling by koelwinkelfasiliteite. Verder vereis die Europese markte nou dat vrugte verskeep moet word teen temperature onder 2°C. Vir hierdie markete word vrugte dikwels gelaai teen omgewingstemperatuur, sodat hulle koelhuisgeriewe kan oorslaan. Omgewingslading is 'n belowende benadering om spanning op fasiliteite, koste en wagtye te verminder. Die verkoelingstempo en eenvormigheid is egter dikwels onvoldoende en kan verbeter word as die verpakking ventilasie 'n beter verspreiding van die lugvloei tydens voorafkoeling en binne die verkoelde houers moontlik maak. Die verkoelingsproses kan dus beter beheer word, wat die bedryf in staat stel om die voorkoms van verkoelingsbeserings te verminder, terwyl dit ook die gewenste koelingsyfers behaal. Die doel van hierdie studie is die ontwikkeling en ontwerp van geïntegreerde verpakkingsisteme vir sitrus. Hierdie verpakkingstelsels moet verseker dat alle verpakkingskomponente (palet, kartonne, velle, ens) effektief saam funksioneer en ventilasiebelyning fasiliteer. Die projek het die huidige verpakkingsisteme wat in die bedryf gebruik word, nagegaan en sal dit nou evalueer ten opsigte van verkoeling. Deur hierdie ontwerpe as 'n maatstaf te gebruik, sal die studie nuwe, geïntegreerde verpakkingsisteme ontwikkel vir 'n meer geoptimaliseerde sitrus-koueketting. Tot dusver is 'n groot deel van die grondwerk vir die studie afgehandel en word daar beoog om nuwe ontwerpe te ondersoek.

5.2.5 **PROGRESS REPORT**: **Investigation of factors contributing towards the non-conformance of in-transit citrus container shipments to cold protocol markets**

Project 1240 (PHI 4-11) (2019/20 – 2020/21) by Tarl Berry, Paul Cronje (CRI), Prof Leila Goedhals-Gerber (Stellenbosch University) and Mitchell Brooke (CGA)

Summary

This multi-seasonal study investigated factors contributing to non-conformance of in-transit cold sterilisation protocols. Non-conformances may occur before the sea leg commences (landside failure to initiate the protocol) or during voyage (water failure and/or quality failure). In the previous season (2019) three objectives were focused on: Firstly, to investigate factors that have been identified in preliminary studies to contribute to non-conformance of citrus container shipments to cold sterilisation treatment protocols. Secondly, to analyse the impact of non-conformances on the quality of fruit and lastly, to determine the correlation between industry specifications for cold sterilisation treatment protocols and the factors contributing to the non-conformance of citrus container shipments. During 2019 the study collected on-site data in Durban over 14-day periods over June and then August. The study also collected commercial shipping data from PPECB on a weekly basis for all cold protocol shipments to China and S. Korea. Primary data were collected through observing and recording the cold sterilisation engagement processes of a number of shipments at the Durban port and then conducting various controlled commercial shipments to China/S. Korea with full temperature and logistic monitoring in collaboration with exporters. Specific attention was given to the performance of pre-cooling operations and the effect of the non-refrigerated container loading on cold treatment compliance during shipping. In addition, the project aimed to ascertain the value of live data feed via cellular loggers for the temperature management, which could enable corrective actions timeously and accurately. To this purpose, GPS temperature sensors were included in various shipments during these two data collection periods. The 2019 data are currently being analysed by the student in order to develop a robust model that could be used to link the occurrence of non-conformances in the cold chain and fruit quality. The project was on track as more detailed and specific measurements were planned for the 2020 season. However, it should be noted that the possibility of executing these plans looks low due to limited opportunity for the student and researchers to visit the Durban port.

Opsomming

In hierdie multi-jaar studie was faktore ondersoek wat bygedra tot n gebrek aan nakoming van koue sterilisasieprotokolle tydens uitvoer van sitrusvrugte. Voorvalle waar protokolle nie aan voldoen word nie kan voorkom voordat die seevaart begin word (oneffektiewe aanvang van die protokol) of tydens die vaart (tempertuuronderbreking en / of kwaliteitsverlies). In die vorige seisoen (2019) was op drie doelwitte gefokus: Eerstens om faktore te ondersoek wat in n voorafgaande studie geïdentifiseer is bydraende tot die mislukking in verskeping van houers in die kouesterlisasieprotokol. Tweedens om die impak hiervan op vrugkwaliteit te ontleed en laastens om die verband te bepaal tussen die voorgeskrewe bedryf-spesifikasies wat tans geld vir koue-sterilisasie-behandelingsprotokolle en die faktore wat bydra tot mislukte versendings. Gedurende 2019 was data in Durban oor 14 dae in Junie en Augustus onderskeidelik versamel. Die studie het ook elke week se kommersiële versendingsdata verskaf deur PPECB versamel vir alle houers verskeep na China en S-Korea. Primêre data was ingesamel in Durban gedurende die kommersiële proses van kouesterilisasie deur 'n aantal besendings uit die Durbanse hawe, te volg en in samewerking met uitvoerders, tempertuur data van hierdie verskillende kommersiële besendings na China / S-Korea te bekom. Spesifieke was aandag geskenk aan die hoe voor-verkoeling uitgevoer was en die effek van vrugte laai in onverkoelde houers om die sukses van die kouebehandeling. Daarbenewens het die projek ten doel gehad om die waarde te bepaal van “lewendige” data verskaf van sellulêre temperatuur data loggers vir die temperatuurbestuur en om moontlike regstellende te kan maak. Vir hierdie doel is GPS-temperatuursensors gedurende hierdie twee data-insamelingsperiodes in verskeie houers wat versend was ingesluit. Hierdie 2019-data word tans deur die student ontleed om te poog om 'n robuuste model te ontwikkel wat gebruik kan word om afwykings in die koueketting en vrugkwaliteit te voorkom. Die projek was op koers en meer gedetailleerde en spesifieke metings vir die 2020-seisoen beplan was. Daar moet egter op gelet word dat die moontlikheid van die uitvoering van hierdie planne laag blyk te wees weens die beperkte geleentheid vir die student en navorsers om die hawe in Durban te besoek.

5.3 PROGRAMME: PRODUCTION AND QUALITY

Programme coordinator: Pieter Raath (CRI)

5.3.1 Programme summary

Research in this programme aims to provide practical recommendations to optimise citrus (1) fertilisation, (2) irrigation and (3) tree manipulation for production of maximum yields of quality fruit, with the least possible use of resources. During the 2019/2020 research period, five new projects were initiated on diverse topics, while one project was ongoing and one was completed. In the one-year investigative project (*The use of novel soil conditioners to improve citrus phosphorus (P) nutrition and tree performance*) that was completed, it was shown that application of P fertiliser has a short-term negative impact on the soil’s biological stability - this was successfully expressed by both nematode profile descriptions (Faunal Profile / Soil Food Web Analysis) and enzyme activity measurements (Alteration Index 3). Furthermore, it was found that the activity of soil inoculants is not sustained at high levels in soil with low organic matter content, due to a lack of substrate - repeated applications are required. The results indicate that the effect of perpetual use of the inoculants might, however, not be beneficial due an imbalance of species, i.e. disturbed soil microbial populations that are created.

The first season’s preliminary results indicate that uptake of micro-nutrients from aerial applications is sufficient to maintain the micronutrient nutrition status of trees, but *not* to mitigate nutrient deficiencies. A novel product, Masterlock®, dramatically increased droplet distribution and the total wetted surface area throughout the tree canopy by increasing droplet size. This product aids in distribution of foliar nutrient sprays, applied either aerially or conventionally, throughout the tree canopy.

A quick response from excessively applied nutrients to both mandarins (cv. Orri) and Valencias (cv. Midknight) was not obtained, although increased concentrations of foliar and fruit N and K were obtained shortly after the onset of the treatments, and throughout the first experimental season. Data generated in this project is providing significant insight into the responsiveness of both mandarin and Valencia trees to mineral nutrition. The role of nutrition to affect fruit set and fruit quality of these two cultivars, is also being elucidated.

Since citrus rootstocks are mainly propagated by seed, techniques to increase successful flower development and pollination are being investigated in a new trial at the Citrus Foundation Block in Uitenhage. The rootstocks used for preliminary evaluation are X639, MXT, Carrizo citrange (CC), Volkameriana (VA), Rough lemon (RL), Swingle citrumelo (SC) and C35 planted in 2015, and Sunki Benecke (SXB) (planted 2010). Of these SC had the largest fruit diameter and X639 the smallest, while VA had the highest seed count per fruit (21) and SXB only 4 per fruit. Leaf analysis indicated significant nutritional differences between cultivars, where the levels for some elements were even below the presently accepted nutritional norms.

Due to the unforeseen impact of the drought in Letsitele, the attempt to re-evaluate the application of GA_3 foliar sprays to improve set of seedless Valencia cultivars was jeopardised since no fruit were set in the two Valencia (Delta and Midknight) cultivars used in the project. Awaiting the new season, two additional research objectives were added, i.e., comparing the macro factors influencing seedless Valencia fruit set in Letsitele with the Eastern Cape, and correlating the effect of soil conditions in Letsitele on root development with fruit set. These additional focus areas will add information that can help address the challenge of obtaining consistent fruit set of seedless Valencia.

The last two chapters of the *Handbook for Fertilisation of Citrus in South Africa* are being finalised. A graphical designer has been appointed, who produced examples of possible cover pages, page layout and illustration styles. Printing of the book is due to be completed by end December 2020.

Outputs of research within this programme are diverse, yet novel. Insights into complex production problems are being obtained, while simultaneously practical, readily-applicable and cost-effective solutions are sought.

Programopsomming

Navorsing in hierdie program is daarop gemik om praktiese aanbevelings te genereer waardeur sitrus (i) bemesting, (2) besproeiing en (3) boom manipulasie verbeter kan word ten einde die hoogs moontlike oeste van goeie vruggehalte verkry kan word; terwyl die impak op hulpbronne verminder. Gedurende die 2019/2020 navorsingsperiode is vyf nuwe projekte, met uiteenlopende onderwperpe, geïnisieer. Een projek was lopend en een projek is afgehandel. Die afgehandelde eenjaar ondersoekende proef (Die gebruik van grondkondisioneerders om sitrus fosfaatvoeding en boomprestasie te verbeter) het getoon dat toediening van fosfaatbemesting ‘n kortstondige negatiewe effek op die grond se biologiese stabiliteit het – dit is suksesvol d.m.v. beide die nematode profiel beskrywings (Fauna Profiel / Grond Voedingsnetwerkbeskrywing) en die ensiem-aktiwiteitsmetings (die sg. Alteration Index 3). Hoë aktiwiteitsvlakke van die innokulante word ook nie gehandhaaf in grond met ‘n lae organiese materiaalinhoud nie. Dit word toegeskryf aan ‘n tekort aan substraat, gevolglik is herhaalde toedienings nodig. Die proef het getoon dat herhaalde gebruik van die innokulante nie noodwendig voordelig is nie a.g.v. ŉ wanbalans wat in die spesies, te wete versteurde mikrobiologiese populasies, geskep word.

Uit die eerste seisoen se voorlopige resultate is gevind dat die opname van mikro-elemente wat met lugbespuitings toegedien is, voldoende is om bome se voedingstatus te onderhou. Dis egter nie geskik om tekorte mee aan te spreek nie. ŉ Nuwe produk, nl. Masterlock®, het druppelverspreiding en die totale benatte oppervlakte reg deur die boom se lower verhoog deurdat dit die druppels vergroot het. Hierdie produk verbeter die verspreiding van blaarvoedingsprodukte wat hetsy d.m.v lugbespuitings of konvensioneeel toegedien word.

ŉ Vinnige reaksie op oormatig toegediende voedingselemente is nie met mandaryne (kultivar Orri) of Valencias (kultivar Midknight) verkry nie. Verskuiwings in die blare en vrugte se N en K konsentrasies is egter reeds kort nadat die behandelings begin het, verkry. Data wat in hierdie projek verkry word, verskaf insig rakende die geneigdheid van beide mandaryn en Valencia bome om op bemesting te reageer. Die rol van bemesting om vrugset en kwaliteit te beïnvloed, word ook uitgeklaar.

Omdat sitrus onderstamme hoofsaaklik van saad vermeerder word, word tegnieke om verhoogde blomontwikkeling en bestuiwing te verkry ondersoek – dit word in ŉ nuwe projek in die Sitrus Moederblok in Uitenhage gedoen. Kultivars wat gebruik word in die voorlopige ondersoek is X639, MXT, Carrizo citrange

(CC), Volkameriana (VA), Rough lemon (RL), Swingle citrumelo (SC) en C35, wat almal in 2015 geplant is, asook Sunki Benecke (SXB) (in 2010 geplant). Van almal het SC die grootste vrugte gehad en X639 die kleinste; terwyl VA die meeste saad per vrug gehad het (21) teenoor SXB met slegs 4/vrug. Blaarontledings het ook groot verskille tussen kultivars getoon, met vlakke van sommige elemente wat selfs onder die huidige aanvaarde norme is.

As gevolg van die onvoorsiene impak van die droogte in Letsitele, is die plan om weer die toediening van GA_3 blaarbespuitings om set van saadlose ‘Valencia’ kultivars te evalueer in die wiele gery. Die gevolg van die droogte was dat geen vrugte in die twee Valencia kultivars (Delta en Midknight) geset het nie. In afwagting op die nuwe seisoen, is twee addisionele faktore tot die navorsing gevoeg, naamlik: ’n vergelyking van die makro-faktore wat die set van saadlose Valencias in Letsitele en die Oos-Kaap beïnvoed, en ‘n poging om die effek van grondtoestande op wortelontwikkeling met vrugset te korreleer. Hierdie twee ekstra fokus areas sal ekstra inligting verskaf wat sal help om die uitdaging van vrugset by Valencias die hoof te bied.

Die laaste twee hoofstukke van die handboek vir Bemesting van Sitrus in Suid-Afrika word gefinaliseer. ’n Grafiese kunstenaar is aangestel was reeds verskeie voorbeelde van die buiteblad, bladuitleg en illustrasies voorberei het. Die druk van boek behoort teen einde Desember 2020 gefinaliseer te word.

Uitsette van navorsing in hierdie program is uiteenlopend, nuut en innoverend. Insig rakende komplekse produksiekwessies word verkry, terwyl praktiese, toepasbare en koste-effektiewe oplossings gesoek word.

5.3.2 **FINAL 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

A one year, exploratory trial was conducted in Addo on a 10-year-old Washington Navel/Carizzo block on a deep Augrabies soil form. The objective was to assess to what extent availability and uptake of P in high pH soil can be enhanced by strip applied P fertiliser, alone or in conjunction with either two soil conditioners (Brimstone 90® and Crestharvest®) or with two microbial soil inoculants (All-Grip® and iNmass®).

Tree nutrition and performance, as well as soil microbiology, were quantified for treatments that all included a once-off 40 kg P application (as double super phosphate) in August 2018, but respectively with different approaches and the addition of various soil conditioners/inoculants, as specified below:

Treatments where P fertilisation and the soil conditioner/inoculant was spread out in the tree row	• Only P fertilisation • P with Crestharvest® at a rate of 20L/ha • P with Kiplant All-Grip® • P with Kiplant iNmass®
Treatments where P fertilisation and the soil conditioner/inoculant was strip-applied at each tree	• Only P fertilisation • P with Brimstone 90® at a rate of 330 kg/ha (300 kg S/ha) • P with Brimstone 90® (330 kg/ha) and Crestharvest® (20L/ha) • P with Kiplant All-Grip® • P with Kiplant iNmass® • P with Kiplant All-Grip® with Kiplant iNmass®

This project was conducted over one season only, and the outcomes of the trial were not conclusive in all respects; but elucidation regarding the following aspects were obtained:

- Despite P-fixation in high pH soils, P-availability is sufficient when the concentration in the soil exceeds the minimum norm and regular, annual P fertilisation is not required.

- Uptake of P and Ca can possibly be enhanced by soil application of fertilisers in conjunction with inoculation of soil with a consortium of bacteria.
- Application of P fertiliser negatively affects the soil's biological stability in the short term, as effectively expressed by both nematode profile descriptions (Faunal Profile / Soil Food Web Analysis) and enzyme activity measurements (Alteration Index 3).
- Application of a combination of Brimstone 90® (330 kg/ha) and Crestharvest® (20L/ha), as well as the soil inoculants Kiplant All-Grip® and Kiplant iNmass®, reduced the negative impact of P-fertilisation and after six months affected the establishment of an improved and more stable soil microbiological community.

Opsomming

ʼn Een jaar ondersoekende proef is uitgevoer in Addo op ʼn 10 jaar oue Washington Navel/Carizzo blok op ʼn Augrabies grondvorm. Die doelwit was om vas te stel tot watter mate P-toeganklikheid en opname in hoë pH gronde bevorder kan word d.m.v. strooktoedienings, alleen of tesame met, hetsy twee gronkonsidioneerders (Brimstone 90® and Crestharvest®) of met twee mikrobiologiese grond-innokulante (All-Grip® and iNmass®).

Bome se voedingstatus en prestasie, asook die grondbiologie, is vir al die behandelings gekwantifiseer. Laasgenoemde het almal ʼn eenmalige toediening van 40 kg P (dubbel superfosfaat) in Augustus 2018 ingesluit, maar ook verskillende benaderings van toediening en die byvoeging van verskeie grondkondisioneerders/innokulante. Dit word hieronder gespesifiseer:

Behandelings waar die P bemesting en die gondkon-disioneerder / innokulant in die boomry uitgestrooi is	• Slegs P bemesting • P met Crestharvest® teen 20L/ha • P met All-Grip® • P met iNmass®
Behandelings waar die P bemesting en die gondkon-disioneerder / innokulant in ʻn trook om die boom gegooi is	• slegs P fertilisation • P met Brimstone 90® teen 330 kg/ha (300 kg S/ha) • P met Brimstone 90® (330 kg/ha) en Crestharvest® (20L/ha) • P met All-Grip® • P met iNmass® • P met All-Grip® tesame met iNmass®

Omrede hierdie projek net oor een seisoen uitgevoer is, is die uitkomste nie in alle opsigte oortuigend nie, maar uitklaring aangaande die onderstaande onderwerpe is wel verky:

- Ten spite van P-fiksering in hoë pH gronde, is P-toeganklikheid voldoende wanneer die konsentrasie in die grond die minimum-norm oorskry. Gereelde, jaarlikse P-bemesting is dan nie nodig nie.
- Opname van P en Ca kan bevorder word deur toediening van kunsmis saam met die innokulasie van die grond met ʼn konsortium van bakterieë.
- Toediening van P-bemesting het ʼn negatiewe impak op die grond se biologiese stabiliteit oor die kort termyn, soos effektief deur middel beide die nematode profiel beskrywing (Fauna Profiel/ Grondvoedselweb analise) en ensiem aktiwiteit metings (Alteration Index 3) geïllustreer.

Toediening van ʼn kombinasie van Brimstone 90® (330 kg/ha) en Crestharvest® (20L/ha), asook die grond-inokulante Kiplant All-Grip® en Kiplant iNmass®, het die negatiewe impak van P-bemesting verminder, en na afloop van ses maande die vestiging van ʼn meer stabiele grondbiologiese omgewing teweeg gebring.

Introduction

Various soil-factors affect the availability of phosphorus (P) for uptake by plant roots, of which the most important is soil pH. In acid soils, P fixation reactions occur with soluble iron and aluminium to form minerals that have very low solubility. In such soils, liming increases the availability of P because the solubility of iron and aluminium compounds is significantly reduced so that less of these two metals are available to react with

P (Conradie, 1994). Soil with a higher pH contains appreciable calcium concentrations that result in calcium fixation of P, similar to the reactions occurring in acid soils with iron and aluminium. Since it is difficult to reduce the soil's pH, especially where free lime is present (Saayman, 1981), management of P nutrition of citrus trees is complex in alkaline soils, e.g. where the pH_{KCl} >6.5 (Coetzee, 2007).

Consequently, in high pH soils citrus producers cannot rely on the accepted soil P norms as guideline whether to fertilise with P. Soil analyses done using Bray II extraction has revealed that many citrus orchards in regions with high soil pH have an elevated P content. This is the consequence of annual P fertilisation to counteract possible P-fixation that occurs in these soils. It is usually done in excess of the crop's requirement, resulting in progressive build-up of P in the soil.

A recent study in the Orange River Region has revealed that in high pH soils with high concentrations of Bray II extractable P, sufficient P is available for uptake to avoid deficiencies without any P fertilisation. The trees seemed to be able to utilise sufficient amounts of the Bray II extractable P. However, additionally applied P did increase the P-concentration of citrus leaves (White, 2018) to more optimal levels.

Due to the detrimental effect that excessive P concentrations in the soil have on Zn- (Coetzee, 2007) and K-nutrition (Saayman, 1981), on soil microbial life (Mengel & Kirkby, 1982) as well as causing eutrophication of water sources (Follet *et al*., 1981), continuous build-up of soil P by superfluous P fertilisation should be avoided. The objective of this study is therefore to explore innovative ways to increase P availability, both from the labile pool of "accumulated P" and from annually applied P, with the goal to reduce fertilisation cost and to the benefit of the environment.

To this end, the following possible ways to mobilize fixated soil P in alkaline soils was explored:
1*. Strip application of P fertiliser in combination with elemental sulphur*: Oxidation of elemental sulphur (S) to produce sulphuric acid ($2S + 3O_2 + H_2O \rightarrow 4H^+ + 2SO_4^{2-}$), which is a recognised method to reduce soil pH (Singer & Munns, 1987), might enhance P-availability. The efficacy of strip application of elemental sulphur in combination with P fertiliser to improve P-uptake and tree P-nutritional status was therefore investigated.
2. *Treat the soil with a phosphinate polymer*: This type of polymer is commonly used as water treatment to assist with calcium phosphate dispersancy in anti-scalant programmes in boiler systems and food plants (Connect Chemicals, 2018). Due to its chemical characteristics, it has the potential to promote the release of P from crystallised tricalcium phosphate, or prevent its formation when fertiliser is applied. Two products, e.g. CrestHarvest 588® (sodium phosphinate/acrylic polymer) and Crestharvest 589® (sodium salt of phosphinocarboxylic acid copolymer) that are marketed as soil conditioners to reduce the negative effects of excessive alkalinity in soil, as well as increase plant available P in soil, were tested.
3. *Inoculation of the soil with a consortium of micro-organisms that were specifically selected to enhance P mobilisation*: An extensive range of soil bacteria and fungi are able to solubilize various forms of precipitated P, viz. strains of Bacillus, Pseudomonas, Penicillium and Aspergillus spp. (Mengel & Kirkby, 1981; Richardson, 2001 & Mohammadi, 2012). The production and release of organic acids by micro-organisms are generally assumed to be a major contributing factor (Mengel & Kirkby, 1981). Furthermore, numerous studies have demonstrated positive plant responses to microbial inoculation in soil (Richardson, 2001). Two commercial products that contain specific strains of either Bacillus and Pseudomonas spp. (Kiplant All-Grip®) or Bacillus and Azospirillum (Kiplant iNmass®), respectively, were therefore evaluated for their effect on citrus tree P nutrition in high pH soils, as well as general tree performance and soil health.

To assess the biological health of soil, changes in the structure or function of the soil food web must be detected. Such changes are a result of environmental distress due to the prevailing agricultural production practices. The nematode faunal analysis was therefore selected since it presents a means of evaluating the function and structure of the soil food web using various indices (Bongers & Ferris, 1999; Ferris *et al*., 2001; Berkelmans *et al*., 2003; Sanchez-Moreno *et al*., 2006).

The use of enzyme activities to assess soil quality was also used in this trial. It is based on the sensitivity of three enzymes to soil management and organic matter decomposition. They are therefore regarded as useful biological soil quality indicators (Adetunji *et al*., 2017). The amount of phosphatase present in the soil varies with the microbial count and the extent of organic materials, mineral and organic fertilizers, tillage and other agricultural practices (Banerjee *et al*., 2012). Furthermore, the method is also relatively easy to perform.

Stated objectives

The objective of this one-year trial was to assess whether the use of the respective products will 1) enhance availability and uptake of P in high pH soils, 2) promote tree performance and 3) positively impact on soil microbial activity. This can be broken up in three parts, viz.:

- Establish possible changes in plant available P (or other nutrients), as expressed in both soil and foliar analyses.
- Quantify the effects of the applied products on tree performance.
- Measure any changes caused by the applied products on the soil biology.

Lastly, due to the recent emphasis on soil health and biology, the trial was also used to establish which method of soil microbiological description is most suitable to assess the impact of specific farming interventions on the soil microbial ecosystem.

Materials and methods

The trial was conducted in a 10 year old Washington Navel/Carizzo block (3 x 6 m spacing), established on Imiti farm in Addo on a deep Augrabies soil form, containing an average of 20% clay. Since September 2018 the treatments, as described in Table 5.3.2.1, were applied in conjunction with a once-off application of 40 kg P per ha (as double superphosphate) in September 2018.

The trial block was initially selected on account of its apparently low P-concentrations, as indicated by the producer's soil analyses, being deficient in Bray II extractable P. The first set of soil analyses (conducted in August 2018) however indicated that, contrary to the producer's analyses results, the experimental block has an exceptionally high soil P concentration (Table 5.3.2.2). Given that it was only a one-year trial, it was consequently decided to shift the focus of the trial to investigate the effect of the treatments in soil with high P-concentrations and to the impact of the treatments on soil biology.

Table 5.3.2.1. Treatments applied to soil of a Washington Navel/Carizzo block in Addo in September 2018, December 2018 and March 2019.

Treatment number	Description of TradeCor products' treatments*
1	Control - P-fertilisation applied, which was spread out in the tree row.
2	Control - Strip applied P-fertilisation only.
3	Strip applied P & product: Brimstone 90® at a rate of 330 kg/ha (300 kg S/ha).
4	P-fertilisation & Crestharvest® at a rate of 20L/ha - spread out in tree row
5	Strip applied P, Brimstone 90® (330 kg/ha) and Crestharvest® (20L/ha)
Treatment number	**Description of Asfert Global products' treatments***
1	Control - P-fertilisation applied, which was spread out in the tree row.
2	Control - Strip applied P-fertilisation only.
3	P-fertilisation and Kiplant All-Grip®, spread out in the tree row.
4	P-fertilisation and Kiplant iNmass®, spread out in the tree row.
5	P-fertilisation and Kiplant All-Grip®, strip applied at each tree.
6	P-fertilisation and Kiplant iNmass®, strip applied at each tree.
7	P-fertilisation and Kiplant All-Grip® with Kiplant iNmass®, strip applied at each tree.

* A once-off application of 40 kg P per ha was applied in August 2018. It was either spread out in the tree row, or strip applied, as per the method of application indicated for each treatment. Application of the abovementioned soil conditioner products was then on top of the fertiliser, followed up by two additional applications of last mentioned in October 2018 and December 2018.
For strip applied P fertiliser, the two subsequent applications of products were done on exactly the same strip as per the fertiliser residue that remained on the soil surface.

Table 5.3.2.2. Initial soil P concentration of the Washington Navel experimental block in Addo, Eastern Cape.

Replication	Plant available P (Bray II-extractable)	Total P (HNO_3-extractable)
	mg/kg	
1	461	521
2	374	411
3	386	395
4	295	440
Average	379 ± 68	442 ± 56

Soil total P and Bray II extractable P were analysed prior to, and four weeks after the treatments commenced (October 2018). Leaf analyses were done of spring flush leaf samples taken four weeks (October 2018) after treatments commenced and of mature leaves from fruit bearing shoots in March 2019. Root samples were also taken for carbohydrate analyses in April 2019.

Tree vegetative growth, expressed as increase in tree volumes, was measured before the treatments commenced (August 2018) and in March 2019.

The soil's biological diversity and health of the soil was assessed prior to application of the P fertilisation and products (August 2018), four weeks after treatments commenced (October 2018) and also six months later (April 2019), using the nematode profile method, a commercial service provided by Nemlab. Nematodes were extracted from the soil samples by means of the Cobb's decanting and sieving method, in combination with a modified Baermann funnel. The extracted nematodes were counted and identified to genus level. The nematodes were then categorised into various feeding groups conforming to Yeates *et al*.'s (1993) guidelines. Coloniser-persister values were also assigned as defined by Bongers (1990). The various indices were calculated.

Total activity of aerobic soil organisms, as well as those involved in P and N mobilisation/mineralisation, was also done on the same samples using an enzyme assay, e.g. urease, phosphatase and ß-glucosidase activity that is determined colourimetrically in soil extracts as per a commercial service provided by ARC Infruitec/Nietvoorbij. In addition, the so-called "Alteration Index Three (AI3)" that quantifies the balance between the three microbially-secreted soil enzymes, and which is sensitive to alterations in soil characteristics caused by management practices were determined for each treatment sample. The AI3 values were calculated with the equation:

$$AI3 = (7.87 \times \beta\text{-glucosidase}) - (8.22 \times \text{phosphatase}) - (0.49 \times \text{urease})$$

where enzyme activities were expressed in micromoles of, respectively, p-nitrophenyl-β-D-glucoside and p-nitrophenylphosphate per gram of soil per hour, and micrograms of urea per gram of soil per hour.

Another set of samples were taken in April 2019 for the same assessments.

Quantification of total production, as well as fruit internal quality, was done during commercial harvest, viz. June 2019.

Statistical analyses of the data were done using STATISTICA® 13.3 software. The trial was set up as a randomised block design and a one-way Anova analysis was done of the various treatments and their effect on the measured parameters. LSD-Fisher post hoc tests were used to report the significance of the treatments.

Results and discussion

The trial was done with the abovementioned two sets of products, and the results obtained for each are reported and discussed separately since their mode of action is different.

Results of TradeCor products

Effect of P-applications on soil P concentration in the root zone: The soil P concentration in the soil was slightly increased after the P-fertilisation (Figure 5.3.2.1). When expressed as Bray II, the increase was less in extent than the increase in total P. This is ascribed to P-fixation in high pH-soils that reduces the extractability of P by Bray II. Despite soil sampling in the direct vicinity of the positions of application, a huge variation in soil P concentration was furthermore obtained for some treatments (T3, T4). This variation is also ascribed to the P-fixation and consequent immobility of P in the soil, often resulting in sampling error, viz. samples being taken outside (or just outside) the zone of P fertilisation. Where strip applications of P is done, a variation in soil analysis results is typical.

Effect of P fertilisation and soil conditioners on tree nutritional status and tree performance: Leaf samples, collected from spring flush in October 2018 (one month after application of the products), as well as mature leaves from fruit bearing shoots sampled in March, showed no difference in foliar nutrient concentrations for any of the treatments (data not shown). The lack of differences in leaf P concentration reiterates the fact that even in high pH soils, a sufficiently high P concentration (> 200 mg/kg Bray II extractable P in both blocks) will ensure adequate P-nutrition. Regular, annual P fertilisation is therefore not required. Furthermore, the fact that there often are no correlations between soil P concentration and the trees' P-nutritional status (Coetzee, 2007) also applies to this situation. The lack of differences in the foliar concentrations of other nutrients between the treatments, in neither the summer flush (October 2018) nor leaves from mature bearing shoots (March 2019), indicate that the impact of the Brimstone 90® and Crestharvest® to affect tree nutrition or availability of nutrients over the short term is negligible.

No significant differences in tree vegetative growth, expressed as changes in tree volume after eight months since the treatments commenced, production and average fruit size, as well as root carbohydrate content (of roots sampled in March 2019) were obtained between any of the treatments and compared to the control (data not shown). This is also ascribed to the short duration of the trial. Any differences that the treatments might possibly induce are expected to realise only after two to three years.

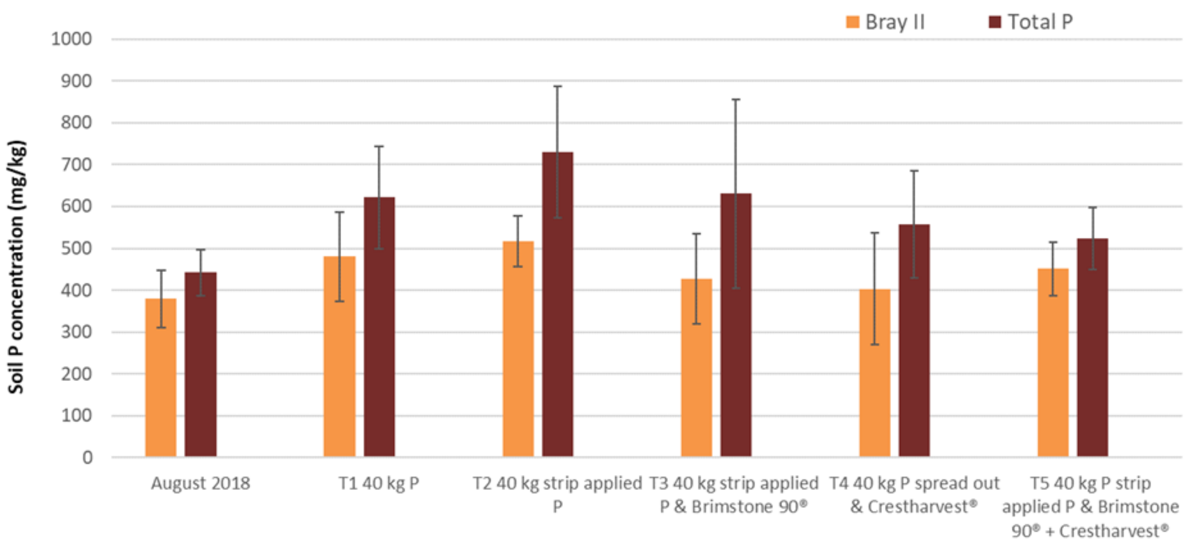

Figure 5.3.2.1. Soil total P and Bray II extractable P of the experimental block, as determined for samples taken four weeks after a once-off application of 40 kg P per ha in September 2018, followed by a treatment of the soil with the soil conditioners. The "August 2018" results are the soil P concentration prior to application of the P-fertilisation.

Effect of P fertilisation and soil conditioners on soil microbiology: Both the nematode profile method (Bongers & Ferris, 1999; Ferris *et al*., 2001; Berkelmans *et al*., 2003; Sanchez-Moreno *et al*., 2006) and enzyme (ß-glucosidase, phosphatase & urease) activity (Dharmakeerthi, & Thenabadu, 1996; Klose & Tabatabai, 1999;

Piotrowska-Dlugosz & Wilczewski, 2014a; Adetunji *et al.*, 2017), are recognised methods used to establish the effect of soil management practices and inputs on soil biology and health.

Comparison of the "Maturity Indexes (MI)", which express the degree of biological stability a soil has on the basis of the number of different species of nematodes in the soil, however clearly showed that the application of P impacted negatively on the soil's biological stability in the short term (Figure 5.3.2.2). This was illustrated by the fact that the MI of the samples taken in Oct 2018 of all the treatments was lower than the average level before the P-applications (Aug 2018). Soil biology, however, was restored to a stable system again after six months, as indicated by the increased MI values of the Apr 2019 samples. This is in accordance with observations by Bongers & Ferris (1999) who found that nematodes respond rapidly to disturbance and enrichment. Any increase in microbial activity leads to changes in the proportion of bacterial feeders in a community, as was found for the Oct 2018 samples (data not shown). According to Bongers & Ferris (1999), enrichment stimulates microbial activity and subsequent succession, which is reflected in an initial decrease in the MI which is then followed by its gradual increase again.

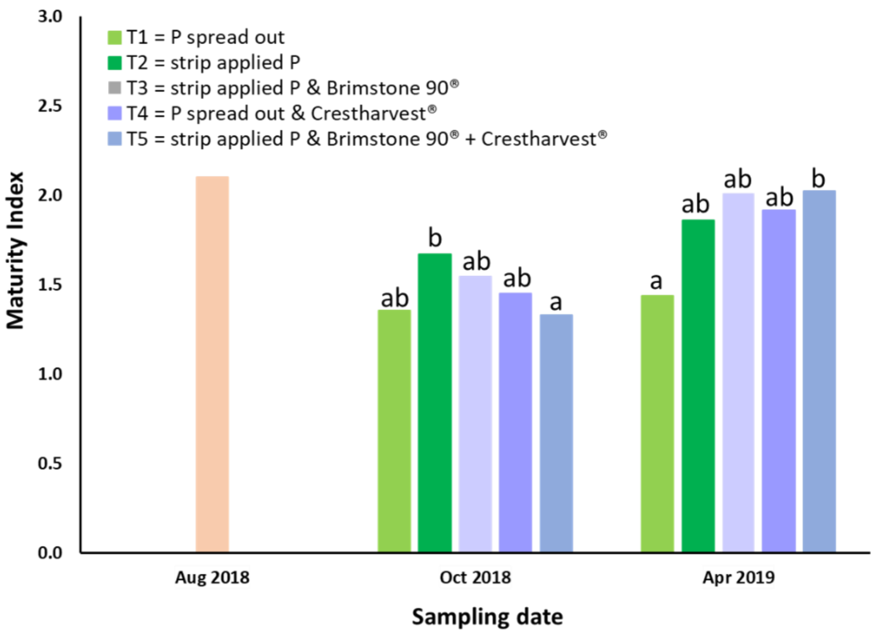

Figure 5.3.2.2. Maturity indices of soils that received a once-off application of 40 kg P per ha in September 2018, followed by a treatment of the soil with the two soil conditioners. The "August 2018" results are the soil P concentration prior to application of the P-fertilisation. Values designated by the same letter do not differ significantly ($p \leq 0.05$).

From the Faunal Profile (Figure 5.3.2.3), a shift occurred after the P application treatments in September 2018 from the top right sector (Aug 2018: black dot) to the top left sector (Oct 2018: blue dots). This indicates a reduction in the structure index (SI) of the soil. The SI is a measure of the number of trophic levels within the soil food web, along with the potential for regulation by predators. A low SI expresses a disturbed or degraded soil ecosystem, whereas a high SI identifies structured or matured conditions (Ferris *et al*., 2001). It can therefore be concluded that the P fertilisation (with or without the soil conditioners) resulted in high disturbance of the Food Web. As a result, the decomposition pathway became bacterial dominated (Table 5.3.2.3) and the low populations of omnivorous and predatory nematodes (Table 5.3.2.3) in these treatments' soil indicate that the soil biology was detrimentally affected (Linsell *et al.*, 2014). In this case by the fertiliser inputs, but on a short term basis, since a shift in the Faunal Profile occurred back to its original state between the Oct 2018 and Apr 2019 (pink dots) sampling times (Figure 5.3.2.3). This is ascribed to an improved balance between bacterial feeders and the other three groups of nematodes (Table 5.3.2.3). The soil's food web (viz. soil biology) could then again be regarded as better structured and more mature, similar to the state prior to when the treatments were started.

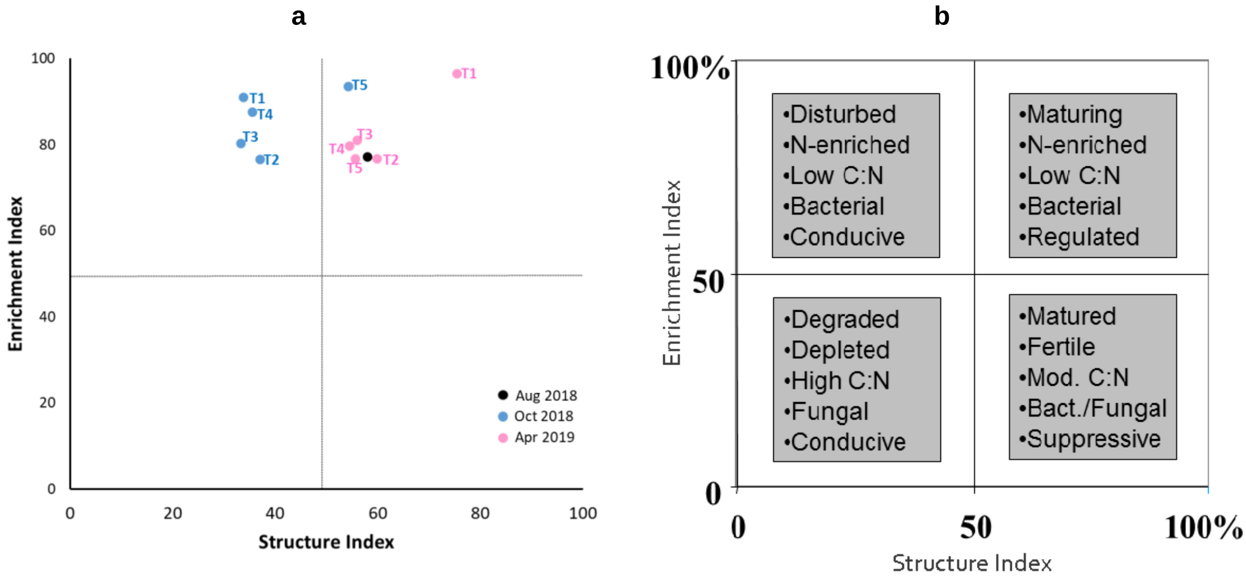

Figure 5.3.2.3. Food web analysis depicting the faunal profile of a Washington Navel/Carizzo block on an Augrabies soil in Addo before (Aug 2018) and after having been treated (sampled in Oct 2018 & Apr 2019) with 40 kg P per ha and three combinations of soil conditioners supplied by Tradecor® (a) and a foodweb analysis interpretation scheme as per Ferris *et al*. (2001) (b).

Table 5.3.2.3. The counts of four of the nematode trophic groups, based on their feeding habits, used as indicator organisms to establish the effect of P fertilisation approach and soil conditioners on the health and stability of the soil food web. Values designated by the same letter do not differ significantly, NS=not significant.

Treatment	Bacterial feeder			Fungal feeder		Predacious		Omnivore	
	Sep 2018	Oct 2018	Apr 2019	Oct 2018	Apr 2019	Oct 2018	Apr 2019	Oct 2018	Apr 2019
1		2273	1063b	58a	13	3a	30b	23a	38ab
2		1963	338a	295b	48	25ab	8ab	40ab	28ab
3	663	3080	1015b	163ab	65	25ab	10ab	68ab	50b
4		2143	2503b	268ab	35	43b	30b	53ab	40b
5		3133	375ab	125ab	43	20ab	0a	100b	20a
Significance		NS	0.05	0.04	NS	0.05	0.05	0.02	0.02

The use of enzyme activities to assess soil quality is based on their sensitivity to soil management, organic matter decomposition, and relative ease of analysis. They are therefore regarded as useful biological soil quality indicators (Adetunji *et al*., 2017). The amount of phosphatase present in the soil varies with the microbial count and the extent of organic materials, mineral and organic fertilizers, tillage and other agricultural practices (Banerjee *et al*., 2012).

When mineral P (PO_4^{3-}) concentration is low, soil, plant roots and micro-organisms increase the secretion of phosphatase to intensify the solubilization and remobilization of phosphate. Phosphatase activity can therefore be used as an indicator of inorganic P availability to plants and micro-organism activity (Adetunji *et al*., 2017). In soil with excessive total P concentrations (viz. the soil of this trial), the availability of the P in this high pH soil will therefore be inversely related to phosphatase activity. Contrary to this logic, all the treatments resulted in an initial increase in the phosphatase activity, with T3 showing a significant increase. This was also found by Piotrowska-Dlugosz & Wilczewski (2014b) who stated that phosphatase activity increases when P fertiliser is added to soils with low organic matter, but no changes in its activity occur when P fertiliser is applied to soils with high organic matter. In the case of this trial, the applied fertiliser, and significant amount of S, on this low C content soil, seemed to cause a spike in phosphatase activity; which then decreased to almost the original levels in the following six months (Apr 2019), as indicated in Figure 5.3.2.4.

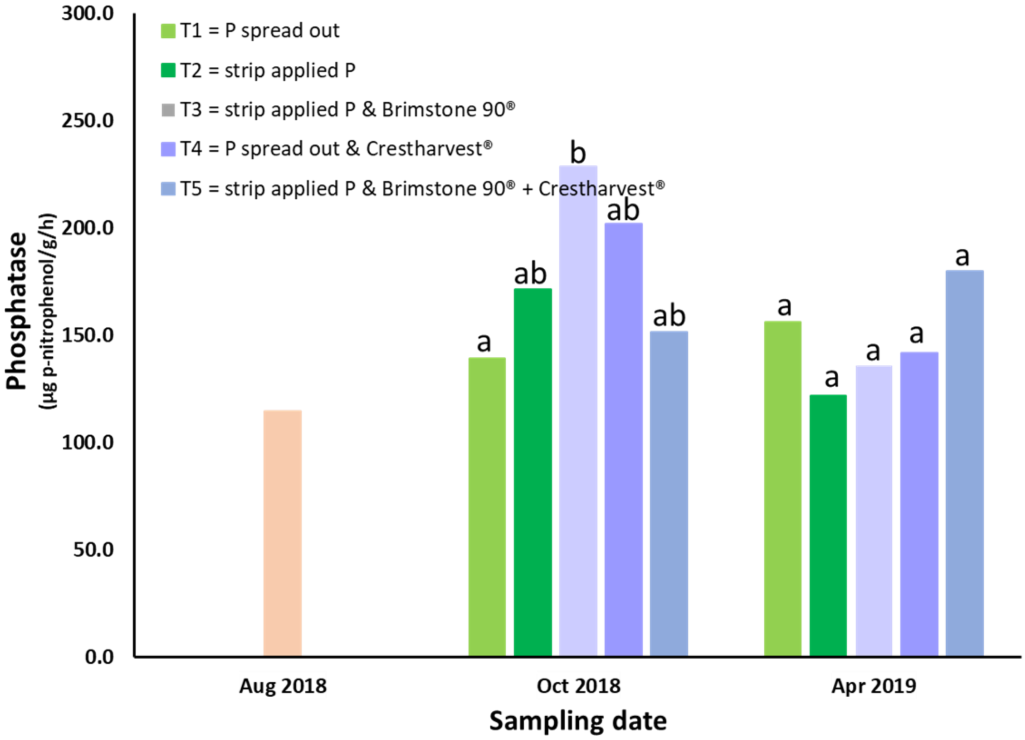

Figure 5.3.2.4. Phosphatase activities of soils that received a once-off application of 40 kg P per ha in September 2018, followed by a treatment of the soil with the two soil conditioners. The "August 2018" results represent the phosphatase activity prior to application of the treatments. Values designated by the same letter do not differ significantly ($p \leq 0.05$).

The urease enzyme is widely distributed in nature, and it originates from bacteria, yeasts, fungi, algae, animal waste and plants (Pirotti & Pidello, 1999). It may also be constitutively synthesized, viz. at a constant rate regardless of physiological demand, in some organisms. Its expression in soil is usually under N regulation (Machuca *et al.,* 2015); as a result, it was expected that the P fertilisation would not affect urease activity. In line with this, none of the treatments affected urease activity, neither directly after application (Aug 2018) nor after six months and three applications of the soil conditioners later (Apr 2019) (data not shown). It can therefore be concluded that neither Brimstone 90 nor Crestharvest 588® has any effect on urease activity (viz. N mineralisation) in soil.

The third enzyme and most common, important and widely used one for indicating soil quality is β-glucosidase (De Almeida *et al*., 2015). This is mainly due to its involvement in C cycling. It is therefore greatly dependent on substrate supply, and the microorganisms that produce this enzyme are mainly aerobic and active in the top soil (Adetunji *et al*., 2017). Application of synthetic fertiliser typically reduces its activity (Meyer *et al*., 2015). Similar to urease, and as expected, no change in β-glucosidase activity was observed for any of the treatments, neither directly after application (Aug 2018) nor six months and three applications of the soil conditioners later (Apr 2019). It can therefore also be concluded that neither Brimstone 90 nor Crestharvest 588® probably affects β-glucosidase activity (viz. total aerobic micro-organisms involved in C cycling and hydrolyses of cellulose) in soil with low organic C content.

The Alteration index three (AI3), which measures the balances between three enzymes, is also regarded as a reliable means of detecting soil quality change due to management practices (Adentunji *et al*., 2019). When the combined effect of the treatments on the soil biology was assessed in terms of the AI3, it was found that application of P fertilisation alone, or in combination with the soil conditioners, improved the soil quality (Figure 5.3.2.4), viz. the Oct 2018 samples of all treatments showed lower AI3 values than the reference sample taken in Aug 2018. Detrimental practices like over-fertilisation, result in AI3 values that are higher than those of control soils (Meyer *et al*., 2014). Furthermore, the improvement was significantly more for both T3 and T4, compared to the other treatments. Six months later the improved AI3 value was maintained, but a shift in the treatments with the best AI3 values occurred, viz. T5 where both the soil conditioners were applied now showed a far better soil quality compared to the other treatments.

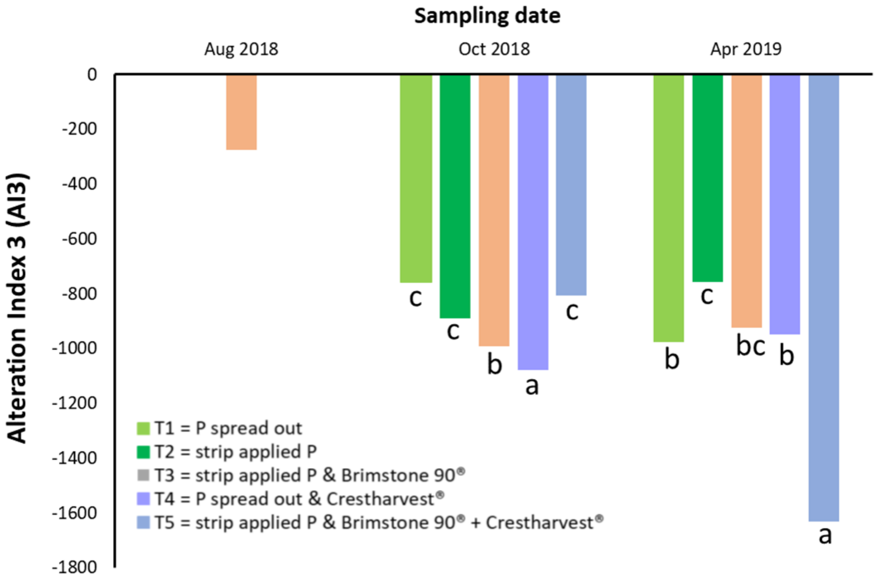

Figure 5.3.2.5. The calculated AI3 values used to establish the effect of P fertilisation approach and two soil conditioners on the health and stability of the soil food web. Values designated by the same letter do not differ significantly ($p \leq 0.05$).

Results of Asfert Global products

Effect of P-applications on soil P concentration in the root zone: Similar to the TradeCor trial/products, the Asfert Global products also did not affect the plant available P (Bray II-extracted), while the total P concentration was increased (Figure 5.3.2.6). The variation in soil P concentration was less for this set of samples. The higher total P concentration values for the strip applied P treatments, however, also indicate the effect of P-fixation that causes immobility of P in the soil.

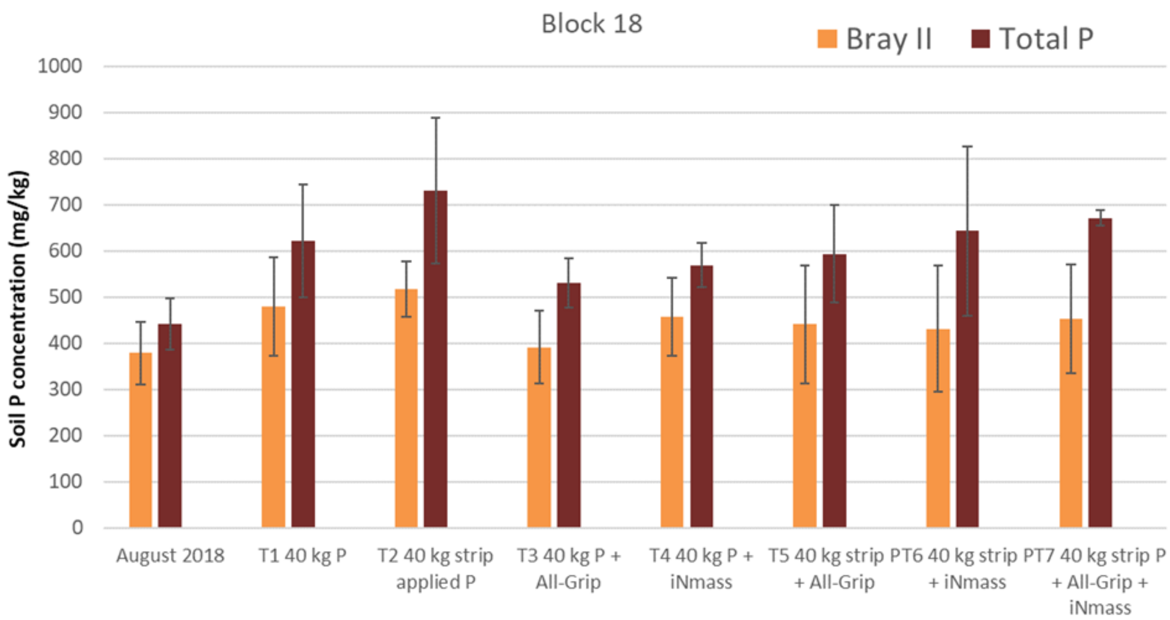

Figure 5.3.2.6. Soil total P and Bray II extractable P of the experimental block, as determined for samples taken four weeks after a once-off application of 40 kg P per ha in September 2018, followed by a treatment of the soil with the two soil inoculants. The “August 2018” results are the soil P concentration prior to application of the P-fertilisation.

Effect of P fertilisation and soil inoculants on tree nutritional status and tree performance: Leaf samples, collected from spring flush in October 2018 (one month after application of the soil conditioners), showed higher leaf P and Ca concentrations for Treatment 7 (strip applied combination of the two commercial microbial inoculants), but only when compared to the control (no soil conditioner) (Figure 5.3.2.7). The increased P and Ca uptake, as expressed in the young spring flush leaves, might have resulted from a positive effect that the combined application of Kiplant All-Grip® and Kiplant iNmass® had on nutrient availability as a consequence of the products maintaining the biological structural index (discussed below). No difference in foliar nutrient concentrations for any of the treatments were however obtained for the mature leaves from fruit bearing shoots sampled in March 2019 (data not shown). The lack of differences in N and P concentration for samples taken in March 2019 is probably due to sufficient, timely N fertilisation and an ample supply of P (excessive P-concentration in the soil). The benefit of microbial inoculants effecting increased availability of the respective nutrients would therefore have been negated.

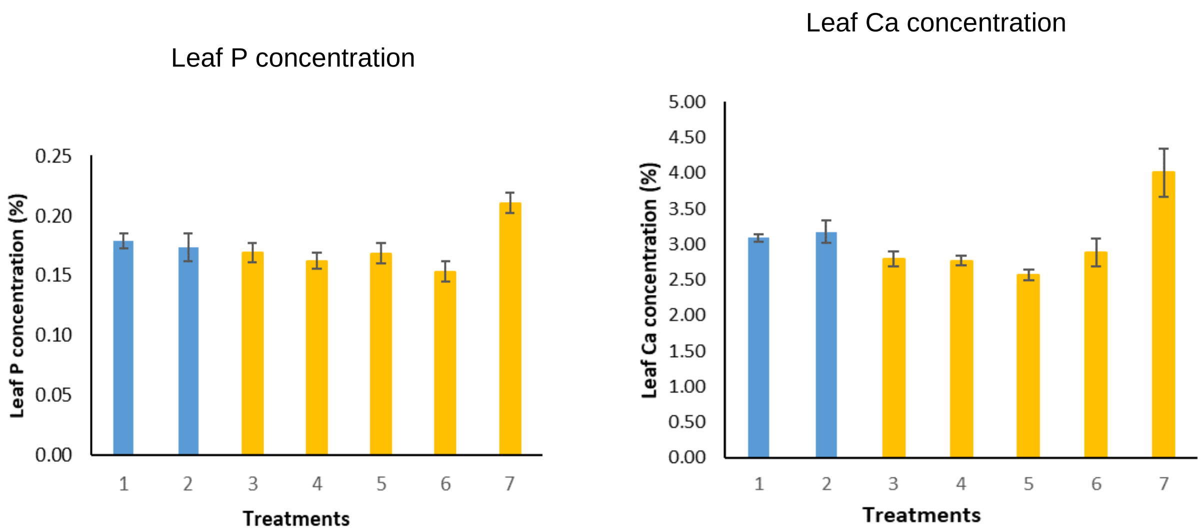

Figure 5.3.2.7. The effect of P fertilisation in combination with Asfert Global soil microbiological inoculants (applied in Sep 2018) on the P and Ca concentration of spring flush leaves, sampled one month after application.

No significant differences in tree vegetative growth, expressed in changes in tree volume after eight months since the treatments commenced, production and average fruit size, as well as root carbohydrate content (of roots sampled in March 2019) were obtained between any of the treatments and the control (data not shown). This is also ascribed to the short duration of the trial. Any differences that the treatments might possibly induce are expected to realise only after two to three years.

Effect of P fertilisation and soil inoculants on soil microbiology: Comparison of the “Maturity Indexes (MI)” showed that the application of P and the products slightly impacted on the soil’s microbial activity in the short term (Figure 5.3.2.2) in that the MI of the samples taken in Oct 2018 of all the treatments was lower than the average level before the P-applications (Aug 2018). It then remained at a stable level over the next six months, as indicated by the Apr 2019 samples. This is probably due to the continued application of the microbial inoculants maintaining the newly established system. According to Bongers & Ferris (1999) enrichment stimulates bacterial activity, and subsequent succession, which is then reflected in a decrease in the MI. In this regard, the fertilisation with P, and in particular the inoculants, would therefore be expected to cause a decreased MI value due to addition of bacteria to the soil. This is what was indeed found over the following six months (Oct 2018 to Apr 2019) for T7 (Figure 5.3.2.8) that consisted of a combination of the two inoculants, which both are bacterial in nature. It is also reflected by the increase in the number of bacterial feeders in the T7 soil of the Apr 2019 samples (Table 5.3.2.4).

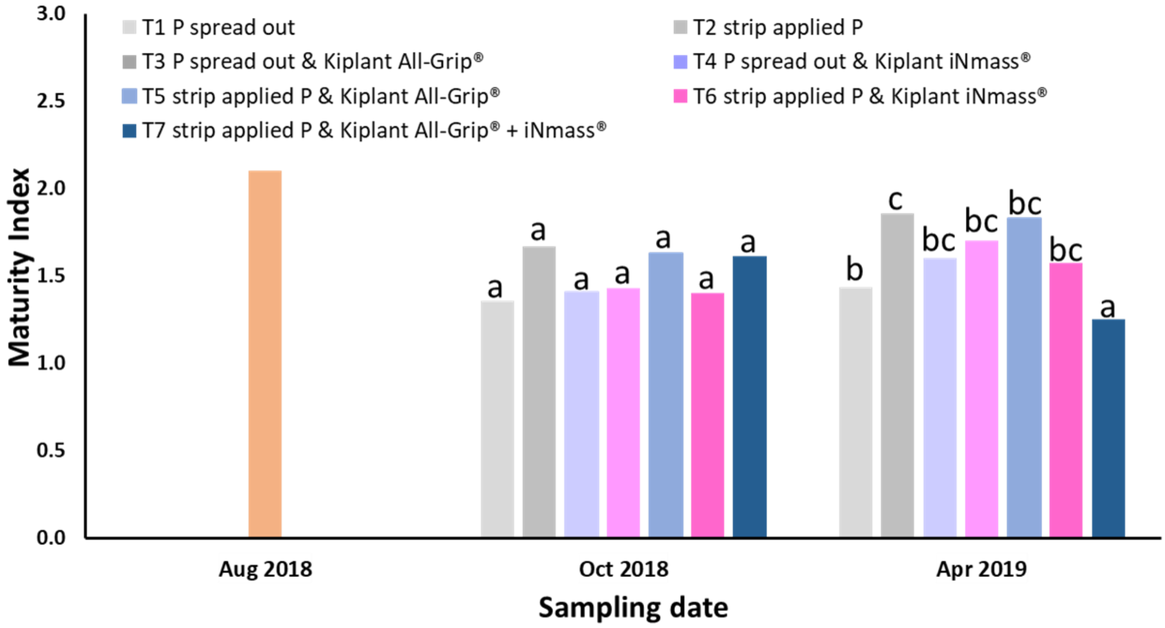

Figure 5.3.2.8: Maturity indexes of soils that received a once-off application of 40 kg P per ha in September 2018, followed by a treatment of the soil with the two types of Asfert Global soil conditioners. The "August 2018" results are the soil P concentration prior to application of the P-fertilisation. Values designated by the same letter do not differ significantly ($p \leq 0.05$).

Table 5.3.2.4. Counts of four of the nematode trophic groups, based on their feeding habits, used as indicator organisms to establish the effect of P fertilisation approach and soil inoculants on the health and stability of the soil food web. Values designated by the same letter do not differ significantly, NS=not significant.

Treatment	Bacterial feeder			Fungal feeder		Predacious		Omnivore	
	Sep 2018	Oct 2018	Apr 2019	Oct 2018	Apr 2019	Oct 2018	Apr 2019	Oct 2018	Apr 2019
1	663	2273	725 b	58	13 a	3 a	30	23	38b
2		1963	338 c	295	48 b	24 b	10	40	28b
3		1883	1427 a	145	50 b	18 b	36	33	68b
4		1825	1075 ab	208	90 b	30 b	19	65	75b
5		1233	678 b	135	48 b	18 b	18	2	50b
6		1818	1170 ab	138	60 b	21 b	20	5	25b
7		953	2105 a	70	95 b	25 b	20	43	5a
Significance		NS	0.05	NS	0.05	0.05	NS		0.05

The Faunal Profile (Figure 5.3.2.10) also showed a shift from the top right sector (Aug 2018: black dot) to the top left sector (Oct 2018: blue dots) after the P application treatments in September 2018. This is due to a reduction in the structure index (SI) of the soil. The lower the SI, the more disturbed or degraded is the soil ecosystem, whereas a high SI identifies structured or matured conditions (Ferris *et al.*, 2001). It can therefore be concluded that the P fertilisation (with or without the soil conditioners) generally resulted in high initial disturbance of the Food Web. The exception was T7, the treatment that contains both microbial inoculants. The high application rate of diverse bacterial species therefore did not result in a quick dramatic increase of the bacterial feeder nematodes (Table 5.3.2.4). The decrease in the structure index of T1 to T6 suggests a decomposition pathway that became more bacterial dominated when the ratio of bacterial feeder numbers were compared to the populations of omnivorous and predatory nematodes (Table 5.3.2.4). This indicates that for all the treatments, except T7, the soil biology was somewhat detrimentally affected after the original application of the treatments (Linsell *et al*., 2014). However, in the course of the next six months (Oct 2018 to Apr 2019) the Faunal Profile was restored for all treatments, except T7, as shown by the shift towards the right on the SI axis (Figure 5.3.2.10). This is ascribed to an improved balance between bacterial feeders and the other three groups of nematodes of T1 to T6 (Table 5.3.2.4). This soil's food web (viz. soil biology) could then again be regarded as better structured and more mature. The exception is T7, where a shift to the left occurred, indicating that the continued application of high numbers of bacterial inoculants stimulated a higher dominance of the bacterial feeders over the long term (Figure 5.3.2.10).

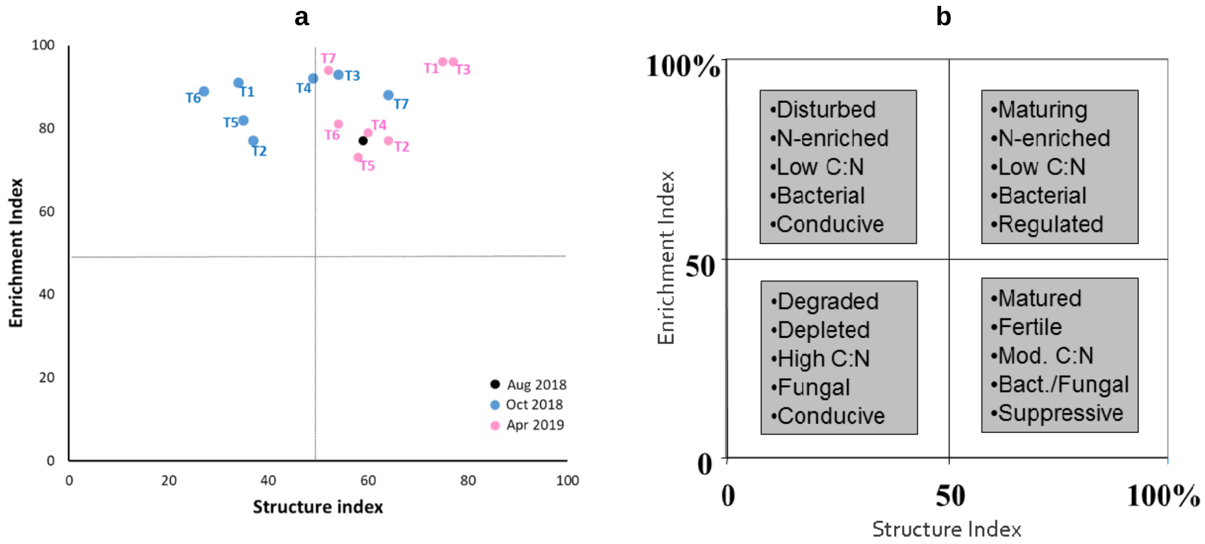

Figure 5.3.2.10. Food web analysis depicting the faunal profile of a Washington Navel/Carizzo block on an Augrabies soil in Addo, before (Aug 2018) and after having been treated (sampled in Oct 2018 & Apr 2019) with 40 kg P per ha and combinations of soil inoculants supplied by Asfert Global® (a) and a foodweb analysis interpretation scheme as per Ferris *et al.* (2001) (b).

In this soil, with excessive total P concentrations, addition of P should have little impact on phosphatase activity, despite the inoculants containing specific strains either *Bacillus* and *Pseudomonas* spp. (Kiplant All-Grip®) or *Bacillus* and *Azospirillum* (Kiplant iNmass®), that are known for high rates of phosphatase production. Addition of mineral P, however, resulted in an initial increase in the phosphatase activity, with the treatments where the P and inoculants were *spread out* (T3 & T4) that showed the largest increase (Figure 5.3.2.11). Treatments, viz. T2, T5 and T6, showed a lower increase than T3, 4 and also T7. This is probably due to suppression of phosphatase activity because of the concentration of P in the sampled zone of treatments 2, 5 and 6. While the high amount of inoculant applied in T7 probably dominated the negative effect of the concentrated P fertiliser application. The overall, general increase in phosphatase activity however correspond to findings by Piotrowska-Dlugosz & Wilczewski (2014b) who stated that phosphatase activity increase when P fertiliser is added to soils with low organic matter, which is the case of this trial's soil. The increase in phosphatase activity, after initial application of the treatments, was followed by a subsequently decrease in the next six months (Oct 2018 to Apr 2019), with no statistical difference between the treatments in Apr 2019. It therefore seems that, once the applied P has been fixated there is a lack of stimulus for soil bacteria or inoculants to produce or maintain high levels of phosphatase in soil with low organic C content.

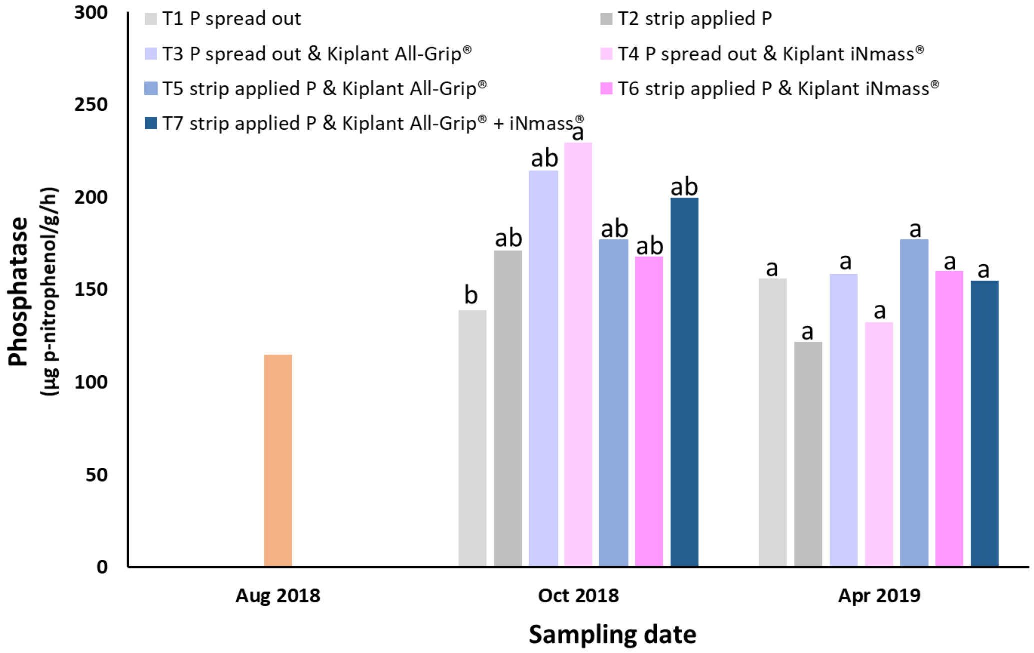

Figure 5.3.2.11. Phosphatase activities of soils that received a once-off application of 40 kg P per ha in September 2018, followed by a treatment of the soil with the two Asfert Global soil inoculants. The "August 2018" results represents the phosphatase activity prior to application of the treatments. Values designated by the same letter do not differ significantly ($p \leq 0.05$).

The urease enzyme's expression in soil is usually under N regulation (Machuca *et al*., 2015); as a result, it was expected that the P fertilisation would only affect urease activity in a negative way. However, inoculation with *Bacillus, Pseudomonas* and *Azospirillum* spp. that produce urease, should cause an increase in the soil's urease activity. Measurement of urease activity one month after application of the P fertiliser and inoculants showed a decrease in urease activity of T1 and also the strip applied treatments, viz. T2, T5 and T6. The P-application, and in particular the concentrated P, therefore seemed to have reduced urease activity. However, the inoculants in the spread out treatments (T3, T4 and T7) maintained the urease activity at a level that was significantly higher than that of the strip applied treatments. It is ascribed to the higher number of bacteria that produce urease, and that they were not affected by the p fertilisation. After six months, the treatments with strip applied inoculants (T5 and T6) now had significantly higher urease activity than the other treatments. Perotti & Pidello (1999) found that competition with native microorganisms and soil NH_4^+-N, applied as fertiliser throughout the season, may affect bacterium capacity, especially that of Azospirillum. The rate of application of the spread out inoculants in treatments 3 and 4 might not have been sufficient to maintain sustained activity of the inoculated bacteria and subsequent urease activity. In the situation where the applied P has been fixated, and soluble concentration of P was very low, either a lack of substrate to maintain their activity, or the competition from native organisms, reduced the activity of the inoculated bacteria., On the other hand, the concentrated application of the inoculants (T5 and T6), as well as the combination of inoculant types (T7), seemed to maintain urease activity at a higher level than the other treatments over the six month period from Oct 2018 to Apr 2019.

Thirdly, β-glucosidase activity, which is mainly involved in C cycling, and therefore greatly dependent on substrate supply (Adetunji *et al*., 2017), was significantly increased by all the treatments that contained the Asfert Global inoculants (Figure 5.3.2.13). It seems that general microbial activity is greatly enhanced by the inoculants, despite the simultaneous application of the mineral P. A decrease in the β-glucosidase activity was, however, observed for all treatments in the period Oct 2018 to Apr 2019. The inoculants therefore did not seem to sustain the same microbial activity throughout the season. This could be due to a decrease in substrate, given the low organic C of the soil or a change in the soil climatic conditions. Despite the much reduced β-glucosidase activity that was measured in the Apr 2019 samples, the strip applied treatments (T5 and T6) as well as T7 showed higher activity than the other treatments. This is also ascribed, like the urease results, to the concentrated nature of the applications. It can therefore also be concluded that both All-Grip® and iNmass® have a positive effect on β-glucosidase activity (viz. total aerobic micro-organisms involved in C cycling and hydrolyses of cellulose) in soil, mainly in the short term, and especially, if applied at high enough rates.

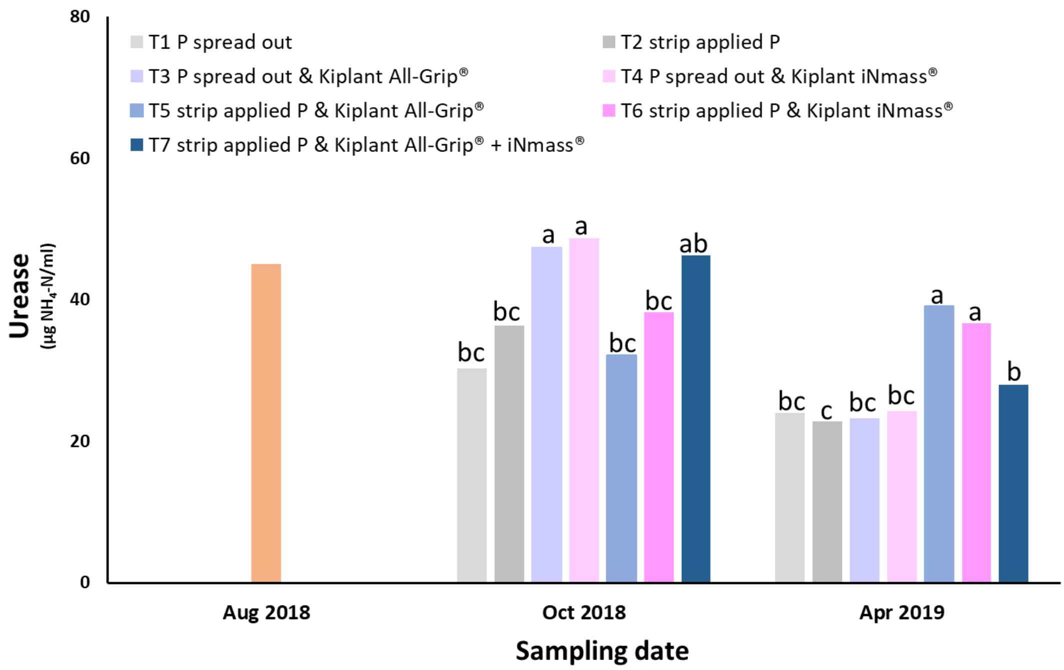

Figure 5.3.2.12. Urease activities of soils that received a once-off application of 40 kg P per ha in September 2018, followed by a treatment of the soil with the two Asfert Global soil inoculants. The "August 2018" results represents the urease activity prior to application of the treatments. Values designated by the same letter do not differ significantly ($p \leq 0.05$).

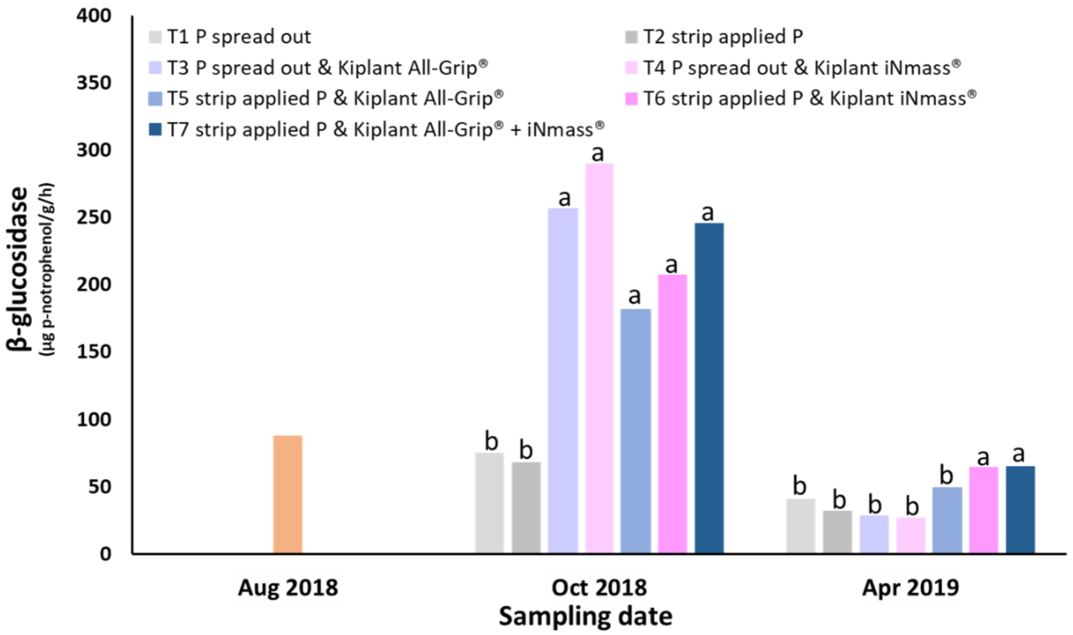

Figure 5.3.2.13. β-glucosidase activities of soils that received a once-off application of 40 kg P per ha in September 2018, followed by a treatment of the soil with the two Asfert Global soil inoculants. The "August 2018" results represents the urease activity prior to application of the treatments. Values designated by the same letter do not differ significantly ($p \leq 0.05$).

The Alteration index three (AI3) measures the balances between the three enzymes and is an expression of change in overall soil quality due to management practices (Adentunji *et al*., 2019). The combined effect of the treatments on the soil biology, in terms of the AI3, showed a dramatic impact brought about by the inoculants after the initial application in Sep 2018, as expressed by the shift in the AI3 values in Aug 2018 to that in Oct

2018 (Figure 5.3.2.14). All the treatments containing inoculants showed higher AI3 values in Oct 2018 than the reference sample taken in Aug 2018. According to Meyer *et al*. (2014) higher values (e.g. less negative, or positive) indicate to a disturbance in the soil biology, leading to imbalanced populations. Six months later, however, the AI3 shifted to lower values than the previous sampling dates, indicating that both the soil inoculants did not maintain its impact on the soil biology the same extent.

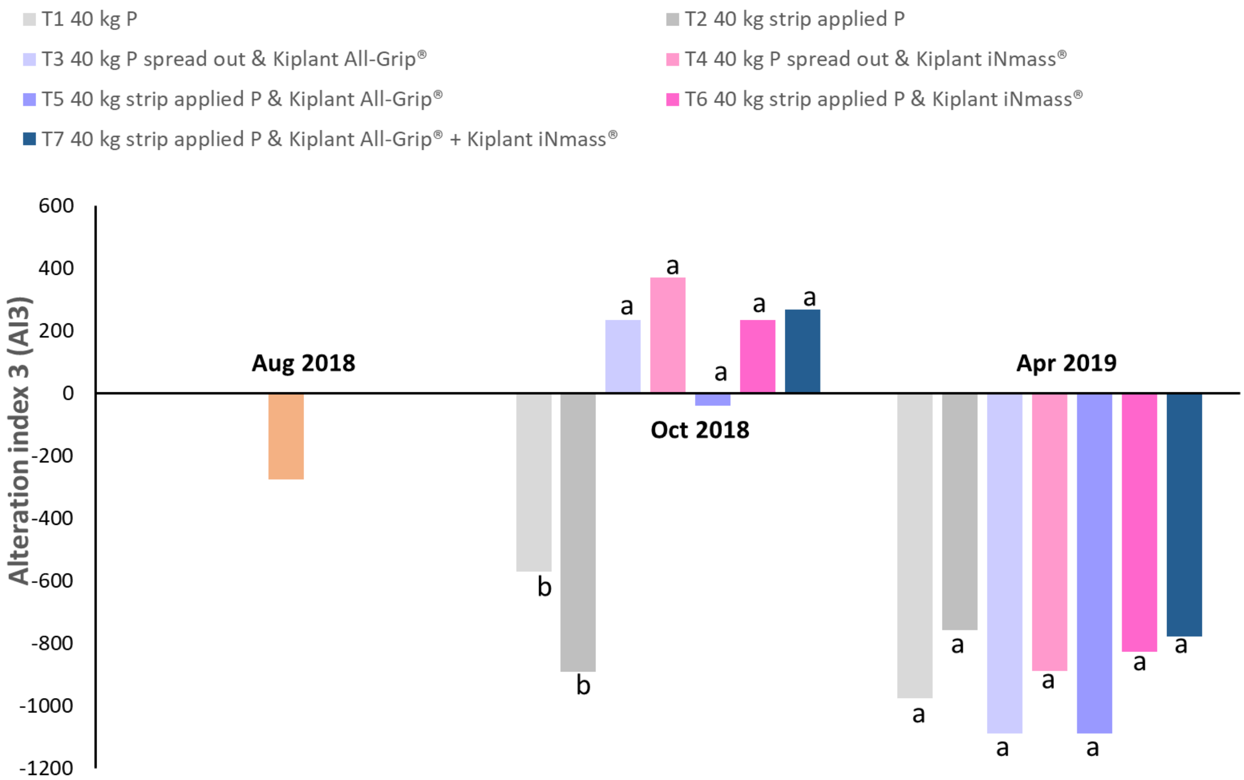

Figure 5.3.2.14. The calculated AI3 values used to establish the effect of P fertilisation approach and soil inoculants on the health and stability of the soil food web. Values designated by the same letter do not differ significantly ($p \leq 0.05$).

Conclusion

This project was exploratory in nature and therefore conducted over one season only. The outcomes of the trial are therefore not conclusive in all respects. It was however successful to confirm the following aspects regarding citrus tree P-nutrition in high pH soils:

- P-fixation of applied fertiliser in high pH soil leads to a discrepancy in the increase in Bray II extracted P compared to total soil P.
- However, despite P-fixation that occurs in high pH soils, P-availability is sufficient when the Bray II or total P concentration in the soil exceeds the minimum norm. The lack of differences in leaf P concentration reiterates the fact that even in high pH soils, a sufficiently high P concentration (> 200 mg/kg Bray II extractable P) will ensure adequate P-nutrition. Regular, annual P fertilisation is therefore not required.
- Uptake of P is not dramatically enhanced by soil application of fertilisers, viz. it is not expressed by increased foliar P concentration within a few weeks, or even a whole season. This is in accordance with comments regarding P nutrition by Coetzee (2007). In this trial it was found that inoculation of soil with a consortium of bacteria, however, might positively affect both P and Ca nutrition in the short term.
- Consequently, due to a lack of short term nutritional differences, effects of P nutrition on tree response should not be expected within the first season.

In addition, the following meaningful observations were made that enhance our understanding of management of soil biology, with the aim to optimise nutrient use:

- Application of P fertiliser negatively impacts on the soil's biological stability in the short term, as expressed by the nematode MI's. This effect, however, is not long-term which shows that soil biology tends to restore itself to a balanced system once the negative impact is terminated.
- In line with the negative impact on the soil's biological stability, application of P fertiliser also results in disturbance of the Soil Food Web, as indicated by the Faunal Profile. The effect is also not long-term.
- The use of the nematode profile can therefore be regarded as helpful to detect disturbances in the soil microbiological stability and therefore the Soil Food Web.
- The use of enzyme activities to assess soil quality with regard to its biology, was also found helpful.
- In soil with low organic matter content, and consequently low phosphatase activity, last mentioned can be stimulated on a short term basis through application of fertiliser P and elemental S (Brimstone 90®). The same positive effect was obtained with both of the soil inoculants. This means that the number or activity of phosphate mobilizing bacteria can be improved on high pH, low soil C soils through application of fertiliser P and Brimstone 90® or with fertiliser P and either All-Grip® or iNmass®. The possible implication is that applied P in a high pH soil will remain at slightly higher plant available levels for a longer period. This could be useful to address serious P-deficiencies in orchards with a high pH soil. The effect obtained with the microbial inoculants used in this trial was even to the extent that foliar P levels can be increased over a short time span.
- Urease activity, e.g. N-mineralising bacteria, is negatively affected by high rates of P-fertilisation, while it is sustained by both the soil inoculants All-Grip® and iNmass® when normal rates of P fertilisation is applied. The beneficial effect of the two inoculants on urease activity levels might assist with N nutrition management of orchards on soil with high organic C contents.
- Both All-Grip® and iNmass® significantly increased β-glucosidase activity, viz. general soil microbiological activity, in the short term. But it was not sustained. This is ascribed to a lack of substrate availability in this low C content soil.
- Application of a combination of Brimstone 90® and Crestharvest® seem to improve soil quality over a period of six months, as expressed by the AI3. And in accordance with its effect on β-glucosidase activity, the AI3 showed a dramatic impact on soil biology caused by both All-Grip® and iNmass®. The effect was a huge shift in the balance of the microbial populations. Last mentioned also lasted for a short period, and finally (after six months) also resulted in a state of improved soil quality.
- In summary, application of the treatments generally resulted in a disturbance of the soil biology, and more specific, the balance of species was disrupted. This was best illustrated by both the Soil Food Web Analysis and the AI3 index respectively.

Lastly, the following observations were made regarding the use and value of soil conditioners and inoculants:

- Brimstone 90®, All-Grip® and iNmass® can assist with P-nutrition on high pH soils.
- A combination of Brimstone 90® and Crestharvest®, All-Grip® and iNmass® seems to have a positive effect on soil quality, but only after six months. This effect might be more pronounced in soil with high C contents.
- Soil inoculant activity, or its effect on the soil, is not sustained at high levels in soil with low organic matter contents, due to a lack of substrate. In this case, practices that increase soil C might assist to obtain longer-term effectiveness of the products. Otherwise, repeated applications of the inoculants are required. The effect of perpetual use of the inoculants might, however, not be beneficial in the long term due to the imbalance in species created, resulting in disturbed soil microbial populations that are bacterially dominated and maintain a high mineral N level.
- Clarity regarding the competition from endemic organisms, adapted to low soil C and the climatic conditions, to limit the longevity of the effect of the inoculants also needs to be obtained.

Given the complexity and instability of the soil microbiological environment, as well as the many factors that affect it, consistent replication of the outcomes of the treatments discussed above cannot be expected in all environments. Furthermore, the role of changing seasonal climatic conditions (and associated production practices and inputs) were not taken into account in this trial – it would have made the extent of the work unmanageable.

Future research

With a general world-wide trend to optimise fertiliser use, and reduce mineral nutrition inputs, the potential use of soil conditioners, and in particular microbial inoculants, needs to be explored further. Since the two methods of establishing soil management practices on soil biology has been found useful, further investigation of the long-term effects, and employment of these (and other) inoculants, is now possible and should be considered.

Technology transfer

None to date.

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5.3.3 PROGRESS REPORT: Evaluation of aerial application and adjuvants of micronutrients

Project 1230 (2019/4 – 2022/3) by P.J. Raath (CRI), P. Strydom (Villa Crop) and N. Taylor (UP)

Summary

A trial to evaluate aerial application of micronutrients, and to test the value of added adjuvants to improve application efficacy and nutrient uptake, was initiated in collaboration with Villa Crop. An experimental orchard was selected on Letaba Estates (Tzaneen) and the first experimental application of foliar Zn and B products, as well as a patented product (Masterlock®) that enhances droplet distribution through the tree canopy, were done in August 2019. Leaf analyses to quantify total Zn and B concentrations were conducted on samples taken two weeks after application. Droplet distribution and size was measured throughout the canopy to ascertain the efficacy of Masterlock® to improve distribution and wetting levels of the spray mixture.

Aerial application of micro-nutrients increased the total foliar nutrient concentration and can be used to maintain the micronutrient nutrition status of trees. The increase, however, was not to the extent that nutrient deficiency can be addressed. When micronutrient deficiencies occur, or trees are at risk of developing deficiencies, nutrients should be applied with conventional spraying. Compared to Ecklomax® (present farm practice), the Max-In products did not increase the foliar total Zn and B concentration when applied aerially, or where Masterlock® was used, despite a far higher total amount per ha and concentration applied with the use of Max-In products. Max-In products, applied conventionally and at the recommended application rate, did surpass the present Farm Practice (using Ecklomax® at the prescribed rate). It must, however, be borne in mind that the total amount of Zn and B, as well as the concentration in the spray mixture, exceeded the Farm Practice by 7 200%. This most probably explains the result instead of an improved efficacy of the Max-In products.

Masterlock® dramatically increased droplet distribution and total wetted surface area throughout the tree canopy. Drop size was also increased which explains the better distribution through the canopy. It should therefore also enhance total tree nutrient uptake.

The trial will be repeated for another season. This time similar amounts of applied Zn and B per hectare will be used. Leaf samples will also be taken at different vertical positions of the canopy, as well as inside vs. outside of the canopy.

Opsomming

ŉ Eksperiment om toediening van spoorlemente deur middel van lugbesputing te evalueer, asook om die waarde van bymiddels se vermoë om toedienings- en opname-effektiwiteit te verhoog, is in samewerking met Villa Crop geïnisieer. ŉ Eksperimentele boord op Letaba Estates, Tzaneen, is gekies. Die eerste eksperimentele toediening van Zn en B blaarvoedingsprodukte, asook ŉ gepatenteerde produk (Masterlock®) wat druppelverspreiding deur die lower verbeter, is in September 2019 gedoen. Blaarontledings om die totale Zn en B konsentrasies te kwantifiseer is op blare gedoen wat twee weke na toediening gemoster is. Druppelverspreiding en grootte is reg deur die boom se lower gemeet sodat die effektiwiteit van Masterlock®, om die verspreiding en benatting van die spuitmengsel te bevorder, bepaal kan word.

Lugbespuiting van spoorelemente het die totale konsentrasie van Zn en B in die blare verhoog en kan gebruik word om die spoorlementvoedingstatus van die bome te handhaaf. Die toename was egter nie groot genoeg om tekorte wat mag voorkom aan te spreek nie. In gevalle waar spoorelementtekorte voorkom, of waar die risiko van ontwikkeling van tekorte groot is, moet liefs gebruik gemaak word van konvensionele spuittoedienings. In vergelyking met Ecklomax® (die huidige plaaspraktyk), het die Max-In produkte nie die totale Zn en B konsentrasie van die blare verhoog as dit met lugbespuitings toegedien word nie, ten spyte dat ŉ baie hoër totale hoeveelhied per hektaar en konsentrasie, in die geval van die Max-in produkte, gebruik is. Waar die Max-in produkte met konvensionele spuite toegedien is, en teen die aanbevole dosis, het dit die huidige plaaspraktyk (waar Ecklomax® teen die aanbevole dosis gebruik is) oorskry in terme van totale konsentrasie Zn en B wat in die blare gemeet is. Dit moet egter beklemtoon word dat die totale hoeveelheid Zn en B, asook die konsentrasie in die spuitmengsel sowat 7 200% hoër was as in die geval van die plaaspraktyk. Dit verklaar heel waarskynlik die resultaat in plaas van ŉ verbeterde opname-effektiwiteit deur die Max-in produkte.

Masterlock® het die druppelverspreiding en totale benatte blaaroppervlak reg deur die lower dramaties verhoog. Druppelgrootte is ook verhoog, wat die beter verspreiding verklaar. Daar word verwag dat die produk dus die opname oor die hele boom sal verhoog.

Die eksperiment sal vir nog ŉ seisoen herhaal word. Hierdie keer sal soortgelyke hoeveelhede Zn en B per hektaar toegedien word. Blaarmonsters sal ook op verskillende vertikale posisies in die lower geneem word, asook binne teenoor buite die lower.

5.3.4 **PROGRESS REPORT: Adaptive Nutrition Management Strategies for Improved Fruit Quality**
Project 1231 (2019/4 – 2022/3) by PJ Raath (CRI) & K Hunter (ARC)

Summary

Collection of the first season's data on the Midknight Valencias in Nelspruit and Orri mandarins in De Wet is almost complete. In addition to leaf, flower and fruit analysis, results of crop load and nutrition treatment effects on fruit set, as well as fruit growth and quality were collected. The research team refrained from doing a wider interpretation and application of the data at this point since a far more pronounced response to the treatments is expected in the following/second season. Furthermore, the data are being used to generate two MSc theses.

From results that were analysed, of which some are reported below, it can be concluded that despite the difficulty in obtaining responses from nutrition treatments, and the complexity of various factors that can affect the measured parameters, the trees are responding to the treatments. From the data generated in this project significant insight into the responsiveness of both mandarin and Valencia trees to mineral nutrition should be obtained. Furthermore, the role of nutrition to affect fruit set and fruit quality of these two cultivars will be further elucidated.

Opsomming

Volledige navorsingsresultate van die Midknight Valencia (Nelspruit) en Orri mandaryne in De Wet is vir die eerste seisoen ingewin. Behalwe vir blaar-, blom- en vrugontledings, is die effek van oeslading en bemestingsbehandelings op vrugset, grootte en kwaliteit ook gemeet. Vir die huidige, weerhou die navorsingspan hul daarvan om ŉ wye interpretasie en toepassing van die data te maak aangesien ŉ groter reaksie op die behandelings in die komense sesisoen verwag word. Verder word die data ook vir twee MSc verhandelings gebruik.

Uit die data wat geanaliseer is, is ŉ klein deel hieronder gerapporteer. Daaruit kan afgelei word dat, hoewel dit moeilik is om reaksies d.m.v. bemestingsbehandelings te verkry, die bome wel op die behandelings reageer. Uit die data wat in hierdie projek gegenereer word sal insig rakende die vatbaarheid van mandarin en Valencia bome om op minerale bemesting te reageer verkry word. Verder sal die rol van bemesting om vrugset en vrugkwaliteit van hierdie twee variëteite te beïnvloed ook uitgeklaar word.

5.3.5 **PROGRESS REPORT: Handbook for Citrus Nutrition in Southern Africa**
Project 1232 (2019/4 – 2021/3) by PJ Raath (CRI)

Summary

Various chapters have been finalised to the point of being ready for publication. The outstanding chapters are in various stages of completion, which should all be ready for final editing by the end of July 2020. A graphical designer has produced examples of possible cover pages, page layout and illustration styles. Printing of the book is due to be completed by end December 2020.

Opsomming

Verskeie hoofstukke is afgehandel tot die punt waar dit gereed is om gepubliseer te word. Die uitstaande hoofstukke is in verskillende stadiums van afhandeling, maar sal gereed wees vir finale redigering teen einde Julie 2020. ŉ Grafiese kunstenaar het verskeie voorbeelde van voorblaaie, bladuitlegte en illustrasie-style voorberei. Finale druk van die boek behoort einde Desember 2020 voltooi te wees.

5.3.6 **PROGRESS REPORT: Studies to improve seed production of rootstock trees**
Project 1264 (RCE 4-2-28) (2020/4 – 2022/3) by J Niemann (CRI) and PJR Cronje (CRI, SU)

Summary

Citrus rootstocks, which play a critical role in determining the success of how citrus is grown under various conditions, are mainly propagated by means of seeds, therefore fruit with a high seed count is critical. Flower development and pollination are important processes preceding fruit development and consequently seed production. Cultural and crop manipulation techniques (nitrogen application, girdling, mechanical pruning and gibberellic acid (GA) applications) are implemented in citriculture to increase flower intensity and fruit set. The tree physiology of rootstocks remains an unknown field, therefore the objective of this project is to determine the horticultural properties of the main rootstock cultivars, to quantify the yield/tree and seed count and to determine if differences in nutritional norms exist. In addition, several vegetative management practices will be implemented to determine the effect thereof on consistent fruit bearing. The trial site is located at the Citrus Foundation Block in Uitenhage and preliminary results will be shown. The cultivars, X639, MXT, Carrizo citrange (CC), Volkameriana (VA), Rough lemon (RL), Swingle citrumelo (SC) and C35 planted in 2015 and Sunki Benecke (SXB) (planted 2010) were selected. Monthly fruit size measurements were conducted for CC, SC, MXT, C35 and X639. SC had the largest fruit diameter and X639 the smallest. The average seed number/fruit was determined from 100 fruit that were harvested from 10 trees respectively (10 fruit/tree). VA had the highest seed count per fruit (21), followed by C35, SC, CC and MXT which had 17/fruit respectively and RL (16/fruit). X639 and SXB had the lowest seed number, 8 and 4/fruit respectively which can be detrimental for seed production. With regard to leaf analysis, nutritional differences between cultivars was apparent. The levels for some elements was either below or above the nutritional norms for certain cultivars.

The yield/tree is yet to be determined for this season, as well as implementation of pruning techniques and GA applications based on yield/tree data.

Opsomming

Sitrus onderstamme speel 'n belangrike rol in die bepaling van die sukses van die groei van sitrus onder verskillende toestande, en word hoofsaaklik deur sade voortgeplant. Vrugte met 'n hoë saadtelling is dus van kritieke belang. Blomontwikkeling en bestuiwing is belangrike prosesse wat lei to vrugvorming en gevolglik saadproduksie. Verskeie kulturele - en gewasmanipulasietegnieke (stikstoftoediening, ringelering, snoei en gibberelliensuur toedienings) word in sitrikultuur toegepas om sodoende blomintensiteit en vrugset te verhoog. Die boomfisiologie van onderstamme is tans 'n onbekende veld, daarom is die doel van hierdie projek om die hortologiese eienskappe van die belangrikste onderstok kultivars te bepaal, die opbrengs/boom- en saadtelling te kwantifiseer, sowel as om vas te stel of daar verskillende voedingsnorme is. Daarbenewens sal verskeie vegetatiewe bestuurspraktyke toegepas word om die effek daarvan op konsekwente vrugdrag te bepaal. Die proefperseel is by die Sitrus Grondvesblok in Uitenhage geleë en slegs voorlopige resultate sal getoon word. Die kultivars, X639, MXT, Carrizo citrange (CC), Volkameriana (VA), Growweskil suurlemoen (GS), Swingle citrumelo (SC) en C35 wat in 2015 geplant is, en Sunki Benecke (SXB) (geplant 2010) is gekies. Maandelikse vruggrootte metings was gedoen vir CC, SC, MXT, C35 en X639. SC het die grootste vrugdeursnee gehad en X639 die kleinste. Die gemiddelde saad inhoud per vrug was bepaal vanaf 100 vrugte wat onderskeidelik van 10 bome (10 vrugte/boom) geoes was. VA het die hoogste saadtelling per vrug (21) gehad, gevolg deur C35, SC, CC en MXT wat onderskeidelik 17 gehad het en GS (16/vrug). X639 en SXB het onderskeidelik die laagste saadgetal gehad, 8 en 4 sade/vrug, wat nadelig kan wees vir saadproduksie. Wat die blaarontleding betref, was daar voedingsverskille tussen kultivars aangedui. Die vlakke van sommige elemente was onder of bo die voedingsnorme vir sekere kultivars. Die opbrengs/ boom moet nog vir hierdie seisoen bepaal word, sowel as die implementering van snoeistegnieke en GA-bespuitings, gebaseer op die opbrengs/ boomdata**.**

5.3.7 PROGRESS REPORT: Re-evaluating fruit set strategies for seedless 'Valencia' in the Letsitele region

Project RCE-2-01 (1209) (2018/9 – 2020/21) by P Cronje, V White (CRI), C van Wyk, K Theron (SU), O P J Stander and Schalk Reynolds (Phillagro SA)

Summary

The objective of this study is to re-evaluate the application of GA_3 foliar sprays in seedless 'Valencia' cultivars in a hot and humid South African citrus growing area, Letsitele, where fruit set in these cultivars has been reported as problematic and fruit yield poor. Different concentrations of GA_3 foliar sprays will be compared with the current standard commercial practice of 2 x 2.5 ppm as the control, as well as in combination with different nitrogen (N) fertilizer regimes, and/or soil applications of uniconazole-P. The treatments' effects on important horticultural plant responses will be determined, *viz.* vegetative growth, flowering, fruit set and fruit yield, as well as important parameters of plant physiological response, *viz.* leaf and root N, and leaf and root carbohydrates, to interpret any possible significant results, or the lack thereof. Due to the unforeseen impact of drought in Letsitele no fruit were set in the two Valencia (Delta and Midknight) orchards used in the project. All treatments were applied in the spring of 2019, however, the climatic stress resulted in a crop failure. Two new research objectives were added: firstly, the macro factors influencing seedless Valencia fruit set in Letsitele as compared to Eastern Cape. Secondly, the influence of soil condition in Letsitele on root development and correlation with fruit set. These additional focus areas will add necessary information to the challenge to realise consistent fruit set of seedless Valencia.

Opsomming

Die doel van hierdie studie is om die toepassing van GA_3 blaarbespuitings in saadlose 'Valencia'-kultivars in 'n warm en vogtige Suid-Afrikaanse sitrus produksie area, Letsitele, te herevalueer waar vrugset as problematiese en vrug opbrengs swak gerapporteer is. Effektiwiteit van verskillende konsentrasies GA_3 blaarbespuitings sal vergelyk word met die huidige standaard kommersiële praktyk van 2 x 2.5 ppm, sowel as in kombinasie met verskillende stikstof (N) bemestingsregimes en / of grondtoediening van unikonazool-P. Die

behandelingseffekte op belangrike plant reaksies sal bepaal word, nl. vegetatiewe groei, blom, vrugset en vrugopbrengs, sowel as belangrike parameters van plantfisiologie, nl. blaar en wortel N en blaar en wortelkoolhidrate, om enige moontlike beduidende resultate te interpreteer. Die onvoorsiene negatiewe impak van die droogte in Letsitele het gelei tot geen vrugset in die Delta en Midknight Valencia boorde in die proef. Al die lente behandelings was toegedien maar die klimaat stremming was te hoog en geen het geslaag om ŉ vrug te set nie. As gevolg hiervan was twee addisionele doelwitte ingesluit in die studie nl. ŉ historiese oorsig oor die set patrone van saadlose Valencias oor die laaste 5 jaar in Letsitele en die Oos Kaap. Tweedens gaan ŉ die wortel ontwikkeling onder verskillende grondtoestande gekyk word om ŉ moontlike korrelasie met set te bepaal. Hierdie twee nuwe areas ingesluit in die projek sal nodig inligting verskaf in die uitdagende oorhoofse doelwit om meer konstante vrugset in Valencias te bewerkstellig.

5.4 PROGRAMME: CULTIVAR EVALUATION

Programme coordinator: Johan Joubert (CRI)

5.4.1 Programme summary

The lemon plantings, and more specifically, Eureka on X639, remain high in all the citrus producing areas, where lemon establishment is possible in any citrus climatic region. One of the most important fruit characteristics remains oblong (longer) fruit shape, followed by prolonged picking window (best prices early in the season) and low seed numbers. Another important aspect on the tree's side is low thorn numbers or smaller thorns on the bearing branches to facilitate the harvesting process. 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. Lisbon and Limoneira will be the next option for commercial plantings, due to compatibility on citrumello and citrange rootstocks and good production and fruit quality. There are several new lemon selections included in the trial sites to challenge Eureka, with a specific goal towards completely seedless fruit along with optimum yield on the trees.

The mandarin trials in the hot citrus production areas (5.4.4, 5.4.7) remain a priority with the reality of good quality fruit with good colour development early in the season and optimum Brix: acid ratios being critical, options are improving with new selections becoming available. The cool and intermediate production areas remain the best mandarin producing options (5.4.6, 5.4.8, 5.4.10, 5.4.14, 5.4.15, 5.4.16, 5.4.17) due to specific climatic requirements (better early colour and acids). The focus remains to plant earlier or later maturing cultivars 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 seedless fruit, that peel easily, has good colour development and excellent flavour. Numerous new experimental options went into trial sites in the main citrus production areas, on different rootstock options, to determine the commercial value of these cultivars, including the hotter production regions (early- and late maturing possibilities).

Star Ruby remains the number one grapefruit planted in South Africa due to excellent internal colour development and very good internal quality with high Brix content. The Star Ruby early and late were included at trial sites to allow the possibility of a longer picking window for red grapefruit. 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).

We need early maturing Valencia options to replace or supplement Turkey because of the rind disorders and shelf life (adding experimental options in trials). The focus on late-maturing Valencia selections increased (5.4.2, 5.4.3, 5.4.5, 5.4.21, 5.4.22) 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, Kobus du Toit Late, McClean SL etc).

Navel prices dropped considerably in the past season due to a 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, 5.4.18, 5.4.19, 5.4.20). The focus will therefore be on lemon, mandarin and Valencia options to fill the requirements in the production cycle of the packhouse programme.

Rootstock evaluations (including semi-commercial trials) will expand the range of new rootstock trials, including the mainstream (commercial), semi-commercial range, as well as several Florida options (with the possibility of HLB tolerance) to evaluate production performance and suitability in 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 and specific conditions and smaller tree volumes (5.4.5, 5.4.11, 5.4.12).

Programopsomming

Die suurlemoen aanplantings, en meer spesifiek Eureka op X639 bly hoog in al die sitrus produserende areas; deurdat suurlemoen vestiging moontlik is in enige sitrus klimaatsone. Een van die belangrikste vrug eienskappe ŉ steeds langwerpige (silindriese) vrug vorm, gevolg deur verlengde plukvenster (beste pryse vroeg in die seisoen) en lae saad getalle. Ander belangrike aspekte is lae en of kleiner dorings op die draetake om die oes proses te vergemaklik. Eureka bly steeds die nommer een suurlemoenseleksie wat deur Sitrusprodusente en verbruikers verkies word om verskillende redes: vrugte van goeie gehalte met 'n hoë sapinhoud; redelike lang vrugvorm; potensiaal om 'n goeie drag met twee tot drie vrugsette asook min dorings. Lisbon en Limoneira is die volgende kommersieleopsie vir aanplantings, a.g.v. verenigbaarheid op citrumelo en citrange onderstamme, asook goeie produksie en vrugkwaliteit. 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.

Die mandaryn proewe in die warm sitrus produksieareas (5.4.4, 5.4.7) bly 'n prioriteit vangoeie vrugkwaliteit (optimale Brix:suur) met goeie kleurontwikkeling vroeg in die seisoen. Die nuwe seleksie brei die opsies uit vir hierdie doelwit.. Sitrus wat in die koel en intermediêre produksieareas verbou word, bly die beste mandaryn produserende opsies (5.4.6, 5.4.8, 5.4.10, 5.4.14, 5.4.15, 5.4.16, 5.4.17) as gevolg van spesifieke klimaatvereistes (beter vroeë kleur en sure). Die fokus bly op die aanplant van vroer of later rypwordende kultivars buiten die Tango, ARCCIT9 (Nadorcott LS) en Nadorcott plukvensters, aangesien groot volumes van hierdie kultivars geplant is. Die beste kwaliteit vrugte wat in hierdie plukvenster geproduseer word, sal in groot aanvraag wees, maar die gebiede met marginale vrugkwaliteit kan problematies wees. Die verbruikers velang vir vrugte met 'n lae, 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, op verskillende onderstam opsies, om die kommersiële waarde van hierdie kultivars te bepaal, insluitend die warmer produksie areas (vroeë en laat rypwordende opsies).

Star Ruby is 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. Die eksperimentele vroee- en laat rypwordende Star Ruby seleksie word in proef persele ingesluit met die moontlikheid om die plukvenster te verleng van die rooi pomelos. Daar is verskeie nuwe rooi pomelo seleksies wat by die proefpersele ingesluit word met laer naringien vlakke (bitter smaak) om die smaak en eet-ervaring te verbeter (5.4.12).

Vroeë Valencia opsies word benodig om die Turkey aan te vul of te vervang, a.g.v. problematiese skildefekte en houvermoë (nuwe opsies word ingesluit in eksperimente). Die fokus op laat rypwordende Valencia-seleksies bly hoog (5.4.2, 5.4.3, 5.4.5, 5.4.21, 5.4.22) in die geskikte sitrusproduksie areas (Letsitele), waar die vraag na Valencias met ŉ lae saadinhoud of totaal saadlose vrugte met goeie produksie toegeneem het. Die probleem vanŉ 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, Kobus du Toit Late, McClean SL ens.).

Die nawel pryse het die afgelope seisoen aansienlik gedaal as gevolg van die kombinasie van rakleeftyd probleme (lae suurvlakke), na-oes prestasie en oop nawel-ente, wat alles bygedra het tot die afname in nuwe nawel aanplantings (5.4.9, 5.4.18, 5.4.19, 5.4.20). Die fokus sal wees op suurlemoen, mandaryn en Valencia opsies om die vereistes in die produksiesiklus van die pakhuisprogram aan te spreek.

Onderstam evaluasies sal uitgebrei word (insluitend semi-kommersiele proewe) met ’n nuwe reeks onderstam proewe, ingesluit die hoofstroom reeks (kommersiele opsies), semi-kommersiele reeks asook verskeie Florida opsies (met moontlike HLB weerstandbiedendheid) om produksie potensiaal te evalueer en geskiktheid vir verskillende grond tipes (pH, 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, were as follows. Valearly, one of the new early maturing (internal quality) cultivars that matures before Turkey. There was still 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 and Millennium would follow, with good internal quality, production and fruit size, followed by McClean SL and Gusocora representing the middle of the Valencia season for this area. Alpha will be next in line, maturing later in this area compared to other areas, but keep the young tree age in mind. 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 1.2 seeds per fruit.

Valearly and Millennium 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 vogtige warm produksie area, is soos volg. Valearly is een van die nuwe vroeë Valencia opsies (vroeg intern ryp) wat voor Turkey inpas. Daar was steeds 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 en Millennium kan dan volg wat goeie interne kwaliteit, produksie en vruggrootte lewer, gevolg deur McClean SL en Gusocora wat dan die middel van die Valencia seisoen vir hierdie area verteenwoordig. Alpha pas dan in, wat later in hierdie area rypword in vergelyking met ander areas, maar hou die jong boom ouderdom in gedagte. Die later seleksies wat kan bydra tot die keuse om die seisoen te verleng, kan bestaan uit Skilderkrans, Kobus du Toit Laat en Jassie (optimum vruggrootte verspreiding) gevolg deur Moosrivier Late 1, wat 1.2 sade per vrug produseer. Lavalle sal dan laaste volg met hoer suurvlakke as die ultra-laat opsie.

Valearly en Millennium 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 Alpha, Delta (control), Gusocora, Jassie, Kobus du Toit Late, Lavalle, McClean SL, Millennium, 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 º	Min Acids	Max Acids	Ratio	Colour
Valencia EU	48	8.5	0.6	1.8%	7.5:1	Colour plate 3 of set no. 34
Midknight	52	9.5	0.85	1.8%	7.5:1	Colour plate 3 of set no. 34
*Turkey	50	10.0	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34

*Interim internal fruit quality standards.

Table 5.4.2.2. List of Valencia selections evaluated at Riverside (Malelane) during 2019.

Selection	Rootstock	Year Planted	Topworked	No. of trees
Alpha	CC	NA	2015	4
Delta	C35/CC/SC	2012	NA	5/5/5
Gusocora	CC	NA	2015	4
Jassie	C35/CC/SC	2012	NA	5/5/5
Kobus du Toit Late	C35/CC/SC	2012	NA	5/5/5
Lavalle	CC	NA	2015	4
McClean SL	C35/CC/SC	2012	NA	5/5/5
Millennium	CC	NA	2015	5
Moosrivier Late 1	C35/CC/SC	2012	NA	5/5/5
Skilderkrans	C35/CC	2012	NA	5/5
Turkey	C35/CC/SC	2012	NA	5/5/5
Valearly	C35/CC/SC	2012	NA	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 2019 season.

<u>Delta (control)</u>
Delta produced a fair to good crop, bearing fruit for the fifth set of evaluations this season. Fruit size range was slightly smaller this season and ranged from medium to large/extra-large (count 88 to 48) with good internal quality values, juice levels above 58, Brix of up to 12.8 and acids above 0.8% (except for C35 with 0.6% on one evaluation). Colour development at peak maturity 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.

<u>Jassie</u>
The trees were bearing another good crop this year on all three rootstock combinations, averaging between 70 and 90 kg per tree. The fruit size was smaller and varied from medium to large, count 72 to 56 (average). The rind texture improved this season, becoming smoother with time. Seed count per fruit was decreased even more this season and varied from 0.9 to 4.5 seeds per fruit. Internal quality improved with tree age and produced better juice levels (above 56%), good Brix (above 11 at maturity) and lower acids (above 0.7). External colour development improved and peaked between T1 and 3 with the final evaluations. Maturity seems to be the middle of July to the beginning 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 56/48) on the trees due to a lighter crop, with 2.1 seeds average. The colour development was very similar on all three rootstocks. The internal quality was good, juice levels above 54%, Brix up to 12.9, and lower acids earlier in the season (below 1 on C35) for the later maturing selection. External colour peaked from T1 to 4. Maturity seems to be middle to end of July, according to Table 5.4.2.3.

McClean SL

The standard McClean will be included in future trials as a control to compare the SL selection's performance, although McClean developed high chimera incidences on the fruit (up to 40%) in commercial plantings. McClean SL produced fairly round fruit with soft fiber strength that peeled easily, containing low rind oil levels. All the fruit evaluated remained 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 (GA_3 sprays are recommended). The fruit size increased and peaked at medium-large to large/extra-large (count 72-56/48). The internal quality improved from good (2017) to very good with high juice levels for the trial site up to 59%, Brix up to 12 and acceptable acid levels (above 0.8%). There was a slight delay in external colour ranging from T1-3. Based on the internal quality results in Table 5.4.2.3, maturity will be mid to end of July.

Moosrivier Late 1

Moos Late 1 developed a high acid level (average 1.2%) when the juice (above 53%, except one sample on CC) and Brix (above 10.7) content was ready for harvesting at Riverside, and the external colour peaked at T1 to 4 (slightly delayed). Moos Late 1 developed 1.2 seeds (cross pollination) per fruit compared to last season's 0.7. Moos Late 1 had promising performance, developed smooth round fruit with deep yellow internal colour, good flavour, peeled easily and had 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 remained the same and varied from medium to large (count 72-48). Internally the Brix content improved (up to 11.2) and the acid level of 0.95 to 1.0% indicated a later maturing Valencia selection. Juice level increased to an average 59%; above the minimum required export figure. There was no delay in external colour (T1-4) 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 the end of July and mid-August on all three rootstocks (Table 5.4.2.3).

Turkey (Control)

Fruit size improved this season, ranging from count 72 to 48/40, 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 1.5 to 3.3 seeds per fruit (lower than 2018). 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 has the qualities of a mid-season orange; for instance, the exceptionally soft rag of the fruit, and the soft rind result in rind problems if managed incorrectly. Turkey should not be harvested over more than four weeks as extending the harvesting season can lead to rind disorders. Based on the internal quality results in Table 5.4.2.3, the estimated maturity will be middle to end of May.

Valearly

Valearly, bearing a good crop (60-80 kg/tree) on the trees, developed low seed numbers (0.0 to 3.0 seeds per fruit) this season. The internal quality of the fruit was good early in the season with medium-high juice (above 50%, except for Swingle – 48%), Brix above 10 (except for Carrizo – one evaluation, and Swingle with two evaluations – 7.8 and 9.2) and acid above 0.8 on C35 and Carrizo. Acids on Swingle remained fairly high till later in the season (1.2%). Compared to 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 T2-4. Estimated maturity according to Table 5.4.2.3, seems to be the end of April.

Additional selection

Alpha, Gusocora, Lavalle and Millennium bore a decent crop for the first time this season and evaluations were possible, all topworked onto Carrizo rootstocks. All four selections were completely seedless. Millennium matured first; end of June with the lowest acid levels and very good Brix, ranging from 11.7 to 12.5. The highest acid level between the four cultivars was on Lavalle; also developing very good Brix and juice content. Colour development remained very similar between T1 and T4 up to peak maturity. There were chimeras present on Millennium and further evaluations will determine the severity and possible cause of the mutations on the fruit. Fruit size peaked on three of the four selections, except for Lavalle with bigger fruit, between count 72 and 56; very good for Valencia production and export.

Conclusions

The internal quality for this season for all the selections evaluated, complied with the export standards at peak maturity of the fruit. 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, but rather the opposite scenario with lower acids in general on most of the fruit samples being evaluated. 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 Turkey and Valearly, more specifically on Swingle rootstock, where the colour development was delayed even after peak quality. The average seed count for this season remained fairly low, including Jassie and Turkey (average 2.5 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 future possibilities to include in new Valencia plantings (optimum Valencia fruit size distribution, high juice levels, low seed counts and late maturing). Fruit size improved on the trees as they mature, between count 72 and up to count 56/48 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 2019 season.

Cultivar	Rootstock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix º	Acid (%)	Ratio	Avg seed	Fruit external colour
Alpha	CC	05/06/2019	77 - 83	72 - 56	60,6	10,5	1,40	7,5	0,0	T3 - 4
Alpha	CC	27/06/2019	75 - 85	72 - 56	58,4	11,3	1,40	8,1	0,8	T1 - 4
Alpha	CC	18/07/2019	74 - 82	72 - 56	61,0	11,1	1,15	9,7	0,0	T1 - 4
Alpha	CC	08/08/2019	77 - 84	72 - 56	62,3	11,2	1,15	9,7	0,0	T1 - 4
Alpha	CC	28/08/2019	73 - 84	72 - 56	51,7	12,1	1,00	12,1	0,0	T1 - 3
Delta	C35	05/06/2019	76 - 88	72 - 48	59,6	10,1	1,20	8,4	0,0	T3 - 5
Delta	C35	27/06/2019	70 - 79	88 - 64	62,7	11,2	1,30	8,6	0,0	T1 - 4
Delta	C35	18/07/2019	79 - 90	64 - 40	60,9	11,0	0,60	18,3	0,0	T1 - 4
Delta	C35	08/08/2019	79 - 87	64 - 48	58,1	10,9	0,90	12,1	0,0	T1 - 4
Delta	CC	05/06/2019	78 - 87	64 - 48	60,3	10,2	1,15	8,9	0,0	T2 - 4
Delta	CC	27/06/2019	74 - 80	72 - 64	54,7	12,8	1,30	9,8	0,0	T1 - 4
Delta	CC	18/07/2019	78 - 84	64 - 56	64,6	11,3	0,85	13,3	0,0	T1 - 4
Delta	CC	08/08/2019	77 - 89	72 - 48	59,6	11,4	0,80	14,3	0,0	T1 - 4
Delta	SC	05/06/2019	80 - 86	64 - 48	56,0	10,2	1,20	8,5	0,0	T2 - 4
Delta	SC	27/06/2019	74 - 84	72 - 56	63,3	11,6	1,30	8,9	0,0	T1 - 4
Delta	SC	18/07/2019	74 - 82	72 - 56	58,5	10,5	0,95	11,1	0,0	T1 - 4
Delta	SC	08/08/2019	77 - 87	72 - 48	60,9	11,0	0,95	11,6	0,0	T1 - 4
Gusocora	CC	05/06/2019	77 - 90	72 - 40	55,3	11,3	1,15	9,8	0,0	T3 - 5
Gusocora	CC	27/06/2019	73 - 83	72 - 56	55,3	11,1	1,15	9,7	0,0	T1 - 4
Gusocora	CC	18/07/2019	73 - 83	72 - 56	60,4	11,8	1,15	10,3	0,0	T1 - 4

Gusocora	CC	08/08/2019	75 - 87	72 - 48	62,4	12,0	1,00	12,0	0,0	T1 - 3
Gusocora	CC	28/08/2019	79 - 84	64 - 56	61,3	11,8	1,00	11,8	0,0	T1 - 3
Jassie	C35	27/06/2019	70 - 80	88 - 64	48,6	10,2	1,20	8,5	3,2	T1 - 4
Jassie	C35	18/07/2019	72 - 81	88 - 64	61,1	11,0	0,95	11,6	1,7	T1 - 4
Jassie	C35	08/08/2019	75 - 82	72 - 56	58,6	11,3	1,05	10,8	4,5	T1 - 4
Jassie	C35	28/08/2019	79 - 85	64 - 56	53,3	12,1	0,90	13,4	3,7	T1 - 4
Jassie	CC	27/06/2019	74 - 84	72 - 56	56,7	9,8	1,15	8,5	2,6	T1 - 4
Jassie	CC	18/07/2019	79 - 83	64 - 56	61,4	11,5	1,05	11,0	1,3	T1 - 4
Jassie	CC	08/08/2019	78 - 87	64 - 48	60,1	11,0	1,00	11,0	3,2	T1 - 4
Jassie	CC	28/08/2019	76 - 85	72 - 56	62,7	12,4	0,90	13,8	3,2	T1 - 3
Jassie	SC	27/06/2019	75 - 80	72 - 64	56,2	14,1	1,20	20,1	0,9	T1 - 3
Jassie	SC	18/07/2019	72 - 84	88 - 56	57,2	11,1	1,00	11,1	4,4	T1 - 3
Jassie	SC	08/08/2019	76 - 85	72 - 56	62,7	11,0	1,05	10,5	3,5	T1 - 4
Jassie	SC	28/08/2019	77 - 81	72 - 64	66,7	12,0	0,80	15,0	3,2	T1 - 4
K du Toit Late	C35	27/06/2019	72 - 79	88 - 64	59,7	11,7	0,95	15,6	0,9	T1 - 3
K du Toit Late	C35	18/07/2019	73 - 84	72 - 56	58,6	12,9	0,85	15,1	0,7	T1 - 4
K du Toit Late	C35	08/08/2019	76 - 86	72 - 48	58,7	10,6	0,95	11,2	3,2	T1 - 4
K du Toit Late	C35	28/08/2019	69 - 84	88 - 56	61,9	11,1	0,95	11,7	2,8	T1 - 4
K du Toit Late	CC	27/06/2019	72 - 83	88 - 56	53,4	10,0	1,10	9,1	0,9	T1 - 4
K du Toit Late	CC	18/07/2019	72 - 85	88 - 56	58,5	10,5	0,85	12,4	3,1	T1 - 4
K du Toit Late	CC	08/08/2019	74 - 84	72 - 56	58,8	11,9	0,80	14,9	2,3	T1 - 4
K du Toit Late	CC	28/08/2019	71 - 82	88 - 56	53,7	11,2	0,75	14,9	2,5	T1 - 4
K du Toit Late	SC	27/06/2019	72 - 76	88 - 72	58,8	10,6	1,25	8,5	2,3	T2 - 4
K du Toit Late	SC	18/07/2019	73 - 80	72 - 64	60,0	11,0	0,85	12,9	2,3	T1 - 4
K du Toit Late	SC	08/08/2019	76 - 87	72 - 48	55,6	10,5	1,00	10,5	1,6	T1 - 5
K du Toit Late	SC	28/08/2019	73 - 87	72 - 48	53,7	10,6	0,80	13,3	3,2	T1 - 4
Lavalle	CC	18/07/2019	80 - 90	64 - 40	65,2	11,8	1,85	6,4	0,0	T1 - 5
Lavalle	CC	08/08/2019	76 - 87	72 - 48	62,5	12,6	1,60	7,9	0,0	T1 - 4
Lavalle	CC	28/08/2019	80 - 91	64 - 40	51,7	12,1	1,25	9,7	0,0	T1 - 4
McClean SL	C35	27/06/2019	74 - 81	72 - 64	58,6	10,4	1,10	9,5	0,0	T1 - 4
McClean SL	C35	18/07/2019	77 - 85	72 -56	59,5	11,4	1,00	11,4	0,0	T1 - 3
McClean SL	C35	08/08/2019	77 - 86	72 - 48	59,9	11,2	0,95	11,8	0,0	T1 - 4
McClean SL	CC	27/06/2019	74 - 84	72 - 56	59,6	9,9	1,50	6,6	0,0	T1 - 3
McClean SL	CC	18/07/2019	73 - 81	72 - 64	52,9	11,9	1,20	9,9	0,0	T1 - 3
McClean SL	CC	08/08/2019	77 - 86	72 - 48	58,7	11,8	1,10	10,7	0,0	T1 - 4
McClean SL	SC	27/06/2019	75 - 85	72 - 56	55,6	10,7	0,90	11,9	0,0	T1 - 4
McClean SL	SC	18/07/2019	74 - 85	72 - 56	66,7	11,9	0,80	14,9	0,0	T1 - 3
McClean SL	SC	08/08/2019	75 - 85	72 - 56	59,2	10,7	0,80	13,4	0,0	T1 - 4
McClean SL	SC	28/08/2019	76 - 89	72 - 48	55,2	11,1	0,80	13,9	0,0	T1 - 4
Millennium	CC	27/06/2019	76 - 82	72 - 56	56,3	11,7	1,05	11,1	0,0	T1 - 4
Millennium	CC	18/07/2019	76 - 85	72 - 56	62,9	11,3	0,80	14,1	0,0	T1 - 3
Millennium	CC	08/08/2019	74 - 85	72 - 56	60,8	12,5	0,75	16,7	0,0	T1 - 4
Millennium	CC	28/08/2019	78 - 90	64 - 40	58,0	12,3	0,70	17,6	0,0	T1 - 4
Moos Late 1	C35	27/06/2019	73 - 84	72 - 56	58,5	8,6	1,25	10,1	0,0	T1 - 3
Moos Late 1	C35	18/07/2019	75 - 84	72 - 56	62,0	11,2	1,20	8,6	3,7	T1 - 3
Moos Late 1	C35	08/08/2019	76 - 85	72 - 56	57,0	11,2	1,15	9,7	0,8	T1 - 4
Moos Late 1	C35	28/08/2019	81 - 90	64 - 40	55,8	11,5	1,20	9,6	1,1	T1 - 4
Moos Late 1	CC	27/06/2019	69 - 84	88 - 56	50,6	10,7	1,10	9,7	0,0	T1 - 3
Moos Late 1	CC	18/07/2019	74 - 88	72 - 48	60,0	11,0	1,35	8,1	0,0	T1 - 4
Moos Late 1	CC	08/08/2019	79 - 84	64 - 56	57,5	12,1	1,10	11,0	1,0	T1 - 4
Moos Late 1	CC	28/08/2019	79 - 89	64 - 48	52,7	10,7	1,05	10,2	1,2	T1 - 4
Moos Late 1	SC	27/06/2019	70 - 81	88 - 64	54,9	11,6	1,55	7,5	0,0	T1 - 4

Moos Late 1	SC	18/07/2019	63 - 67	125-105	64,1	12,2	1,40	8,7	1,4	T1 - 4
Moos Late 1	SC	08/08/2019	74 - 87	72 - 48	59,8	10,9	1,20	9,1	1,4	T1 - 4
Moos Late 1	SC	28/08/2019	74 - 84	72 - 56	56,0	11,4	1,25	9,1	1,7	T1 - 4
Skilderkrans	C35	27/06/2019	76 - 81	72 - 64	61,2	9,4	1,35	7,0	0,2	T1 - 4
Skilderkrans	C35	18/07/2019	75 - 84	72 - 56	59,9	11,4	1,15	9,9	0,0	T1 - 4
Skilderkrans	C35	08/08/2019	75 - 83	72 - 56	60,0	11,1	1,00	11,1	0,0	T1 - 4
Skilderkrans	C35	28/08/2019	77 - 86	72 - 48	56,8	9,7	1,00	9,7	0,0	T1 - 4
Skilderkrans	CC	27/06/2019	75 - 82	72 - 56	58,8	10,2	1,10	9,3	0,0	T1 - 3
Skilderkrans	CC	18/07/2019	75 - 88	72 - 48	61,4	11,4	1,05	10,9	0,0	T1 - 4
Skilderkrans	CC	08/08/2019	74 - 79	72 - 64	59,9	10,8	1,05	10,3	0,0	T1 - 4
Skilderkrans	CC	28/08/2019	78 - 88	64 - 48	56,4	11,1	0,95	11,7	0,0	T1 - 4
Skilderkrans	SC	27/06/2019	77 - 81	72 - 64	56,7	8,8	1,25	7,0	0,0	T1 - 4
Skilderkrans	SC	18/07/2019	73 - 85	72 - 56	57,6	11,2	1,25	9,0	0,0	T1 - 4
Skilderkrans	SC	08/08/2019	73 - 84	72 - 56	61,2	11,0	1,15	9,6	0,0	T1 - 4
Skilderkrans	SC	28/08/2019	74 - 88	72 - 48	59,6	11,4	1,00	11,4	0,1	T1 - 4
Turkey	C35	25/04/2019	79 - 85	64 - 56	58,3	9,5	1,25	7,6	2,5	T5 - 6
Turkey	C35	14/05/2019	74 - 86	72 - 48	56,2	10,7	1,00	10,7	2,4	T3 - 5
Turkey	C35	05/06/2019	77 - 82	72 - 56	58,5	11,2	0,95	11,8	2,3	T2 - 3
Turkey	C35	27/06/2019	77 - 81	72 - 64	53,4	11,5	0,95	12,1	1,5	T1 - 3
Turkey	C35	18/07/2019	77 - 84	72 - 56	57,6	11,2	0,65	17,2	3,2	T1 - 3
Turkey	CC	25/04/2019	75 - 85	72 - 56	53,4	9,8	1,40	7,0	1,7	T4 - 6
Turkey	CC	14/05/2019	75 - 89	72 - 48	50,3	11,1	1,00	11,1	2,8	T3 - 5
Turkey	CC	05/06/2019	81 - 90	64 - 40	56,9	11,6	0,90	12,9	1,9	T2 - 4
Turkey	CC	27/06/2019	75 - 84	72 - 56	54,2	11,0	0,85	12,9	2,7	T1 - 4
Turkey	CC	18/07/2019	77 - 88	72 - 48	60,0	11,7	0,70	16,7	5,3	T1 - 4
Turkey	SC	25/04/2019	80 - 90	64 - 40	53,6	10,0	1,15	8,7	3,0	T5 - 6
Turkey	SC	14/05/2019	74 - 89	72 - 48	56,7	11,3	1,05	10,8	3,3	T3 - 5
Turkey	SC	05/06/2019	76 - 87	72 - 48	56,1	11,2	0,95	11,8	2,7	T2 - 4
Turkey	SC	27/06/2019	80 - 84	64 - 56	55,1	7,8	0,80	9,8	1,5	T1 - 3
Turkey	SC	18/07/2019	75 - 85	72 - 56	62,5	12,0	1,00	12,0	1,3	T1 - 3
Valearly	C35	25/04/2019	75 - 88	72 - 48	51,0	10,9	9,00	1,2	3,0	T5 - 6
Valearly	C35	14/05/2019	70 - 85	88 - 56	55,6	10,8	0,80	13,5	0,4	T3 - 5
Valearly	C35	05/06/2019	77 - 85	72 - 56	56,5	11,7	0,90	13,0	0,3	T2 - 4
Valearly	C35	27/06/2019	75 - 85	72 - 56	59,2	10,6	0,85	7,9	4,3	T1 - 3
Valearly	C35	18/07/2019	73 - 84	72 - 56	52,6	12,3	0,60	20,5	0,0	T1 - 3
Valearly	CC	25/04/2019	79 - 90	64 - 48	51,3	9,9	0,85	11,6	2,0	T5 - 6
Valearly	CC	14/05/2019	77 - 90	72 - 40	55,0	10,1	0,70	14,4	0,0	T3 - 5
Valearly	CC	05/06/2019	75 - 80	72 - 64	51,5	10,6	0,75	14,1	0,8	T2 - 4
Valearly	CC	27/06/2019	75 - 83	72 - 56	60,3	10,8	0,70	15,4	0,0	T1 - 3
Valearly	CC	18/07/2019	73 - 84	72 - 56	55,6	10,7	0,50	21,4	0,7	T1 - 4
Valearly	SC	25/04/2019	73 - 90	72 - 40	47,7	7,8	1,70	4,6	0,0	T7 - 8
Valearly	SC	14/05/2019	75 - 81	72 - 64	50,7	9,2	1,30	7,1	1,8	T6 - 7
Valearly	SC	05/06/2019	77 - 84	72 - 56	54,3	10,2	1,30	7,8	3,0	T3 - 5
Valearly	SC	27/06/2019	74 - 81	72 - 64	53,6	10,0	1,25	8,0	0,0	T1 - 3
Valearly	SC	18/07/2019	72 - 82	88 - 56	62,8	11,5	1,20	9,6	1,0	T1 - 4

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 these 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 follow next with completely seedless fruit and very good Brix: acid ratios. Midknight 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 an 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 large to extra-large fruit size, pebbly rind texture, delayed colour development and good yield.

A series of experimental/semi-commercial selections have also been included in the hot production areas. The selection range follows from mid-, to late-maturing options. The mid-season starts with Skilderkrans. Jassie with optimum fruit size as well as good internal quality and Kobus Du Toit late mature more towards the end of the Valencia season with medium to large fruit size. Moosrivier Late 1 could 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. Midknight 1 en 2 vul die middel van die Valencia seisoen met goeie interne kwaliteit vrugte, groot vruggrootte, 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). Lavalle is huidiglik die laatste rypwordende Valencia seleksie wat kommersieel aangeplant word, met groot tot ekstra-groot vruggrootte, growwe tekstuur, vertraagde kleurontwikkeling en goeie produksie.

Daar is ʼn reeks experimentele/semi-kommersiele seleksies wat ook vir die warm produksie areas ingesluit is. Hier volg die seleksies van middel, tot laat rypwordende kultivars. Die mid-seisoen kan begin word met Skilderkrans. Jassie met optimum vruggrootte asook goeie interne kwaliteit en Kobus Du Toit Laat word meer aan die einde van die Valencia seisoen ryp met medium tot groot vruggroote. Laat in die seisoen kan aangevul word met 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, Lavalle 1, McClean, McClean SL, Midknight 1&2, Moosrivier Late 1, Skilderkrans, Turkey, Valearly and Val Late at Bosveld Citrus (Letsitele) and Groep 91 (Letsitele).

Table 5.4.3.1. List of Valencia selections evaluated at Bosveld Citrus (Letsitele) during the 2019 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
Lavalle 1	SC	2009
McClean SL	C35/CC/SC	2011
Midknight 1	SC	2009
Midknight 2	SC	2009
Moosrivier Late 1	C35/CC/SC	2011
Skilderkrans	C35/CC/SC	2011
Turkey	C35/CC/SC	2011
Valearly	C35/CC/SC	2011
Val Late	SC	2009

Table 5.4.3.2. List of Valencia selections evaluated at Groep 91 (Letsitele) during the 2019 season.

Selection	**Rootstock**	**Planted**
Bennie 1	CC/SC	2006
Bennie 2	CC/SC	2006
Kobus du Toit Late	CC/SC/Sunki 812/X639	2013
Jassie	CC/SC	2013
McClean	CC/SC/Sunki 812/X639	2013
McClean SL	CC/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

Results and discussion

Alpha

Fruit production on the Alpha trees was good this season in the Letsitele area, despite the terrible drought conditions with limited water supply. 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 2018, juice levels peaked at 62%, Brix was above 10 and acids were fairly high (between 1.3 and 1.5%). Fruit size decreased slightly and varied from count 88 to 56, still excellent for Valencia production and export. External colour was delayed on Swingle (typical characteristic on this rootstock) and peaked from T1 to T4. Maturity seems to be the end of June to the middle of July (Table 5.4.3.3).

Bennie 1 and 2

Bennie was evaluated at two trial sites this season: Bosveld and Groep 91. There was a good crop on the Benny trees at both sites despite the remaining drought conditions and severe high temperatures. Fruit size peaked between count 72 and 56 (Bosveld) and 88 up to 56 (Groep 91); fruit size was smaller at Groep 91 compared to Bosveld. 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 58.2%), Brix (average 11.1), acid (1.4%) and seed counts (average 2.3 seeds per fruit). External colour on both selections by the time of harvest varied between T1 and T4 (better colour on SC this season). Based on ratios, Benny 1 and 2 mature end of June to the beginning of July (Tables 5.4.3.3 & 5.4.3.4).

Delta (control)

Delta on all three rootstocks (C35, CC, SC), as control cultivar, 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 58%, Brix of 13.9 and acid content of 1.1%. The external colour of the fruit was between T1 and T4. Maturity is middle to the end of June (Table 5.4.3.3).

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 48 (lighter crop load on the trees). The external colour peaked between T1 and T4 (maturity) and the average seed count was 0.3 seeds per fruit (lower). Swingle developed a juice content of 61%, Brix of 11.2 and acids of 1.3%. Maturity is end of July to middle August (Table 5.4.3.3).

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, with 2.2 seeds average (lower compared to 2018). The colour development was very similar on all the rootstock combinations this season (T1 to T3/4). The internal quality was good, juice levels above 55%, Brix up to 14 (lower levels at Groep 91 – average 11) and good acids for the later maturing selection. Maturity seems to be middle to end of July to middle August according to Tables 5.4.3.3. & 5.4.3.4.

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 (60%), Brix (11.2) and acid (1.0) complied with export requirements. The external colour varied from T1 to T5, correlating with the internal quality and Brix:acid ratio of 12; although delayed. Fruit size was bigger and peaked between counts 88 and 56, optimal fruit size for export Valencia (medium to large). There was a good crop on the trees, bearing in mind that Swingle induces good yields and internal quality. It is apparent that Gusocora maturity is middle to end of July (Table 5.4.3.3).

Jassie

Fruit size at Bosveld on C35, Carrizo and Swingle was slightly smaller (peaked between count 88 and 56) compared to Groep 91, also on Carrizo and Swingle. Production was good on all the rootstock combinations. Internal quality was good with juice levels of 57%, Brix up to 15 (C35) at Bosveld (Groep 91 peaked at 12.4) and average acid levels of 1.5. Seed count dropped considerably this year and varied from 0.8 to 3.6 (avg. 1.6) 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 the middle of July to the beginning of August in this area (Tables 5.4.3.3. & 5.4.3.4).

Lavalle 1

The seed production this season decreased and Lavalle produced no seeds per fruit at Bosveld. The internal quality complied with export requirements and acid level was above 1.0% at the final evaluation in the middle of August. 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 was split fruit on some of the trees evaluated, but this varies from season to season (seen mainly in 2013). From the ratio on this date, it is apparent that Lavalle 1 maturity is the end of August to middle of September (Table 5.4.3.3).

McClean and McClean SL

Both selections were planted and evaluated this season on Carrizo, Swingle, X639 and Sunki 812 at Groep 91 to compare the performance of McClean SL with the old clone Mclean selection. McClean SL was planted on C35, Carrizo and Swingle at the Bosveld trial site with good crop production and remained completely seedless (Bosveld) or very low seeded (Groep 91); 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 third crop now; the Carrizo combination followed by Swingle were the best for 2019 (Sunki 812 was best 2018). Fruit size peaked from count 88 to 56 (excellent for Valencia production). External colour varied from T1-3/4 at Bosveld (optimum), but with delayed development at Groep 91 (T2-5 on SC). At both sites juice was 51% and above (as high as 63% on Carrizo), Brix improved to as high as 13.7 (over mature) and acids were above1.0% (peak maturity) towards the end of the season, resulting in good Brix:acid ratios (above 12:1). Maturity seems to be the end of June to the middle of July (Tables 5.4.3.3. & 5.4.3.4).

Midknight 1 & 2

Midknight 1 and 2 bore an average to good yield on the two rootstocks Carrizo and Swingle. The fruit size this year was very uniform and ranged between count 72 and 56/48, juice content was around 58% at Groep 91 and 63% at Bosveld, Brix levels lower around 10.5 (peak maturity) and acids around 1.1/1.2%. The two Midknight selections performed very similarly this season. Midknight 1 and 2 developed low seed numbers in the fruit, ranging from seedless up to 2.1 seeds per fruit. The characteristic Midknight die-back was more visible on Midknight 1 compared to Midknight 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 the middle of July to the end of July (Table 5.4.3.3. & 5.4.3.4).

Moosrivier Late 1

Moos Late 1 developed a better fruit size distribution this season in general (medium to large size count 88-56/48) on Carrizo, C35 and Swingle. 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 (from 1.45 up to 1.75% towards peak maturity) indicating a late to ultra-late maturing Valencia selection. The seed count per fruit varied from 1.2 to 2.6 (lower compared to 2018). When internal quality was taken into consideration, estimated maturity is end of July to middle/end of August. (Tables 5.4.3.3. & 5.4.3.4).

Skilderkrans

Skilderkrans at Group 91 is the back-up site (was evaluated) and the trial block at Bosveld bore fruit on C35, Carrizo and Swingle. Fruit size was smaller this season and more favourable for good quality fruit; varied from medium to large (count 88-56/48). Internally the Brix content was good (up to 14.6 at Bosveld) and high acid level (Groep 91) of 1.1 to 1.7% (peak maturity) indicated a later maturing Valencia selection. Juice level increased to an average 58% later in the season; above the minimum required export figure. There was a delay in external colour at Groep 91 (CC: T2-4 and SC: T 3-5); Bosveld was better on all three of the rootstocks evaluated (between T1 and 3/4). The fruit developed a smooth rind, fibre strength was fairly soft and the fruit shape was round. Ratios were lower at Groep 91 this season due to the higher acid levels on both Carrizo and Swingle, delaying peak maturity to end of July and mid-August. Bosveld indicated a peak maturity by the end of July (Tables 5.4.3.3. & 5.4.3.4).

Turkey

Turkey was planted on three rootstocks at both trial sites: 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 with highest levels at Bosveld (average 12), lower acid levels at Bosveld compared to Groep 91 and higher Brix:acid ratio. The average seed count per fruit decreased to 1.6 and peaked at 1.4 seeds. The external colour (between T1 and T3/4) in the middle of June was similar to all three rootstocks and both trial sites. 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 (Tables 5.4.3.3. & 5.4.3.4).

Valearly

Valearly, bearing an average to good crop, remained low-seeded this season (0.3 seeds per fruit). The internal quality of the fruit was fair to good with juice levels above 50% at Groep 91 (below 50% at Bosveld) early in the season, higher Brix on Swingle (avg.11.9) compared to Carrizo (11.4) at Groep 91 and acid of 0.9. Compared with the other early maturing selections (Turkey and Weipe), Valearly seems to be at least two weeks earlier, similar to Weipe, with 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 (100 kg per tree; 555 trees/ha – 56 t/ha) and fruit size peaked from medium to large (count 88 to 64/56), optimal Valencia export quality. Acid levels were above 1.5% (average) when the third evaluation was completed, indicating the late maturity qualities of the selection. The juice content was similar to 2018 this season at 57% and Brix 11.6 with the last evaluation. Seed count went up from 1.2 seeds per fruit to 1.5. 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 higher – 62%) and fruit size peaked smaller between counts 88 to 56 (dropped one count size on either side).

Bennie 1 and 2 produced similar fruit quality this season, as well as yield production and medium to large fruit size (peaked from count 88 to 56 between the two trial sites). Delta was the control cultivar for the trial; fruit size peaked between count 72 and 48 (similar to 2018 due to the lighter crop) with good internal quality, reaching juice levels of 59%, Brix of 13.9 and acid content of 1.1%.

Du Roi was evaluated on Swingle this season with a bigger fruit size ranging from count 88 to 48 (up by one count size compared to 2018 season). Kobus du Toit Late performed well with good fruit size and promising juice and Brix levels. Gusocora performed well on Swingle (delayed colour development), meeting the export standards (acid levels improved).

Jassie produced an excellent internal quality (high Brix and acid) on Carrizo and Swingle, with medium to large fruit size (count 88-56) due to the crop load on the trees. Lavalle 1 was ultra-late, maturing middle of August (acids at 0.95%) on Swingle rootstock; colour peaked from T 2 to 5.

McClean SL remained completely seedless at Bosveld and very low seeded at Groep 91 with good internal quality and optimum fruit size (count 88-56).

Fruit quality on Midknight 1 was better, with higher Brix than Midknight 2 (Bosveld trial site). The external colour was similar on both Midknight selections this season (slight delay on Midknight 2); range between T1 and T4/5.

Turkey performed best in combination with Carrizo when Brix:acid ratio and yield production were considered. Valearly produced a better crop on the trees, fairly low juice levels and a delayed colour development compared to Turkey this season. Future evaluations will determine the value of this cultivar for the citrus industry.

Table 5.4.3.3. Internal fruit quality data for Valencia orange selections at Bosveld Citrus (Letsitele) during the 2019 season.

Cultivar	Root stock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix º	Acid (%)	Ratio	Avg seed	Fruit external colour
Alpha	SC	16/06/2019	73 - 80	88 - 64	51,9	8,9	1,45	6,1	0,0	T3 - 5
Alpha	SC	12/07/2019	74 - 83	72 - 56	59,6	10,0	1,25	8,0	0,0	T2 - 5
Alpha	SC	01/08/2019	72 - 80	88 - 64	62,4	10,8	1,35	8,0	0,1	T1 - 4
Bennie	SC	19/06/2019	76 - 82	72 - 56	58,0	10,1	1,30	7,8	1,2	T2 - 4
Bennie	SC	12/07/2019	74 - 85	72 - 56	60,3	9,6	1,00	9,6	0,0	T2 - 4
Bennie	SC	01/08/2019	74 - 92	72 - 40	58,7	10,9	1,10	9,9	0,0	T1 - 4
Bennie	SC	21/08/2019	81 - 87	64 - 48	60,5	10,3	1,00	10,3	1,3	T1 - 4
Delta	C35	19/06/2019	72 - 85	88 - 56	54,8	11,8	1,10	10,7	0,0	T1 - 4
Delta	C35	11/07/2019	73 - 83	72 - 56	55,6	12,6	1,05	12,0	0,0	T1 - 4
Delta	C35	31/07/2019	76 - 83	72 - 56	56,9	13,1	0,95	13,8	0,0	T1 - 5
Delta	CC	19/06/2019	73 - 80	72 - 64	54,6	12,4	0,85	14,6	0,0	T2 - 4
Delta	CC	11/07/2019	70 - 82	88 - 56	54,8	13,9	0,85	16,4	0,0	T1 - 4
Delta	CC	31/07/2019	74 - 80	72 - 64	58,2	13,5	1,00	13,5	0,0	T1 - 4
Delta	SC	19/06/2019	73 - 83	72 - 56	54,6	11,1	1,65	6,7	0,0	T2 - 4

Delta	SC	11/07/2019	64 - 72	105 - 88	57,5	13,1	1,15	11,4	0,0	T1 - 4
Delta	SC	31/07/2019	74 - 84	72 - 56	57,2	13,4	1,20	11,2	0,0	T1 - 4
Du Roi	SC	12/07/2019	72 - 85	88 - 56	62,9	11,1	1,45	7,7	0,0	T2 - 5
Du Roi	SC	01/08/2019	78 - 87	64 - 48	60,8	11,2	1,35	8,3	0,9	T2 - 5
Du Roi	SC	21/08/2019	74 - 80	72 - 64	60,2	11,2	1,20	9,3	0,0	T1 - 4
K du Toit Late	C35	31/07/2019	77 - 80	72 - 64	63,2	12,9	1,25	10,3	2,2	T1 - 5
K du Toit Late	C35	21/08/2019	75 - 80	72 - 64	59,4	13,3	1,10	12,1	3,1	T1 - 4
K du Toit Late	CC	11/07/2019	67 - 76	105 - 72	57,5	13,4	1,45	9,2	1,3	T2 - 5
K du Toit Late	CC	31/07/2019	72 - 80	88 - 64	61,9	13,9	1,30	10,7	1,2	T1 - 4
K du Toit Late	CC	21/08/2019	74 - 80	72 - 64	60,5	13,3	1,15	11,6	3,1	T1 - 4
K du Toit Late	SC	11/07/2019	72 - 78	88 - 64	64,6	12,8	1,40	9,1	0,8	T1 - 4
K du Toit Late	SC	31/07/2019	76 - 84	72 - 56	60,0	12,4	1,15	10,8	0,0	T1 - 4
K du Toit Late	SC	21/08/2019	77 - 83	72 - 56	61,5	13,4	1,30	10,3	1,9	T1 - 3
Gusocora	SC	19/06/2019	74 - 79	72 - 64	56,1	10,6	1,00	10,6	0,0	T3 - 5
Gusocora	SC	12/07/2019	72 - 81	88 - 64	62,8	10,8	1,10	9,8	0,0	T1 - 4
Gusocora	SC	01/08/2019	71 - 82	88 - 56	61,1	12,3	0,85	14,5	0,0	T1 - 5
Jassie	C35	11/07/2019	69 - 81	88 - 64	58,0	14,5	1,90	7,6	1,3	T1 - 4
Jassie	C35	31/07/2019	74 - 81	72 - 64	50,9	15,0	1,90	7,9	1,8	T2 - 4
Jassie	C35	21/08/2019	72 - 79	88 - 64	60,7	15,6	1,40	11,1	3,6	T1 - 4
Jassie	CC	11/07/2019	69 - 82	88 - 56	57,6	13,5	1,50	9,0	1,2	T1 - 4
Jassie	CC	31/07/2019	76 - 81	72 - 64	56,8	11,0	1,40	7,9	1,3	T1 - 3
Jassie	CC	21/08/2019	75 - 80	72 - 64	58,2	14,2	1,10	12,9	2,0	T1 - 4
Jassie	SC	11 /07/2019	69 - 80	88 - 64	52,3	12,9	1,35	9,6	2,5	T1 - 5
Jassie	SC	31/07/2019	78 - 85	64 - 56	57,6	13,0	1,25	10,4	0,8	T1 - 3
Jassie	SC	21/08/2019	79 - 85	64 - 56	59,4	13,7	1,35	10,1	3,1	T1 - 4
Lavalle	SC	21/08/2019	78 - 87	64 - 48	61,8	10,8	0,95	11,4	0,0	T2 - 5
McClean SL	C35	19/06/2019	74 - 85	72 - 56	54,5	12,1	1,30	9,3	0,0	T1 - 4
McClean SL	C35	11/07/2019	72 - 83	88 - 56	54,3	13,5	1,35	10,0	0,0	T1 - 5
McClean SL	C35	31/07/2019	74 - 89	72 - 48	52,9	12,9	1,25	10,3	0,0	T1 - 4
McClean SL	CC	19/06/2019	70 - 80	88 - 64	60,4	12,7	1,40	9,1	0,0	T1 - 3
McClean SL	CC	11/07/2019	68 - 80	88 - 64	63,6	13,5	1,55	8,7	0,0	T1 - 4
McClean SL	CC	31/07/2019	71 - 80	88 - 64	61,3	13,3	1,10	12,1	0,0	T1 - 4
McClean SL	SC	11/07/2019	65 - 80	105 - 64	51,4	13,5	0,95	14,2	0,0	T2 - 5
McClean SL	SC	31/07/2019	78 - 85	64 - 56	55,1	13,7	0,90	15,2	0,0	T1 - 3
Midknight 1	SC	19/06/2019	75 - 79	72 - 64	62,1	10,2	1,05	9,7	1,0	T1 - 4
Midknight 1	SC	12/07/2019	73 - 83	72 - 56	63,3	10,8	1,10	9,8	0,0	T1 - 4
Midknight 1	SC	01/08/2019	76 - 83	72 - 56	71,0	11,9	0,90	13,2	0,0	T1 - 4
Midknight 2	SC	19/06/2019	74 - 82	72 - 56	62,2	10,5	1,20	8,8	0,0	T1 - 5
Midknight 2	SC	12/07/2019	75 - 82	72 - 56	58,0	10,3	1,15	9,0	0,0	T1 - 5
Midknight 2	SC	01/08/2019	75 - 87	72 - 48	60,5	10,8	1,05	10,3	0,0	T1 - 4
Moos Late 1	C35	11/07/2019	72 - 78	88 - 64	56,8	14,6	1,85	7,9	1,8	T1 - 4
Moos Late 1	C35	21/08/2019	73 - 84	72 - 56	57,6	13,9	1,55	9,0	2,3	T1 - 4
Moos Late 1	CC	11/07/2019	73 - 82	72 - 56	55,2	13,8	1,60	8,6	1,5	T1 - 4
Moos Late 1	CC	21/08/2019	74 - 80	72 - 64	56,6	14,1	1,45	9,7	2,4	T1 - 4
Moos Late 1	SC	11/07/2019	73 - 82	72 - 56	58,6	12,5	1,75	7,1	1.4	T2 - 5
Moos Late 1	SC	21/08/2019	76 - 85	72 - 56	55,4	13,1	1,50	8,7	1,6	T1 - 3
Skilderkrans	C35	19/06/2019	73 - 84	72 - 56	59,2	12,1	1,10	11,0	0,0	T1 - 4
Skilderkrans	C35	11/07/2019	70 - 80	88 - 64	58,5	12,7	1,65	7,7	0,0	T1 - 3
Skilderkrans	C35	31/07/2019	75 - 86	72 - 48	61,3	11,4	1,45	7,9	1,0	T1 - 4
Skilderkrans	C35	21/08/2019	76 - 86	72 - 48	57,3	13,9	1,25	11,1	1,5	T1 - 4
Skilderkrans	CC	19/06/2019	74 - 82	72 - 56	55,7	13,0	1,65	7,9	0,0	T1 - 4

Skilderkrans	CC	31/07/2019	79 - 85	64 - 56	58,5	13,3	1,05	12,7	0,0	T1 - 3
Skilderkrans	CC	21/08/2019	73 - 85	72 - 56	59,0	14,6	1,25	11,7	0,0	T1 - 4
Skilderkrans	SC	19/06/2019	75 - 84	72 - 56	56,3	12,3	1,65	7,5	0,0	T1 - 4
Skilderkrans	SC	11/07/2019	69 - 80	88 - 64	62,1	12,1	1,30	9,3	1,0	T1 - 5
Skilderkrans	SC	31/07/2019	79 - 91	64 - 40	59,9	13,1	1,40	9,4	0,0	T1 - 4
Skilderkrans	SC	21/08/2019	79 - 88	64 - 48	59,9	13,3	1,05	12,7	0,0	T1 - 4
Turkey	C35	17/04/2019	79 - 88	64 - 48	49,7	10,8	1,05	10,3	0,0	T7 - 8
Turkey	C35	10/05/2019	75 - 80	72 - 64	55,0	11,9	0,95	12,5	2,3	T5 - 7
Turkey	C35	29/05/2019	75 - 88	72 - 56	54,2	12,5	0,95	13,2	1,2	T3 - 5
Turkey	C35	19/06/2019	74 - 82	72 - 56	57,9	13,1	0,95	13,8	0,0	T2 -4
Turkey	CC	17/04/2019	77 - 87	72 - 48	47,2	11,7	1,25	9,4	0,3	T7 - 8
Turkey	CC	10/05/2019	75 - 82	72 - 56	51,9	12,6	1,00	12,6	3,5	T5 - 7
Turkey	CC	29/05/2019	71 - 85	88 - 56	54,7	12,6	1,00	12,6	0,0	T2 - 5
Turkey	CC	19/06/2019	71 - 77	88 - 72	58,0	13,7	0,90	15,2	2,3	T1 - 4
Turkey	SC	17/04/2019	73 - 90	73 - 40	45,8	10,9	1,15	9,5	0,7	T7 - 8
Turkey	SC	10/05/2019	75 - 80	72 - 64	52,6	12,3	1,15	10,7	4,5	T5 - 7
Turkey	SC	29/05/2019	72 - 84	88 - 56	54,1	12,6	1,10	11,5	2,4	T2 - 5
Turkey	SC	19/06/2019	72 - 76	88 - 72	58,3	12,9	0,85	15,2	0,0	T1 - 4
Turkey	SC	11/07/2019	71 - 80	88 - 64	57,1	13,3	0,80	16,6	0,0	T1 - 4
Valearly	C35	17/04/2019	79 - 93	64 - 40	39,7	11,1	0,90	12,3	0,0	T7 - 8
Valearly	C35	10/05/2019	78 - 88	64 - 48	48,8	11,9	0,75	15,9	0,0	T5 - 7
Valearly	C35	29/05/2019	74 - 85	72 - 56	48,4	13,4	0,90	14,9	0,0	T3 - 5
Valearly	C35	19/06/2019	77 - 80	72 - 64	49,1	13,7	0,90	15,2	0,0	T1 - 4
Valearly	C35	11/07/2019	70 - 80	88 - 64	52,1	14,2	0,85	16,7	0,0	T1 - 4
Valearly	CC	17/04/2019	74 - 80	72 - 64	49,6	10,5	0,90	11,7	1,0	T7 - 8
Valearly	CC	10/05/2019	76 - 85	72 - 56	52,6	11,5	0,75	15,3	0,0	T5 - 7
Valearly	CC	29/05/2019	75 - 84	72 - 56	51,7	12,0	0,70	17,1	0,6	T1 - 4
Valearly	CC	19/06/2019	70 - 80	88 - 64	44,6	11,1	0,85	13,1	1,0	T1 - 3
Valearly	CC	11/07/2019	69 - 77	88 - 72	44,1	12,7	0,60	21,2	0,0	T1 - 3
Val late	SC	12/07/2019	73 - 78	72 - 64	52,8	11,0	1,30	8,5	1,5	T3 - 5
Val late	SC	01/08/2019	75 - 82	72 - 56	58,6	11,3	1,05	10,8	1,3	T1 - 5
Val late	SC	21/08/2019	74 - 83	72 - 56	58,6	11,6	1,05	11,0	1,6	T1 - 4

Table 5.4.3.4. Internal fruit quality data for Valencia orange selections at Groep 91 (Letsitele) during the 2019 season.

Cultivar	Rootstock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix º	Acid (%)	Ratio	Avg seed	Fruit external colour
Bennie 1	CC	29/05/2019	70 - 80	88 - 64	60,0	10,2	1,80	5,7	5,0	T4 - 7
Bennie 1	CC	19/06/2019	70 - 81	88 - 64	53,3	11,5	1,90	6,1	2,4	T1 - 5
Bennie 1	CC	11/07/2019	66 - 75	105 - 72	25,7	12,7	1,80	7,1	0,0	T1 - 4
Bennie 1	CC	01/08/2019	75 - 86	72 - 48	61,3	12,5	1,50	8,3	1,5	T1 - 4
Bennie 1	SC	29/05/2019	77 - 80	72 - 64	56,8	11,3	1,80	6,3	3,0	T3 - 5
Bennie 1	SC	19/06/2019	75 - 80	72 - 64	59,6	11,7	1,35	8,7	1,7	T1 - 4
Bennie 1	SC	11/07/2019	74 - 83	72 - 56	58,1	12,9	1,90	6,8	3,5	T1 - 5
Bennie 1	SC	01/08/2019	78 - 85	64 - 56	64,6	12,7	1,85	6,9	1,5	T1 - 3
Bennie 1	SC	21/08/2019	74 - 85	72 - 56	56,8	13,6	1,55	8,8	1,5	T1 - 4
Bennie 2	CC	29/05/2019	72 - 85	88 - 56	56,5	10,9	1,90	5,7	3,0	T4 - 6
Bennie 2	CC	19/06/2019	73 - 85	72 - 56	55,3	11,1	1,65	6,7	0,0	T3 - 5
Bennie 2	CC	11/07/2019	71 - 84	88 - 56	61,5	11,9	1,75	6,8	2,2	T1 - 4
Bennie 2	CC	01/08/2019	71 - 84	88 - 56	60,3	12,2	1,35	9,0	1,5	T1 - 4
Bennie 2	SC	29/05/2019	74 - 90	72 - 40	59,0	11,4	1,65	6,9	3,3	T4 - 6

Bennie 2	SC	19/06/2019	74 - 83	72 - 56	59,1	10,9	1,15	9,5	1,6	T2 - 5
Bennie 2	SC	11/07/2019	75 - 82	72 - 56	55,2	11,8	1,35	8,7	0,5	T1 - 4
Bennie 2	SC	01/08/2019	75 - 83	72 - 56	59,2	12,5	1,40	8,9	1,0	T1 - 4
Bennie 2	SC	21/08/2019	79 - 86	64 - 48	61,1	12,3	1,20	10,3	3,1	T1 - 4
K du Toit Late	CC	11/07/2019	71 - 76	88 - 72	59,7	11,0	1,30	8,5	1,9	T1 - 4
K du Toit Late	CC	01/08/2019	71 - 76	88 - 72	63,8	11,2	1,15	9,7	1,3	T2 - 5
K du Toit Late	CC	21/08/2019	74 - 80	72 - 64	54,5	10,4	0,95	10,9	2,8	T1 - 4
K du Toit Late	SC	11/07/2019	74 - 79	72 - 64	64,0	11,1	1,30	8,5	4,7	T1 - 5
K du Toit Late	SC	01/08/2019	75 - 87	72 - 48	56,8	11,3	1,00	11,3	3,3	T2 - 5
K du Toit Late	SC	21/08/2019	77 - 84	72 - 56	56,2	11,3	0,95	11,9	3,8	T2 - 5
K du Toit Late	X639	11/07/2019	67 - 80	105 - 64	59,7	11,5	1,20	9,6	3,5	T1 - 5
K du Toit Late	X639	01/08/2019	73 - 82	72 - 56	61,2	11,2	1,15	9,7	1,3	T2 - 5
K du Toit Late	X639	21/08/2019	74 - 83	72 - 56	56,6	11,3	0,95	11,9	1,2	T1 - 4
K du Toit Late	US812	11/07/2019	65 - 75	105 - 72	59,7	11,9	1,20	9,9	1,7	T1 - 4
K du Toit Late	US812	01/08/2019	72 - 75	88 - 72	57,5	12,2	1,25	9,8	3,6	T2 - 5
Jassie	CC	11/07/2019	73 - 83	72 - 56	59,3	11,6	1,35	8,6	1,0	T2 - 5
Jassie	CC	01/08/2019	77 - 87	72 - 48	61,6	12,7	1,05	12,1	1,5	T1 - 5
Jassie	CC	21/08/2019	75 -82	72 - 56	55,8	12,4	1,15	10,8	0,0	T1 - 4
Jassie	SC	11/07/2019	69 - 80	88 - 64	49,6	13,2	1,50	8,8	0,0	T2 - 5
Jassie	SC	01/08/2019	75 - 82	72 - 56	54,3	12,5	1,80	6,9	2,2	T2 - 4
Jassie	SC	21/08/2019	75 - 82	72 - 56	57,2	11,9	1,30	9,2	2,5	T3 - 5
McClean	CC	19 /06/2019	75 -82	72 - 56	55,6	11,1	1,35	8,2	1,9	T3 - 5
McClean	CC	11/07/2019	72 - 78	88 - 64	65,1	11,9	1,45	8,2	0,0	T1 - 4
McClean	CC	01/08/2019	75 - 84	72 - 56	56,8	11,9	1,30	9,2	1,3	T1 - 4
McClean	SC	19 /06/2019	71 - 83	88 - 56	54,4	10,8	1,25	8,6	3,7	T2 - 5
McClean	SC	11/07/2019	71 - 80	88 - 64	63,4	11,6	1,20	9,7	0,8	T2 - 5
McClean	SC	01/08/2019	74 - 85	72 - 56	56,3	11,6	1,30	8,9	2,1	T2 - 5
McClean	X639	19/08/2019	72 - 83	88 - 56	59,3	10,1	1,50	6,7	0,0	T3 - 5
McClean	X639	11/07/2019	72 - 80	88 - 64	58,7	11,1	1,50	7,4	1,2	T1 - 5
McClean	X639	01/08/2019	74 - 86	72 - 48	58,2	11,6	1,05	11,0	1,3	T1 - 4
McClean	US812	19/06/2019	70 - 79	88 - 64	58,5	11,2	1,55	7,2	1,7	T3 - 5
McClean	US812	11/07/2019	70 - 81	88 - 64	56,9	11,6	1,45	8,0	0,0	T2 - 5
McClean	US812	01/07/2019	74 - 81	72 - 64	56,3	11,8	1,25	9,4	1,4	T2 - 5
McClean SL	CC	19/06/2019	74 - 85	72 - 56	60,9	9,9	1,20	8,3	0,0	T3 - 5
McClean SL	CC	11.07/2019	70 - 76	88 - 72	62,1	10,7	1,10	9,7	0,0	T1 - 4
McClean SL	CC	01/08/2019	79 - 87	64 - 48	60,1	11,7	1,00	11,7	0,0	T1 - 5
McClean SL	SC	19/06/2019	77 - 85	72 - 56	54,6	10,9	1,25	8,7	0,0	T1 - 4
McClean SL	SC	11/07/2019	72 - 82	88 - 56	59,0	10,7	1,20	8,9	0,0	T1 - 5
McClean SL	SC	01/08/2019	82 - 85	56	58,0	11,2	1,10	10,2	0,5	T2 - 5
McClean SL	X639	19/06/2019	75 - 83	72 - 56	55,3	9,9	1,10	9,0	0,0	T2 - 4
McClean SL	X639	11/07/2019	71 - 83	88 - 56	63,3	12,0	1,15	10,4	0,0	T1 - 4
McClean SL	X639	01/08/2019	77 - 89	72 - 48	58,5	11,7	0,90	13,0	0,0	T1 - 4
McClean SL	US812	19/06/2019	75 - 84	72 - 56	56,1	11,5	1,50	7,7	1,2	T3 - 5
McClean SL	US812	11/07/2019	72 - 83	88 - 56	54,1	11,2	1,35	8,3	1,0	T1 - 5
McClean SL	US812	01/08/2019	80 - 88	64 - 48	58,9	11,6	1,40	8,3	0,0	T1 - 5
Midknight 1	CC	19/06/2019	75 - 86	72 - 48	50,9	11,0	1,15	9,6	0,0	T1 - 4
Midknight 1	CC	11/07/2019	74 - 85	72 - 56	53,8	11,2	1,05	10,7	2,1	T1 - 4
Midknight 1	CC	01/08/2019	76 - 88	72 - 48	62,0	11,7	1,05	11,1	0,0	T1 - 4
Midknight 1	SC	19/06/2019	74 - 86	72 - 48	57,8	10,9	1,35	8,1	0,0	T2 - 4
Midknight 1	SC	11/07/2019	75 - 84	72 - 56	60,8	11,0	1,05	10,5	0,0	T1 - 4
Midknight 1	SC	01/08/2019	77 - 90	72 - 48	60,5	11,5	1,30	8,8	0,0	T1 - 4
Moos Late 1	CC	01/08/2019	68 - 77	88 - 72	61,6	12,8	2,00	6,4	1,1	T2 - 5
Moos Late 1	CC	21/08/2019	71 - 80	88 - 64	61,1	12,5	1,45	8,6	2,6	T2 - 4

Moos Late 1	SC	01/08/2019	74 - 85	72 - 56	58,2	11,9	2,20	5,4	2,3	T2 - 5
Moos Late 1	SC	21/08/2019	72 - 86	88 - 48	59,7	12,9	1,75	7,4	1,9	T2 - 4
Skilderkrans	CC	19/06/2019	72 - 82	88 - 56	57,1	10,8	1,60	6,8	0,0	T3 - 6
Skilderkrans	CC	11/07/2019	74 - 84	72 - 56	60,6	11,8	1,85	6,4	0,0	T2 - 5
Skilderkrans	CC	01/08/2019	79 - 85	64 - 56	58,9	11,1	1,35	8,2	0,0	T1 - 5
Skilderkrans	CC	21/08/2019	77 - 83	72 - 56	57,6	12,4	1,35	9,2	0,0	T2 - 4
Skilderkrans	SC	19/06/2019	72 - 79	88 - 64	51,9	10,8	2,40	4,5	0,0	T3 - 5
Skilderkrans	SC	11/07/2019	70 - 80	88 - 64	64,8	12,0	2,35	5,1	2,3	T2 - 5
Skilderkrans	SC	21/08/2019	74 - 83	72 - 56	50,7	13,4	1,65	8,1	2,5	T3 - 5
Turkey	C35	03/05/2019	67 - 73	105 - 72	57,9	11,1	1,10	10,1	1,2	T5 - 7
Turkey	C35	19/06/2019	75 - 84	72 - 56	56,8	11,2	1,00	11,2	0,8	T1 - 4
Turkey	C35	11/07/2019	72 - 84	88 - 56	59,8	11,7	0,95	12,3	3,2	T1 - 4
Turkey	CC	17//04/2019	69 - 82	88 - 56	51,2	9,8	1,35	7,3	1,7	T7 - 8
Turkey	CC	03/05/2019	69 - 79	88 - 64	55,7	9,9	1,60	6,2	4,5	T5 - 7
Turkey	CC	29/05/2019	70 - 84	88 - 56	59,9	11,2	1,50	7,5	2,6	T3 - 5
Turkey	CC	19/06/2019	74 - 79	72 - 64	15,8	13,1	1,10	11,9	2,2	T1 - 5
Turkey	CC	11/07/2019	71 - 79	88 - 64	59,4	12,1	1,05	11,5	0,0	T1 - 4
Turkey	SC	03/05/2019	76 - 80	72 - 64	59,1	10,8	1,35	8,0	1,2	T5 - 7
Turkey	SC	17/04/2019	74 - 94	72 - 40	53,6	9,9	1,25	7,9	2,0	T7 - 8
Turkey	SC	29/05/2019	72 - 85	88 - 56	56,8	11,6	1,05	11,0	3,0	T3 - 5
Turkey	SC	19/06/2019	74 - 79	72 - 64	60,3	11,8	1,00	11,8	0,0	T2 - 4
Turkey	SC	11/07/2019	72 - 84	72 - 56	58,4	11,3	0,95	11,9	1,7	T1 - 3
Val Early	CC	17/04/2019	69 - 82	88 - 56	50,4	9,3	1,00	9,3	0,0	T7 - 8
Val Early	CC	03/05/2019	70 - 80	88 - 64	55,9	11,4	0,95	12,0	0,0	T5 - 7
Val Early	CC	29/05/2019	74 - 85	72 - 56	52,1	10,8	0,80	13,5	0,0	T2 - 5
Val Early	CC	19/06/2019	74 - 83	72 - 56	54,6	11,1	0,80	13,9	0,0	T1 - 4
Val Early	SC	17/04/2019	74 - 84	72 - 56	50,8	9,8	0,95	10,3	1,0	T7 - 8
Val Early	SC	03/05/2019	74 - 80	72 - 64	56,5	10,1	0,90	11,2	0,0	T5 - 7
Val Early	SC	29/05/2019	72 - 86	88 - 48	48,9	11,9	0,85	14,0	0,0	T3 - 5
Val Early	X639	17/04/2019	84 - 87	56 - 48	50,3	9,9	0,85	11,6	0,0	T4 - 8
Val Early	X639	03/05/2019	75 - 83	72 - 56	59,9	9,9	0,85	11,6	0,8	T5 - 7
Val Early	US812	17/04/2019	71 -94	88 - 40	52,4	9,8	0,95	10,3	0,0	T7 - 8
Val Early	US812	03/05/2019	76 - 82	72 - 56	51,1	10,0	0,95	10,5	1,0	T5 - 8

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

RHM, Etna, Sirio and Tango mature 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 with high juice levels, fit in before Furr that followed with the highest seed count per fruit for this trial. Next will be African Sunset and Orah, developing the highest seed count per fruit. The mid-maturing mandarins are represented by Valley Gold and Mor 26, which developed high Brix levels compared to the other selections (up to Brix of 14). 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 and good external colour (T1). Tahoe Gold followed, with an alternate bearing pattern on the trees, an on-year 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 (1.7% acid) were 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 avoid rind disorders.

Opsomming

RHM, 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, met hoë sap vlakke, pas in voor Furr, wat daarna volg met die hoogste saadtelling per vrug vir hierdie proef. Volgende is African Sunset en Orah, met die hoogste saad telling per vrug. Die middel van die mandaryne word verteenwoordig deur Valley Gold en Mor 26 met die hoë Brix vlakke in vergelyking met die ander seleksies (tot Brix van 14). 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, asook goeie eksterne kleur ontwikkeling (T1). Tahoe Gold volg, met 'n altenerende drag patroon op die bome, aan seisoen hierdie jaar. 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 (1.7% 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) from the Limpopo region, and Riverside (Malelane).

The following cultivars were evaluated:

Table 5.4.4.1. List of Mandarin Hybrid selections evaluated at Bosveld Citrus (Letsitele) during the 2019 season.

Selection	Rootstock	Topwork
Saint Andre	CC	2015
Samba	CC	2015
Sugar Belle	CC	2015
Tanor Late 2	CC	2015

Table 5.4.4.2. List of Mandarin Hybrid selections evaluated at Mahela (Letsitele) during the 2019 season.

Selection	Rootstock	Planted
Etna	CC	2014
Gold Nugget	CC	2013
Saint André	CC	2014
Samba	CC	2014
Sirio	CC	2014
Tahoe Gold	CC	2013
Tango	CC	2013
Tasty 1	CC	2014
Yosemite Gold	CC	2013

Table 5.4.4.3. List of Mandarin Hybrid selections evaluated at Moriah (Hoedspruit) during the 2019 season.

Selection	Rootstock	Topwork
ARCCIT 9 LS	C35	2015
Furr (Clemcott)	MxT	2011
IRM 2	C35	2015
Leanri	C35	2015
Mor 26	MxT	2011
Nova SL ARC	C35	2014
Orah	MxT	2011
Orri	C35	2015
RHM	C35	2015
Saint Andre	C35	2014
Samba	C35	2014
Tambor	MxT	2011
Winola	MxT	2011

Table 5.4.4.4. List of Mandarin Hybrid selections evaluated at Overbrug (Hoedspruit) during the 2019 season.

Selection	Rootstock	Planted
African Sunset (B24)	C35	2011
ARCCIT 9 LS	CC	2011
Dina (Edit x Nona)	CC	2011
IRM 2	CC	2011
Nadorcott ARC	CC	2011
Valley Gold	CC	2011

Table 5.4.4.5. List of Mandarin Hybrid selections evaluated at Riverside (Malelane) during the 2019 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 higher instance of quality and rind issues.

<u>African Sunset (B24)</u>
There was enough fruit on the trees to complete two evaluations at the Overbrug trial site. 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 good to very good at the Overbrug site (high juice – up to 63%), Brix 15 to 15.5, and fairly high acid levels (1.40%). The fruit was low seeded at the combination trial site where crosspollination was possible. External colour improved ranging from T1 to T3, bearing in mind the hot production areas. Based on the internal quality results in Table 5.4.4.5, the estimated maturity will be middle to the end of May.

Etna
Etna bore a good crop with large to extra-large fruit (count 1 to 1XX) and good internal quality for a hot production area. External colour was better at peak maturity (varied from T2 to 4). The average seed count was 0.7 seeds per fruit this season due to cross-pollination (lower than in 2018). Maturity seems to be the 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. The external colour development on the fruit was good for the Hoedspruit area (T1-3). Internally the fruit quality was very good, developing high juice (up to 64%) and Brix (up to 13.4) levels with good acids. Another quality of the fruit is the high seed count (high self-and cross pollination). Maturity seems to be middle to the end of May to the 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 cultivar 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 58%), high Brix (up to 12.8) and lower acid (above 0.8%) levels and an improved external colour (T1 - 4). 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, the estimated maturity will be the end of May to the middle of June.

Mor 26
Mor 26 produced an average (Moriah) crop on the trees for the 2019 season. The fruit size was more stable this season compared to 2018 and peaked between count 1X and 1XX, large to extra-large fruit. The external colour development was yellow and peaked at T1-3. The internal quality was good with high juice levels of up to 54%, Brix up to 14 and acceptable acid levels (1.0%). There were on average less seed in the fruit (1.0 seeds. 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
The crop on both selections was good this season to evaluate (management improvement). The fruit shape was very 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 ranged from count 2 and 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 0.1 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 the information was limited due to only two evaluations (beginning to middle of June).

Orah
Orah, producing a good crop with medium to large/extra-large (avg. count 1-1XX/1XXX) fruit size. The average seed count in the fruit went down from 2018 to 5.3 seeds per fruit, one of the selection characteristics (high seed numbers). Internal quality was good, the Brix levels were above 12.0 by time of harvest, good juice levels (above 65%) and acceptable acids (0.95%). Early external colour development ranged from T1 to T3 (only two evaluations). 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 Carrizo rootstock produced a good third crop with good internal quality on the large fast growing thornless trees at Mahela and Bosveld (Letsitele). The trees at Moriah planted on C35 bore their first crop this season, producing large fruit size due to the young tree age (from count 3 up to count 1 XXX). Colour

development was early in the season (deep orange) with a smooth rind texture on the fruit. Fruit were completely seedless this season in the combined trial blocks (future evaluations will confirm low seed numbers) and peaked from medium to large fruit size at Bosveld and Mahela (average count 2 to 1XX). Internal quality was good with high juice and Brix levels, and lower acids (average 0.7%). Based on the internal quality results in Table 5.4.4.5, the 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 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 1X-1XXX). The internal quality was good with juice levels up to 57%, Brix above 13 and acceptable acid levels (above 1.25% - middle July). 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 Mahela and Riverside. The fruit size decreased due to the heavy crop and peaked from medium to extra-large (count 3-1XX) and the fruit shape was similar to that of a Minneola tangelo fruit. The external colour was better developed between T1-3 when the internal quality was optimum. Tahoe produced fruit with soft fibre strength that peeled easily, and there was on average 0.3 seeds per fruit evaluated (crosspollination in a mixed orchard). The internal quality was good with juice levels of as high as 62%, Brix averaged 11 and acid levels were acceptable by the time of harvest (1.0%). Based on the internal quality results in Table 5.4.4.5, the 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 seedless fruit this season (0.7 seeds per fruit 2018), 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 63%, Brix above 12 and acids above 0.8 at the last evaluation. Fruit size peaked from count 1XX to count 1XXX, very large for mandarin cultivars. Based on the internal quality results in Table 5.4.4.5, estimated maturity will be the 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 were 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 thornless 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 60%), Brix levels lower (average 9.2) and peaked at 12.1 for the Mahela site, 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 Moriah and Overbrug trial site. The internal quality was good with Brix averaging 15 and acid levels around 1.5% (high this season at Overbrug) and external colour between T1 and 3 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 Moriah 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 middle of June to the beginning of July 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 good 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 reasons 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-1XXX), similar to Tahoe Gold, due to the light crop on the trees. The internal quality improved this season at Riverside with higher juice (above 60%), good Brix (above 13 at Mahela) and good acid levels. The external colour developed along with the internal quality towards the end of the evaluations (T1-3). Based on the internal quality results in Table 5.4.4.5, the estimated maturity will be mid-June to mid-July.

Additional selections

The internal quality of Tasty (Bruce) was below average this season with low juice levels (44.1%), higher Brix (above 11) and acids were very low (0.50%), questioning the potential of the cultivar in the hot production areas. The fruit size peaked from count 1 to count 1XX/XXX with low seed numbers (0.6 seeds/fruit).

Sirio produced large to extra-large fruit (count 1X/XX to 1XXX) on the trees due to a fair crop (large fruit size in hot areas and young trees) with average internal quality (low juice levels) in the hot production areas. The fruit was low seeded (average 0.6 seeds per fruit) with three evaluations and one evaluation seedless.

Dina (Edit x Nova) bore fruit on the Overbrug (Hoedspruit) trial trees this year. The tree shape is very upright with a dark bark colour. Fruit size varied from count 2 to 1XX/XXX (large fruit size compared to 2018), and seedless fruit in the crosspollination environment. The internal quality was good on the young trees; juice was above 52%, Brix levels in the 50's and better acid levels (remained on the low but stable side).

Leanri cropped the second time on the trees this season at Moriah, the fruit size varied from large to extra-large (1XX-1XXX) with good internal quality; high juice levels and good flavoured fruit, good Brix (average 12.8) and fairly low acids (average 0.8%).

One of the new ultra-late selections to include at the Bosveld trial site will be Sugar Belle, bearing fruit for the second time this year. Middle July the acids were still above 1.7%, keeping the Brix: acid ratio at 9.7 due to a Brix of 16 at peak colour development (T1-3). Juice content was above 60% with the third evaluation and fruit size medium to large (count 2 to 1XX).

Nova SL was included as a control for Saint André in the trial, the fruit is fairly difficult to peel and low numbers of seed were discovered in the fruit with the last evaluation. External colour was late and the fruit size varied between count 1 and count 1XX/XXX (large to extra-large fruit). Nova SL (ARC) produced a course rind texture on the fruit with medium to large fruit size (count 2 to count 1XX). The acid levels in the fruit were similar compared to Saint André (slightly lower) and the external colour development better (T1 to 2).

Tanor Late cropped fruit at Bosveld in Letsitele and was at peak maturity by the middle of July (late maturing selection). Fruit size was extra-large (count 1XXX) and completely seedless.

IRM 2, ARCCIT 9 LS, Orri and RHM bore their first crop at Moriah Estate this season. IRM 2, ARCCIT 9 LS and Orri cropped large to extra-large fruit on the trees with acceptable internal quality and low seed numbers in the crosspollination trial block. RHM performed well on C35 and developed good internal qualities with lower acid levels. The fruit size varied from count 2 to 1XXX.

Conclusion

There was an improvement in the external colour delay in the hot areas that was 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. Leanri, Orri, IRM 2, 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 the packout 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 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.

Dina (Edit x Nova), Tanor Late, Sugar Belle and Leanri were evaluated for the second time; and Etna, Sirio and Tasty 1 for the third 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), Mahela (Letsitele), Moriah (Hoedspruit), Overbrug (Hoedspruit) and Riverside (Malelane) during the 2019 season.

Bosveld										
Cultivar	**Rootstock**	**Date harvested**	**Fruit size (mm)**	**Count**	**Juice (%)**	**Brix º**	**Acid (%)**	**Ratio**	**Avg seed**	**Fruit external colour**
Saint Andre	CC	27/03/2019	69 - 76	1X - 1XX	50,7	10,7	0,60	17,8	0,5	T7 - 8
Saint Andre	CC	17/04/2019	73 - 78	1XX 1XXX	53,7	10,1	0,65	15,5	0,3	T4 - 7
Saint Andre	CC	10/05/2019	73 - 80	1XX - 1XXX	47,3	10,1	0,55	18,4	0,0	T3 - 6
Samba	CC	27/03/2019	59 - 66	2 - 1	60,6	11,3	0,90	12,6	0,0	T6 - 8
Samba	CC	10/05/2019	65 - 71	1 - 1X	58,9	11,8	0,70	16,9	0,0	T1 - 4
Samba	CC	29/05/2019	60 - 70	2 - 1X	56,5	11,9	0,70	17,0	0,0	T1 - 3
Sugar Belle	CC	29/05/2019	69 - 73	1X - 1XX	29,1	13,5	2,15	6,3	1,7	T1 - 3
Sugar Belle	CC	19/06/2019	62 - 72	2 - 1XX	58,4	14,7	2,05	7,2	0,0	T1 - 3
Sugar Belle	CC	11/07/2019	69 - 70	1X	60,0	16,0	1,65	9,7	1,5	T1 - 3
Tanor Late	CC	11/07/2019	81 - 100	1XXX	47,9	12,6	1,05	12,0	0,0	T1 - 3
Mahela										
Cultivar	**Rootstock**	**Date harvested**	**Fruit size (mm)**	**Count**	**Juice (%)**	**Brix º**	**Acid (%)**	**Ratio**	**Avg seed**	**Fruit external colour**
Etna	CC	17/04/2019	58 - 79	1 - 1XXX	59,8	8,9	0,65	13,7	1,3	T4 - 6
Etna	CC	03/05/2019	65 - 77	1 - 1XX	65,7	10,1	0,65	15,5	0,0	T2 - 4
Gold Nugget	CC	17/04/2019	66 - 79	1 - 1XXX	45,1	8,8	1,70	5,2	0,0	T6 - 8
Gold Nugget	CC	29/05/2019	67 - 75	1 - 1XX	49,5	11,2	0,90	12,4	0,0	T4 - 6
Gold Nugget	CC	19/06/2019	65 - 74	1 - 1XX	57,6	12,8	0,85	15,1	0,0	T1 - 4
Saint Andre	CC	03/05/2019	66 - 82	1 - 1XXX	53,4	10,5	0,60	17,5	0,9	T1 - 3
Samba	CC	27/03/2019	63 - 72	2 - 1XX	60,3	9,9	0,85	11,6	0,0	T7 - 8
Samba	CC	17/04/2019	64 - 72	1 - 1XX	56,0	9,8	0,70	14,0	0,0	T3 - 5
Samba	CC	03/05/2019	62 - 75	2 - 1XX	59,3	11,5	0,75	15,3	0,0	T1 - 3
Sirio	CC	17/04/2019	77 - 88	1XX - 1XXX	50,0	10,3	0,95	10,8	0,0	T4 - 7
Sirio	CC	03/05/2019	68 - 79	1X - 1XXX	49,3	11,0	0,85	12,9	0,6	T1 - 3
Sirio	CC	29/05/2019	71 - 80	1X - 1XXX	51,8	10,4	0,80	13,0	0,8	T1 - 3
Sirio	CC	19/06/2019	69 - 74	1X - 1XX	51,4	12,2	0,95	12,8	1,2	T1 - 3
Tahoe Gold	CC	17/04/2019	70 - 91	1X - 1XXX	61,7	8,4	1,00	8,4	0,2	T4 - 6
Tahoe Gold	CC	29/05/2019	72 - 88	1XX - 1XXX	55,5	10,5	0,90	11,7	0,5	T1 - 4
Tahoe Gold	CC	19/06/2019	72 - 84	1XX - 1XXX	58,3	10,9	1,05	10,4	0,0	T1 - 3
Tango	CC	17/04/2019	63 - 74	2 - 1XX	52,6	8,8	0,90	9,8	0,0	T6 - 7

Tango	CC	03/05/2019	65 - 75	1 - 1XX	62,6	9,6	0,70	13,7	0,0	T5 - 6
Tango	CC	29/05/2019	62 - 75	2 -1XX	50,8	10,8	0,65	16,6	0,0	T2 - 4
Tango	CC	19/06/2019	64 - 70	1 - 1X	60,8	10,2	0,65	15,7	0,0	T1 - 4
Tango	CC	12/07/2019	65 - 70	1 -1X	58,2	12,1	0,65	18,6	0,0	T1 - 3
Tango	CC	01/08/2019	64 - 73	1 - 1XX	50,4	12,0	0,55	21,8	0,0	T1 - 3
Tasty 1	CC	29/05/2019	69 - 88	1X - 1XXX	44,9	9,5	0,40	23,8	1,3	T1 - 3
Tasty 1	CC	19/06/2019	71 - 80	1X - 1XXX	44,1	11,9	0,70	17,0	0,3	T1 - 3
Tasty 1	CC	12/07/2019	69 - 77	1X - 1XX	43,3	11,8	0,50	23,6	0,0	T1 - 3
Yosemite Gold	CC	19/06/2019	75 - 80	1XX - 1XXX	50,0	11,2	1,05	10,7	0,0	T1 - 4
Yosemite Gold	CC	12/07/2019	71 - 80	1X - 1XXX	49,1	13,0	1,05	12,4	0,0	T1 - 3
Yosemite Gold	CC	01/08/2019	78 - 84	1XXX	48,1	13,7	1,00	13,7	0,0	T1 - 3

Moriah

Cultivar	Rootstock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix º	Acid (%)	Ratio	Avg seed	Fruit external colour
Furr	MxT/C35	30/05/2019	72 - 84	1XX - 1XXX	55,6	12,9	1,00	12,9	1,5	T1 - 3
Furr	MxT/C35	20/06/2019	69 - 80	1X - 1XXX	64,3	13,4	1,10	12,2	2,2	T1 - 3
IRM 2	C35	30/05/2019	67 - 82	1 - 1XXX	57,9	10,8	1,20	9,0	3,0	T1 - 3
IRM 2	C35	20/06/2019	72 - 75	1XX - 1XX	49,0	12,6	0,80	15,8	0,0	T1 - 3
Leanri	C35	28/03/2019	72 - 80	1XX - 1XXX	60,1	11,3	0,90	12,6	0,0	T6 - 7
Leanri	C35	17/04/2019	72 - 82	1XX - 1XXX	70,5	12,6	0,75	16,8	0,0	T2 - 4
Leanri	C35	30/04/2019	73 - 84	1XX - 1XXX	52,1	13,6	0,70	19,4	0,0	T1 - 4
Leanri	C35	30/05/2019	71 - 82	1X - 1XXX	61,4	13,7	0,80	17,1	0,8	T1 - 3
Mor 26	MxT/C35	20/06/2019	69 - 76	1X - 1XX	54,1	13,9	1,00	13,9	1,0	T1 - 3
ARCCIT 9 LS	C35	30/05/2019	67 - 78	1 - 1XXX	59,1	11,3	0,85	13,3	1,9	T1 - 3
ARCCIT 9 LS	C35	20/06/2019	64 - 71	1 - 1XXX	62,5	12,1	0,90	13,4	1,1	T1 - 3
Nova ARC	C35	30/03/2019	61 - 74	2 - 1XX	58,8	13,5	0,80	16,9	0,0	T1 - 2
Nova ARC	C35	17/04/2019	66 - 76	1 - 1XX	61,8	12,0	0,75	16,0	0,0	T1 - 4
Nova ARC	C35	30/04/2019	64 - 75	1 - 1XX	55,2	13,0	0,80	16,3	0,0	T1 - 3
Orri	C35	30/05/2019	69 - 76	1X - 1XX	54,4	12,5	1,00	12,5	1,6	T1 - 2
Orri	C35	20/06/2019	69 - 79	1X -1XXX	55,8	13,6	0,75	18,1	1,3	T1 - 3
Orah	C35	30/05/2019	70 - 78	1X - 1XXX	59,6	11,7	0,70	16,7	7,3	T1 - 3
Orah	C35	20/06/2019	67 - 80	1 - 1XXX	65,4	12,5	0,95	13,2	3,2	T1 - 3
RHM	C35	28/03/2019	62 - 70	2 - 1X	57,7	10,7	0,75	14,3	0,8	T6 - 8
RHM	C35	17/04/2019	64 - 73	1 - 1XX	54,8	11,0	0,65	16,9	0,0	T2 - 4
RHM	C35	30/04/2019	64 - 79	1 - 1XXX	55,1	12,8	0,60	21,3	0,4	T2 - 4
RHM	C35	30/05/2019	71 - 78	1X - 1XXX	49,7	13,1	0,55	23,8	0,0	T1 - 3
Saint Andre	C35	28/03/2019	67 - 69	1 - 1X	53,5	11,7	0,80	14,6	0,0	T7 - 8
Saint Andre	C35	17/04/2019	65 - 78	1 - 1XXX	39,4	11,4	0,70	16,3	0,0	T4 - 6
Saint Andre	C35	30/04/2019	75 - 84	1XX- 1XXX	51,5	12,8	0,65	19,7	0,0	T2 - 4
Saint Andre	C35	30/05/2019	67 - 79	1 - 1XXX	53,5	12,3	0,65	18,9	1,3	T1 - 3
Samba	C35	28/03/2019	57 - 71	3 - 1X	59,5	11,3	0,70	16,1	0,0	T4 - 6
Samba	C35	17/04/2019	66 - 75	1 - 1XX	56,7	11,1	0,70	15,9	0,0	T1 - 4
Samba	C35	30/04/2019	60 - 79	2 - 1XXX	57,0	12,9	0,70	18,4	0,0	T1 - 3
Tambor	C35	20/06/2019	76 - 84	1XX - 1XXX	63,3	12,3	0,80	15,4	0,0	T1 - 3
Winola	C35	30/05/2019	61 - 70	2 - 1X	60,8	11,4	1,65	6,9	0,0	T1 - 3
Winola	C35	20/06/2019	65 - 76	1 - 1XX	63,3	12,7	1,50	8,5	0,0	T1 - 3

Overbrug

Cultivar	Rootstock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix º	Acid (%)	Ratio	Avg seed	Fruit external colour
African Sunset	C35	30/05/2019	67 - 82	1 - 1XXX	62,7	14,9	1,50	9,9	0,6	T1 - 3

African Sunset	C35	20/06/2019	68 - 78	1X - 1XXX	50,9	15,5	1,30	11,9	1,2	T1 - 3
Dina	CC	30/04/2019	80 - 100	1XXX	51,3	12,4	0,80	15,5	0,0	T2 - 5
Dina	CC	30/05/2019	84 - 95	1XXX	54,1	13,4	0,85	15,8	0,0	T3 - 4
Dina	CC	20/06/2019	70 - 79	1X - 1XXX	55,0	14,0	0,95	14,7	0,0	T2 - 3
Dina	CC	12/07/2019	60 - 74	2 - 1XX	57,1	15,8	1,25	12,6	0,0	T1 - 3
IRM 2	CC	30/05/2019	67 - 77	1 - 1XX	59,4	14,0	1,00	14,0	1,8	T1 - 4
IRM 2	CC	20/06/2019	63 - 74	2 - 1XX	57,3	14,6	1,00	14,6	0,8	T1 - 3
IRM 2	CC	12/07/2019	70 - 78	1X - 1XXX	57,4	14,0	0,90	15,6	1,5	T1 - 3
Nadorcott ARC	CC	10/04/2019	60 - 78	2 - 1XXX	59,7	15,8	1,05	15,0	0,0	T1 - 3
Nadorcott ARC	CC	30/04/2019	66 - 70	1 - 1X	61,4	18,4	1,25	14,7	0,0	T1 - 3
Nadorcott ARC	CC	30/05/2019	61 - 72	2 - 1XX	59,1	11,6	1,50	7,7	0,0	T4 - 6
Nadorcott ARC	CC	20/06/2019	65 - 79	1 - 1XXX	61,3	13,7	1,70	8,1	0,1	T2 - 4
Nadorcott ARC	CC	12/07/2019	65 - 75	1 - 1XX	62,1	14,9	1,20	12,4	0,0	T1 - 4
ARCCIT 9 LS	CC	10/04/2019	64 - 75	1 - 1XX	58,1	16,0	1,30	12,3	0,0	T1 - 3
ARCCIT 9 LS	CC	30/04/2019	64 - 73	1 - 1XX	60,8	17,2	1,25	13,8	0,0	T1 - 3
ARCCIT 9 LS	CC	30/05/2019	64 - 76	1 - 1XX	58,8	12,0	1,65	7,3	0,0	T4 - 6
ARCCIT 9 LS	CC	20/06/2019	59 - 76	2 - 1XX	60,5	13,2	1,75	7,5	0,0	T2 - 4
ARCCIT 9 LS	CC	12/07/2018	64 - 73	1 - 1XX	57,8	14,5	1,45	10,0	0,0	T1 - 3
Valley Gold	C35	30/05/2019	69 - 75	1X - 1XX	61,4	16,2	2,25	7,2	0,0	T1 - 4
Valley Gold	C35	20/06/2019	69 - 76	1X - 1XX	57,6	17,0	1,70	10,0	0,0	T1 - 3
Riverside										
Cultivar	**Rootstock**	**Date harvested**	**Fruit size (mm)**	**Count**	**Juice (%)**	**Brix º**	**Acid (%)**	**Ratio**	**Avg seed**	**Fruit external colour**
Gold Nugget	CC	25/04/2019	76 - 81	1XX - 1XXX	49,5	9,3	1,20	7,8	0,0	T5 - 7
Gold Nugget	CC	14/05/2019	67 - 72	1 - 1XX	55,0	9,6	0,80	12,0	0,0	T3 - 5
Gold Nugget	CC	05/06/2019	67 - 79	1 - 1XXX	54,5	10,1	0,75	13,5	0,0	T1 - 4
Gold Nugget	CC	27/06/2019	65 - 74	1 - 1XX	55,0	10,2	0,80	12,8	0,0	T1 - 3
Tahoe Gold	CC	14/05/2019	57 - 76	3 - 1XX	65,2	11,0	1,30	8,5	0,3	T3 - 6
Tahoe Gold	CC	05/06/2019	64 - 73	1 - 1XX	66,2	11,8	1,25	9,4	0,3	T1 - 3
Tahoe Gold	CC	18/07/2019	63 - 72	2 - 1XX	56,4	12,4	1,20	10,3	0,0	T1 - 3
Tango	CC	20/03/2019	65 - 72	1 - 1XX	36,5	12,1	1,25	9,7	0,0	T7 - 8
Tango	CC	25/04/2019	62 - 72	2 -1XX	54,1	9,9	1,20	8,3	0,0	T1 - 5
Tango	CC	14/05/2019	64 - 77	1 - 1XX	62,6	10,0	1,05	9,5	0,0	T2 - 4
Tango	CC	05/06/2019	66 - 79	1 - 1XXX	57,4	10,6	0,90	11,8	0,0	T1 - 3
Shasta Gold	CC	27/06/2019	73 - 82	1XX - 1XXX	51,6	11,4	1,60	7,1	0,0	T1 - 3
Shasta Gold	CC	18/07/2019	68 - 79	1X - 1XXX	57,1	13,5	1,25	10,8	0,0	T1 - 3
Yosemite Gold	CC	25/04/2019	65 - 86	1 - 1XXX	48,7	9,1	1,40	6,5	0,0	T4 - 6
Yosemite Gold	CC	05/06/2019	77 - 86	1XX - 1XXX	59,4	11,2	1,05	10,7	0,1	T1 - 3
Yosemite Gold	CC	27/06/2019	70 - 76	1X - 1XX	69,7	11,0	1,15	9,6	0,0	T1 - 3

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 fifth season to evaluate the NGB trial site and the fourth season for the Alicedale site due to fruit numbers on the trees, and meaningful data collected. Valearly and Turkey 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. 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 (120 kg per tree). Lavalle will end off the Valencia season in the warm dry production areas.

Opsomming

Hierdie was die vyfde seisoen wat die NGB proef ge-evalueer is en die vierde seisoen vir Alicedale as gevolg van voldoende vrugte aan die bome, betekenisvolle data kon versamel word. Valearly en Turkey 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 (120 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 the list below at Alicedale (Tshipise) and NGB (Weipe).

Table 5.4.5.1. List of Valencia selections evaluated at Alicedale (Tshipise) during 2019.

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/RL/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
McClean 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 2019.

Selection	Rootstock	Topwork
Delta	X639	2012
Jassie	X639	2012
Kobus du Toit Late	X639	2012
McClean 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 2019 (determine yield production) and all cultivar combinations will be evaluated in the next season.

<u>Alpha</u>
The fruit was completely seedless on all four rootstock combinations with medium to large fruit size (count 64 to 40). Sunki 812 matured first, Brix:acid ratio above 13.5 and high juice content (above 61%). It is apparent that Alpha's maturity is middle to end of July (Table 5.4.5.3).

<u>Bennie</u>
There was a good crop on Bennie 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 58%), Brix (average 11.5) and acid (0.8%) and fairly low seed counts (average 0.7 seeds per fruit). External colour by the time of harvest varied between T1 and T4. Based on ratios, maturity end of June to the beginning of July (Table 5.4.5.3).

<u>Delta</u>
The Delta (control) trees were 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 ranged from count 72 to 48, medium to large fruit and optimum Valencia requirements. Internal quality was good with high juice (up to 57%), higher Brix (12.5) and fairly low acid levels through the season (average 0.80%). Based on the internal quality results in Table 5.4.5.3, the estimated maturity will be mid-June to mid-July.

<u>Gusocora</u>
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 57%), Brix (up to 12.7) and acid above 0.8 complied with export requirements. The external colour (delayed) varied from T1 to T6, correlating with the internal quality and Brix:acid ratio (8:1 for maturity). Fruit size was bigger and peaked between counts 72 and 40, optimal fruit size for export Valencia (medium to large). There was a good crop on the trees. It is apparent that Gusocora maturity is middle to end of July (Table 5.4.5.3).

<u>Henrietta</u>
Henrietta was evaluated on all four rootstock combinations at Alicedale, Tshipise this season. Juice levels peaked above 58% average with higher Brix (up to 11.6) and acids 0.9 (Sunki 812). The external colour development improved and peaked between T1 and T4 for the season. Average seeds per fruit increased slightly to 0.5 seeds per fruit (0.4 seeds for 2018). Based on the internal quality results in Table 5.4.5.3, estimated maturity will be mid-July to mid-August.

<u>Jassie</u>
Jassie seems to be one of the most promising new Valencia selections being tested and evaluated in the different citrus production and climatic areas. Fruit size distribution was excellent even with the high yield on the trees; the counts were from 64 to 40. Fruit quality was good with high juice (average 56%), Brix of up to 10.7 (X639) and fairly high acid levels (above 0.7%) at the final evaluation, indicating the late characteristics of the cultivar. The seed counts varied from 1.8 up to 3.3 seeds per fruit (average 2.4 seeds per fruit - lower). Based on the internal quality results in Table 5.4.5.3, the estimated maturity will be mid-July to mid-August.

<u>Kobus du Toit Late</u>
There was an external colour delay that improved on the fruit during the season, ranging from T1 to T5/6 up until the last evaluation. Fruit average size varied from medium to large, count 72 to 36. 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 was even lower this season for a seeded selection (average 1.7 seeds per fruit). Acid levels were above 0.85% the entire season, except for on X639 at both sites. Maturity, based on the internal quality results in Table 5.4.5.3, is estimated to be the end of June to the middle of July for these hot production areas.

<u>Lavalle</u>
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 1.1% at the final 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).

<u>Louisa</u>
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 (58 to 57%) and higher Brix levels (up to 11.6). 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.

<u>McClean SL</u>
Compared to all the other Valencia trial sites, McClean SL remained completely seedless (except one evaluation at NGB on X639 – 1.5 seeds). This year all the combinations with McClean SL were bearing fruit (120 kg per tree average) at Alicedale to evaluate, indicating the 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 59%, Brix 11, acid 0.8). Maturity (Table 5.4.5.3) is estimated to be the end of June to middle of July.

<u>Moosrivier Late 1</u>
Moosrivier produced fruit with medium to large/very large fruit size (count 72 to 40) at the NGB trial site on X639 rootstock. Internal quality improved and was very good with high juice (average 58.2%), average Brix (11.5) and improved acids (above 1.0) after completion of the evaluations. Seed count per fruit varied from 0.0 to 2.6 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.

<u>Skilderkrans</u>
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 11.0) and the acid level of 0.80 to 1.0% (peak maturity) indicated a later maturing Valencia selection. Juice level increased to an average 57% later in the season; above the minimum required export figure. There was an improvement in external colour at Alicedale on all four of the rootstocks evaluated, except on RL (T1-5). 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 the end of July and mid-August on Swingle and C35 (Table 5.4.5.3).

<u>Turkey</u>
Turkey cropped fruit on all four rootstock combinations at Alicedale performing well on RL, X639, Sunki 812 and C35 with high juice (above 58%) and average Brix (above 9.6) 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 0.2 and 0.4 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.

<u>Weipe</u>

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. Fruit size was medium to large/extra-large (count 72-48), internal quality was average (juice below 50% - except on C35, acid 0.70%) with higher Brix level (up to 10.7). Colour development ranged from T2 to T4. 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 juice (47%) and acid level of 0.80%, and a good Brix (up to 11.5). The fruit size varied from count 72 to 40 (medium to large) and there were no seeds per fruit.

Rhode Red developed high numbers of Chimeras on the fruit and future evaluations will determine the cultivar's potential due to instability.

Conclusions

Bennie matures well on the trees and reduces rind pitting problems. The recommendation will be to harvest the fruit from middle July onwards (stronger rind). Gusocora seems to have delayed colour development at peak maturity and degreening might be an option.

Valearly colour was delayed at peak maturity (T2-4) and resulted in low acids (0.80) 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 selections' stability.

All the selections evaluated developed seeds in their fruit, except for Alpha, Delta, Gusocora, Louisa, Lavalle, 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 2019 season.

Alicedale										
Selection	**Root-**	**Date**	**Size**	**Count**	**Juice**	**Brix º**	**Acid**	**Ratio**	**Ave.**	**Colour**
	stock	**harvested**	**mm**		**(%)**		**(%)**		**seed**	
Alpha	RL	23/08/2019	80-93	64 -40	55,8	11,3	0,87	13,0	0,0	T1-4
Alpha	X639	23/08/2019	82-94	56 – 40	52,3	11,6	0,87	13,3	0,0	T1-4
Alpha	Sunki 812	23/08/2019	82-92	56 – 40	60,7	12,3	0,90	13,7	0,0	T1-4
Alpha	C35	23/08/2019	79-94	64 – 40	55,2	11,4	0,87	13,1	0,0	T1-4
Bennie	RL	23/08/2019	80-95	64 – 36	54,5	11,4	0,79	14,4	0,8	T1-4
Bennie	X639	23/08/2019	80-95	64- 36	58,5	11,5	0,75	15,3	0,4	T1-4
Bennie	Sunki 812	23/08/2019	84-93	56 – 40	60,8	11,9	0,82	14,5	0,6	T1-4
Bennie	C35	23/08/2019	82-95	56 – 36	57,0	11,3	0,65	17,4	0,8	T1-4
Delta	RL	15/08/2019	76-87	72 – 48	55,1	11,9	0,87	13,7	0,0	T1-5
Delta	X639	16/08/2019	77-87	72 – 48	56,6	12,4	0,70	17,7	0,0	T1-5
Delta	Sunki 812	15/08/2019	73-85	72 – 56	57,3	13,0	0,87	14,9	0,0	T1-5
Delta	C35	15/08/2019	75-86	72 – 48	56,7	12,5	0,75	16,7	0,0	T1-5
Gusocora	RL	23/08/2019	75-89	72 – 48	53,6	12,1	0,72	16,8	0,0	T2-5
Gusocora	X639	23/08/2019	74-90	72 – 40	53,8	12,0	0,72	16,7	0,0	T2-6
Gusocora	Sunki 812	23/08/2019	75-88	72 – 48	56,8	12,7	0,80	15,9	0,0	T2-4
Gusocora	C35	23/08/2019	82-96	56 – 36	56,0	12,7	0,70	18,1	0,0	T1-4
Henrietta	RL	23/08/2019	77-90	72 – 40	55,8	11,3	0,87	13,0	0,3	T1-4
Henrietta	X639	23/08/2019	75-93	72 – 40	57,3	11,6	0,87	13,3	0,4	T1-4

Henrietta	Sunki 812	23/08/2019	77-92	72 – 40	59,6	11,2	0,87	12,9	0,3	T1-4
Henrietta	C35	23/08/2019	82-99	56 – 36	58,5	10,4	0,85	12,2	1,0	T1-4
Jassie	RL	23/08/2019	78-91	64 – 40	53,8	10,4	0,75	13,9	1,8	T1-5
Jassie	X639	23/08/2019	80-92	64 – 40	55,5	10,7	0,70	15,3	2,1	T1-5
Jassie	Sunki 812	23/08/2019	81-93	64 – 40	56,1	10,3	0,80	12,9	3,3	T2-5
Jassie	C35	23/08/2019	80-99	64 – 36	58,5	10,5	0,82	12,8	2,5	T2-5
K du Toit Late	RL	23/08/2019	78-100	64 – 36	56,5	10,2	0,85	12,0	2,5	T2-6
K du Toit Late	X639	23/08/2019	79-93	64 – 40	57,9	10,6	0,80	13,3	1,3	T2-5
K du Toit Late	Sunki 812	23/08/2019	79-97	64 – 36	59,7	11,5	0,95	12,1	2,1	T2-5
K du Toit Late	C35	23/08/2019	76-85	72 – 56	59,0	11,5	0,95	12,1	0,8	T1-5
Lavalle	RL	03/0/20219	78-90	64 – 40	59,0	9,8	1,10	8,9	0,0	T2-5
Lavalle	X639	03/0/20219	72-89	88 – 48	59,8	11,0	1,15	9,6	0,0	T2-5
Lavalle	Sunki 812	03/0/20219	78-89	64 – 48	56,2	11,6	1,20	9,7	0,0	T2-5
Lavalle	C35	03/0/20219	81-93	64 – 40	58,4	10,3	1,05	9,8	0,0	T2-5
Louisa	RL	03/0/20219	77-90	72 – 40	55,1	11,0	1,10	10,0	0,0	T2-6
Louisa	X639	03/0/20219	76-87	72 – 48	58,5	11,4	1,00	11,4	0,0	T2-5
Louisa	Sunki 812	03/0/20219	73-87	72 – 48	58,5	11,6	1,00	11,6	0,0	T2-5
Louisa	C35	03/0/20219	78-87	64 – 48	56,6	10,5	0,95	11,1	0,0	T2-5
McClean SL	RL	03/0/20219	72-85	72 – 56	55,9	10,8	0,85	12,7	0,0	T2-6
McClean SL	X639	03/0/20219	70-88	88 – 48	55,2	10,8	0,69	15,7	0,0	T2-6
McClean SL	Sunki 812	03/0/20219	75-93	72 – 40	56,1	11,7	0,80	14,6	0,0	T2-6
McClean SL	C35	03/0/20219	73-87	72 – 48	58,8	10,8	0,85	12,7	0,0	T2-6
Rhode Red	RL	23/07/2019	75-94	72 – 40	56,6	9,5	0,85	11,2	0,7	T3-6
Rhode Red	X639	05/08/2019	74-88	72 – 48	56,2	9,3	0,85	10,9	0,8	T2-6
Rhode Red	Sunki 812	05/08/2019	74-92	72 – 40	59,4	9,9	0,77	12,9	0,8	T3-6
Rhode Red	C35	03/0/20219	75-94	72 - 40	58,6	11,0	1,00	11,0	0,9	T3-6
Skilderkrans	RL	05/08/2019	72-87	88 – 48	58,7	10,4	0,77	13,5	0,0	T2-5
Skilderkrans	X639	03/08/2019	71-85	88 – 56	53,8	9,3	0,90	10,3	0,0	T1-4
Skilderkrans	Sunki 812	03/08/2019	72-89	88 – 48	57,6	9,5	0,85	11,2	0,0	T1-5
Skilderkrans	C35	03/08/2019	77-92	72 – 40	58,6	11,0	1,00	11,0	0,0	T1-5
Turkey	RL	31/06/2019	73-80	72 – 64	52,6	11,5	1,00	11,5	0,3	T3-4
Turkey	X639	31/06/2019	73-80	73 – 64	57,8	9,9	0,75	13,2	0,4	T3-4
Turkey	Sunki 812	31/06/2019	75-82	72 – 56	58,3	9,6	0,87	11,0	0,3	T3-4
Turkey	C35	31/06/2019	75-85	72 – 56	57,0	11,3	0,92	12,3	0,2	T3-4
Weipe	RL	01/06/2019	74-80	72 – 64	44,5	9,8	0,65	15,1	0,0	T2-3
Weipe	X639	01/06/2019	75-84	72 – 56	48,1	10,7	0,65	16,5	0,0	T3-4
Weipe	Sunki 812	01/06/2019	77-84	72 – 56	47,8	10,1	0,70	14,4	0,0	T3-4
Weipe	C35	01/06/2019	78-89	64 – 48	51,3	10,4	0,72	14,4	0,0	T2-3

NGB										
Selection	**Root-**	**Date**	**Size**	**Count**	**Juice**	**Brix °**	**Acid**	**Ratio**	**Ave.**	**Colour**
	stock	**harvested**	**mm**		**(%)**		**(%)**		**seed**	
Delta	X639	18/06/2019	75 - 86	72 - 48	55,7	10,9	1,05	10,4	0,0	T3 - 5
Delta	X639	11/07/2019	74 - 82	72 - 56	56,1	11,2	0,90	12,4	0,0	T1 - 5
Delta	X639	30/07/2019	76 - 85	72 - 56	55,2	11,6	0,85	13,6	0,0	T1 - 5
Jassie	X639	11/07/2019	74 - 84	72 - 56	59,0	11,2	1,00	11,2	1,2	T2 - 5
Jassie	X639	30/07/2019	75 - 82	72 - 56	61,6	12,3	0,85	14,5	0,0	T1 - 4
Jassie	X639	20/08/2019	74 - 82	72 - 56	56,9	12,1	0,85	14,2	1,4	T1 - 4
K du Toit Late	X639	11/07/2019	74 - 82	72 - 56	59,5	11,0	0,95	11,6	0,0	T2 - 5
K du Toit Late	X639	20/08/2019	80 - 87	64 - 48	56,6	12,4	0,80	15,5	0,0	T1 - 4

Moos Late 1	X639	11/07/2019	73 - 85	72 - 56	65,2	11,1	1,20	9,3	2,6	T2 - 5
Moos Late 1	X639	30/07/2019	78 - 84	64 - 56	50,7	11,7	1,20	9,8	0,0	T1 - 5
Moos Late 1	X639	20/08/2019	82 - 90	56 - 40	58,7	11,7	0,95	12,3	0,0	T1 - 4
McClean SL	X639	18/06/2019	75 - 88	72 - 48	59,2	11,1	1,30	8,5	1,5	T3 - 5
McClean SL	X639	11/07/2019	79 - 85	64 - 56	56,1	11,2	0,80	14,0	0,0	T2 - 5
McClean SL	X639	30/07/2019	77 - 86	72 - 48	54,7	11,4	0,85	13,4	0,0	T1 - 4
Rhode Red	X639	18/06/2019	72 - 79	88- 64	50,4	10,4	1,15	9,0	0,0	T4 - 6
Rhode Red	X639	11/07/2019	72 - 79	88 - 64	59,2	11,2	1,00	11,2	2,7	T3 - 5
Rhode Red	X639	30/08/2019	75 - 81	72 - 64	60,6	11,6	0,90	12,9	1,7	T2 - 5
Skilderkrans	X639	11/07/2019	80 - 85	64 - 56	57,5	11,4	0,95	12,0	0,0	T2 - 5
Skilderkrans	X639	30/07/2019	78 - 89	64 - 48	57,8	11,5	0,85	13,5	0,0	T1 - 5
Skilderkrans	X639	20/08/2019	80 - 90	64 - 40	53,2	11,9	0,95	12,5	0,0	T1 - 4
Valearly	X639	18/06/2019	78 - 94	64 - 40	46,6	10,9	0,80	13,6	0,0	T2 - 4
Valearly	X639	11/07/2019	74 - 89	72 - 48	46,7	11,5	0,80	14,4	0,0	T2 - 4
Weipe	X639	05/06/2019	74 - 84	72 - 56	53,3	11,8	0,85	13,9	0,0	T3 - 5
Weipe	X639	05/06/2019	77 - 85	72 - 56	46,4	12,4	0,85	14,6	0,0	T2 - 4
Weipe	X639	05/06/2019	77 - 82	72 - 56	47,1	11,0	0,85	12,9	0,0	T2 - 4
Weipe	X639	05/06/2019	75 - 87	72 - 48	44,7	10,5	0,80	13,1	0,0	T1 - 4
Weipe	X639	05/06/2019	77 - 84	72 - 56	59,3	10,3	0,85	12,1	0,0	T2 - 5
Weipe	X639	05/06/2019	75 - 83	72 - 56	45,5	11,6	0,80	14,5	0,0	T3 - 5
Weipe	X639	05/06/2019	78 - 83	64 - 56	48,2	10,6	0,85	12,5	0,0	T2 - 4
Weipe	X639	05/06/2019	78 - 85	64 - 56	46,2	10,6	0,70	15,1	0,0	T2 - 4
Weipe	X639	05/06/2019	75 - 85	72 - 56	48,2	11,5	0,85	13,5	0,0	T1 - 4

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 area). The results indicated that RHM with higher seed counts and low acid levels, followed by Leanri matures first. Tango was next in line with an increase in fruit size and fair to good internal quality. Tango, Gold Nugget, Tahoe Gold and Yosemite Gold were completely seedless this season. Dina 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 this season. Gold Nugget, followed by Shasta Gold were the last selections 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 klimaatsone (intermediere areas). Die resultate het aangedui dat RHM met hoër saadtellings en lae suurvlakke, gevolg deur Leanri die vroegste ryp geword het. Tango is volgende, met ′n toename in vruggrootte en gemiddelde tot goeie interne kwaliteit. Tango, Gold Nugget, Tahoe Gold en Yosemite Gold was total saadloos gewees hierdie seisoen. Dina 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 laatste 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 2019 season.

Selection	Rootstock	Planted
African Sunset (B24)	Sunki 812	2013
Dina (Edit x Nova)	Sunki 812	2013
Gold Nugget	CC/C35/X639	2013
IRM 1 & 2	Sunki 812	2013
Leanri	Sunki 812	2013
Meirav 63	Sunki 812	2013
Meirav 119	Sunki 812	2013
Mor 15	Sunki 812	2013
Mor 26	Sunki 812	2013
RHM	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 fourth 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.

<u>African Sunset (B24)</u>
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 was good with average juice (53%), Brix above 9 and acceptable acid levels. External colour was delayed between T4 and 6 for this intermediate production area.

<u>Gold Nugget</u>
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 cultivar, 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 was found 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 1X-1XXX) due to young trees. The internal quality was good (best on CC and X639), low to acceptable juice (from 44 up to 60%) was captured throughout the season; Brix and acid levels were better (avg. 12.7 and 1.0%). The external colour improved this season and peaked between T1 and T3/4. Based on the internal quality results in Table 5.4.6.2, the 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 (IRM 1 better Brix) and acids above 1.0% on IRM 1, above 0.75% on IRM 2. There was a slight colour delay on the IRM 2 fruit at peak maturity. Seed counts very low and peaked at 1.0 for both selections (cross pollination).

Mor 15 and 26

Mor 15 was planted as control for the 26 selection at Moosrivier. Mor 2 had no fruit on the trees this season. The fruit size stabilized and peaked between count 1X and 1XXX, large to extra-large fruit. The external colour development was yellow and peaked between T1 and T4. The internal quality improved to good with juice levels of up to 63% (peak maturity), Brix up to 12 and fairly high acid levels (avg. 0.8%). There were on average 2.5 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 1X-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 seedless. The internal quality improved from good to very good; juice up to 59%, Brix lower up to 11 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 0.2 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 thornless with a 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 54%), Brix was average (up to 11) and the acid levels below 1.0% during the season (X639 outperformed C35 and Sunki 812 this season). Fruit size varied on young trees and peaked at count 1 to 1XXX (large to extra-large). Based on the internal quality results in Table 5.4.6.2, estimated maturity will be end of April to the middle of May (slightly 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 10.2 and acid levels around 0.9% and external colour between T2 and 4 when the evaluation was completed. Fruit size peaked from count 1XX 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 and CC (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 channeled into fruit set and crop. Fruit size varied from large to very large (count 1X-1XXX), similar to Shasta Gold and Tahoe Gold. The internal quality was average to good, developing higher juice and acid levels (above 1.3%) with lower Brix for the season (above 10). External colour developed along with the internal quality towards the end of the evaluations (T3 to T5). 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 Dina (Edit x Nova) was average to good this season with better juice levels (average 51%) and no granulation problems in the fruit compared to Nova. Brix (average 11.2) and similar acids (1.0%), indicating the early-mid maturing characteristics of the selection in the intermediate production areas, with seedless fruit this season. The fruit size peaked from count 2 to count 1XX (similar to 2017).

Leanri developed a fairly large fruit size between count 1 and 1XXX, slightly smaller than the hot production areas. 2019 was the second crop on the trees and internal quality was very good on Sunki 812 (average juice 55%, Brix 10.2, acid 1.0%). Seed numbers were fairly low; from completely seedless up to 4.2 seeds per fruit.

Meirav 63 and 119 (experimental) developed a deep orange rind colour (T1 with peak maturity). Internal quality improved; juice content was average to good (average above 47%), Brix of 12.3 and acids above 0.8%. The fruit evaluated was seedless at the Moosrivier trial site.

Shani SL bore a fair crop with large to extra-large fruit size (count 1 to count 1XX) and deep orange rind colour (T1-T35). Internal quality was good early in the season with good juice (up to 53%), Brix (13.3) and acids (average 1.2%) levels. The fruit was seedless this season.

RHM cropped fruit for the second time this season with average seed numbers on one evaluation (7.5 seeds per fruit) and are prone to cross-pollination. There was a delayed colour development (T4 to 7) from the first evaluation with low acids (average 0.60%), indicating peak maturity Brix: acid ratio over 12. Future evaluations will determine the optimum quality of the fruit evaluated.

Conclusion

The delay in external colour development improved this season due to the 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. Tahoe Gold had the largest fruit size, followed by Shasta Gold, Yosemite Gold, and then Tango. The smaller fruit size was produced on Gold Nugget this year, 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 third evaluation of IRM 1&2, Leanri, Meirav 119, Mor 15, 26 and UC 5; the fourth evaluation of Dina (Edit x Nova) and Meirav 63 at Moosrivier; so, information is becoming available and future evaluations will improve recommendations on these cultivars (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 with deep orange colour development and Dina (Exit x Nova). RHM continued with the delayed external colour development on the fruit and lower acids.

Table 5.4.6.2. Internal fruit quality data for Mandarin hybrid selections at Moosrivier (Marble Hall) during the 2019 season.

Cultivar	Rootstock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix º	Acid (%)	Ratio	Avg seed	Fruit external colour
African Sunset	Sunki 812	13/06/2019	83 - 91	1XXX	52,4	9,3	0,75	12,4	0,0	T4 - 6
African Sunset	Sunki 812	04/07/2019	92 - 105	1XXX	41,0	8,9	0,65	13,7	0,0	T2 - 5

African Sunset	Sunki 812	25/07/2019	81 - 91	1XXX	40,3	10,3	0,70	14,7	0,0	T1 - 4
Dina (Edit x Nova)	Sunki 812	16/04/2019	62 - 69	2 - 1X	54,4	10,2	0,85	12,0	0,0	T5 - 8
Dina (Edit x Nova)	Sunki 812	13/06/2019	66 - 74	1 - 1XX	47,1	12,1	1,05	11,5	0,0	T1 - 3
Gold Nugget	C35	13/06/2019	70 -- 76	1X - 1XX	45,5	12,2	0,95	12,8	0,0	T1 - 3
Gold Nugget	C35	04/07/2019	69 - 88	1X -1XXX	44,4	12,8	1,10	11,6	0,0	T1 - 4
Gold Nugget	CC	13/06/2019	72 - 79	1XX - 1XXX	45,0	11,6	1,00	11,6	0,0	T1 - 4
Gold Nugget	CC	25/07/2019	71 - 77	1X - 1XX	60,1	14,1	1,10	12,8	0,0	T1 - 3
Gold Nugget	X639	13/06/2019	70 - 77	1X - 1XX	53,6	12,6	1,05	12,0	0,0	T1 - 3
IRM 1	Sunki 812	13/06/2019	70 - 75	1X - 1XX	52,3	11,4	1,10	10,4	0,0	T1 - 4
IRM 1	Sunki 812	25/07/2019	69 - 80	1X - 1XXX	57,3	13,5	1,05	12,9	1,5	T1 - 3
IRM 1	Sunki 812	14/08/2019	68 - 77	1X - 1XX	61,3	13,6	1,05	13,0	1,5	T1 -2
IRM 2	Sunki 812	13/06/2019	70 - 79	1X - 1XXX	48,3	11,1	0,75	14,8	1,3	T2 - 4
IRM 2	Sunki 812	04/07/2019	62 - 74	2 - 1XX	58,2	11,9	0,80	14,9	0,8	T1 - 4
IRM 2	Sunki 812	25/07/2019	69 - 76	1X - 1XX	54,9	12,9	0,75	17,2	0,7	T1 - 3
Leanri	Sunki 812	13/04/2019	71 - 87	1X - 1XXX	56,8	8,6	0,90	9,6	4,2	T7 - 8
Leanri	Sunki 812	29/04/2019	67 - 82	1 - 1XXX	53,9	10,6	0,95	11,2	0,0	T3 - 5
Meirav 63	Sunki 812	13/06/2019	66 - 77	1 - 1XX	49,4	12,0	0,85	14,1	0,0	T1 - 4
Meirav 63	Sunki 812	04/07/2019	69 - 80	1X - 1XXX	51,6	12,4	0,80	15,5	0,0	T1 - 3
Meirav 119	Sunki 812	13/06/2019	69 - 74	1X - 1XX	40,2	11,8	0,85	13,9	0,0	T1 - 3
Meirav 119	Sunki 812	04/07/2019	67 - 78	1 - 1XXX	46,4	13,0	0,80	16,3	0,0	T1 - 3
Mor 15	Sunki 812	13/06/2019	68 - 75	1X - 1XX	55,8	11,4	0,80	14,3	0,7	T1 - 4
Mor 15	Sunki 812	04/07/2019	69 - 79	1X - 1XXX	56,2	10,0	0,80	12,5	1,0	T1 - 4
Mor 15	Sunki 812	25/07/2019	71 - 81	1X- 1XXX	55,8	11,6	0,75	15,5	0,0	T1 - 3
Mor 26	Sunki 812	13/06/2019	70 - 84	1X - 1XXX	53,1	10,8	0,90	12,0	0,0	T1 - 4
Mor 26	Sunki 812	04/07/2019	68 - 77	1X - 1XX	62,5	11,6	0,80	14,5	0,8	T2 - 5
RHM	Sunki 812	29/04/2019	66 - 85	1 - 1XXX	61,6	9,2	0,75	12,3	7,5	T6 - 7
RHM	Sunki 812	02/05/2019	67 - 75	1 - 1XX	57,3	9,1	0,50	18,2	0,0	T4 - 6
Shani SL	Sunki 812	04/07/2019	66 - 71	1 - 1X	53,1	13,7	1,40	9,8	0,0	T1 - 3
Shani SL	Sunki 812	25/07/2019	68 - 77	1X - 1XX	49,1	12,8	1,05	12,2	0,0	T1 - 3
Tahoe Gold	C35	22/05/2019	77 - 90	1XX - 1XXX	56,2	8,9	0,70	12,7	0,0	T3 - 5
Tahoe Gold	C35	13/06/2019	74 - 80	1XX - 1XXX	56,4	9,2	1,00	9,2	0,0	T1 - 4
Tahoe Gold	C35	04/07/2019	71 - 80	1X - 1XXX	56,0	10,7	0,70	15,3	0,0	T1 - 3
Tahoe Gold	CC	22/05/2019	76 - 91	1XX - 1XXX	52,9	9,2	0,85	10,8	0,0	T2 - 4
Tahoe Gold	CC	13/06/2019	74 - 83	1XX - 1XXX	43,5	10,9	0,95	11,5	0,0	T1 - 4
Tahoe Gold	CC	04/07/2019	78 - 85	1XXX	52,0	10,6	0,85	12,5	0,0	T1 - 3
Tahoe Gold	Sunki 812	13/06/2019	70 - 78	1X - 1XXX	58,5	10,3	0,90	11,4	0,0	T1 - 3
Tahoe Gold	X639	22/05/2019	71 - 78	1X - 1XXX	58,4	9,6	0,95	10,1	0,0	T3 - 5
Tango	C35	29/04/2019	73 - 86	1XX - 1XXX	48,6	8,6	0,70	12,3	0,0	T4 - 7
Tango	C35	22/06/2019	66 - 83	1 - 1XXX	52,8	9,2	0,75	12,3	0,2	T2 - 4
Tango	C35	13/06/2019	67 - 74	1 - 1XX	51,2	10,3	0,75	13,7	0,0	T1 - 3
Tango	CC	29/04/2019	70 - 82	1X - 1XXX	50,5	8,1	0,75	10,8	0,0	T4 -6
Tango	CC	22/05/2019	67 - 80	1 - 1XXX	54,3	9,2	0,75	12,3	0,0	T2 - 4
Tango	CC	13/06/2019	68 - 73	1X - 1XX	52,5	10,6	0,80	13,3	0,0	T1 - 3
Tango	X639	29/04/2019	65 - 83	1 - 1XXX	52,0	8,5	0,85	10,0	0,0	T4 - 6
Tango	X639	22 /05/2019	64 -75	1 - 1XX	52,0	9,6	0,85	11,3	0,0	T2 - 5
Tango	X639	13/062019	66 - 79	1 - 1XXX	48,4	10,7	0,80	13,4	0,0	T1 - 4
Yosemite Gold	C35	13/06/2019	71 - 84	1X - 1XXX	50,4	10,2	1,45	7,0	0,0	T3 - 5
Yosemite Gold	CC	04/07/2019	74 - 82	1XX - 1XXX	55,6	10,0	1,30	7,7	0,0	T3 - 5

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)

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 2019 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 the 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 followed with good external colour development and 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 mature last, ending off the mandarin season for the hot areas.

Evaluations on the latest additions indicated that Goldup would be very early maturing in the hot areas, followed by RHM with lower acids. Dina, Meirav and Leanri will be next in line, cropping large fruit on the trees with good internal quality. Taylor Lee follows; developing low seed number and good internal quality as well as colour on the fruit. The Nadorcott selections will be next, cropping a good crop on the trees; ARCCIT 9 LS had no seeds in the fruit.

Picking periods should not be longer than 3 - 4 weeks to maintain good internal quality and avoid rind disorders.

Opsomming

Die kwaliteit van die Mandaryn Hibried vrugte het aansienlik verskil tussen die verskillende produksie areas, wat ŉ 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 2019 seisoen vir hierdie warm produksie areas het steeds aangedui dat Tango die vroegste ryp geword het met kleiner vruggrootte en goeie interne kwaliteit (suurvlakke daal vinnig in begin van seisoen). Daarna het Tahoe Gold gevolg, met beter eksterne vrug kleur. 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 ŉ gemiddelde tot goeie interne kwaliteit, asook goeie eksterne kleur ontwikkeling. 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 kleur ontwikkeling en 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 en eindig die mandaryn seisoen vir die warm produksie area.

Evaluasies op die nuutste toevoegings het aangedui dat Goldup die vroegste ryp word in die warm area, gevolg deur RHM met lae suur vlakke. Dina, Meirav en Leanri is volgende in lyn, wat groot vrugte op die bome dra en goeie interne kwalieit. Taylor Lee pas nou in; lae saad tellings, goeie interne kwaliteit en eksterne kleur ontwikkeling. Die Nadorcott seleksies pas nou in met `n goeie oes op die bome; ARCCIT 9 se vrugte was totaal saadloos gewees.

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, 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 2019 season.

Selection	Rootstock	Topworked
Dina (Edit x Nova)	X639	2015
Etna	X639	2013/2014
Furr (Clemcott)	X639	2013/2014
Gold Nugget	X639	2010
Goldup	X639	2015
IRM 2	X639	2015
Leanri	X639	2015
Meirav 119	X639	2015
Mor 26	X639	2013/2014
Nadorcott ARCCIT 9	X639	2015
Nova	X639	2013/2014
Nova SL (ARC)	X639	2013/2014
Page	X639	2013/2014
RHM	X639	2015
Saint Andre	X639	2013/2014
Samba	X639	2013/2014
Sirio	X639	2013/2014
Tahoe Gold	X639	2010
Tango	X639	2010
Taylor Lee	X639	2015

Table 5.4.7.2. List of Mandarin Hybrid selections evaluated at NGB (Weipe) during the 2019 season.

Selection	Rootstock	Topworked
Etna	X639	2015
Furr	X639	2015
Meirav	X639	2015
Nova	X639	2015
Saint André	X639	2015
Shasta Gold	X639	2011
Tahoe Gold	X639	2011
Yosemite Gold	X639	2011
Gold Nugget	X639	2011

Tango	X639	2011
Tanor Late	X639	2015

Results and discussion

More information was available at Alicedale (new and existing trial site) due to older trees with better crops compared on the younger topworked mandarin trees at NGB. 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 cultivar, 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/x-large (count 2-1XXX) and the fruit on all the trees was completely seedless. The internal quality of the fruit improved from good/very good (2018) to good and developed juice (average 52.5%), Brix (11.7) and acid levels above 1.0% average and an external colour between T1 and T3. Future evaluations will determine the feasibility of Gold Nugget in hot areas. Based on the internal quality results in Table 5.4.7.3, the 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 many 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 was picked before the late evaluations took place.

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 bigger this season and peaked from medium to large (count 2-1XXX) 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 61%, Brix averaging 11.1 and acid levels improved (1.3%). Based on the internal quality results in Table 5.4.7.3, estimated maturity was the middle of May to the middle of June.

Tango

Tango remained completely seedless at NGB and Alicedale this season. 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 thornless 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 56%), Brix improved for this selection (average 11.4), acid levels (below 1.0) 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 the 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). There was very limited fruit on the trees to evaluate this season.

Additional selections (fourth crop)

Etna bore a good crop with medium to very large fruit (count 2 to 1XXX) and average/good internal quality (high juice and Brix at NGB, and average acids of 0.75). The external colour was delayed at peak maturity (T2 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 (average 0.9%) early in the season and lower number of seeds in the fruit.

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. The external colour was late and the fruit size increased between count 2 and 1XXX (medium to large/extra-large). Nova SL (ARC) produced a course rind texture on the fruit with similar fruit size, medium to large (count 2 to count 1XX). The acid levels in the fruit were similar compared to Nova (control) and the external colour development delayed this season at peak maturity.

Orri trees were very aggressively growing, producing a light crop on the trees this season and no evaluation was possible. Page had a fair yield on the trees (50 kg/tree) with large/extra-large fruit size (count 1X to 1XX/1XXX). Internal quality was fair to good, juice content was high (52 to 65%), acid levels dropped early in the season (0.6 with first evaluation middle of April) with delayed colour development (T3 to T5). The fruit was seedless compared to last season and the rind texture was very smooth with deep orange colour development.

Saint André originated from a Furr orchard in the Eastern Cape. The fruit matures after Nova (2 weeks) with good internal quality juice (54.3%) and acids (average 0.7%), but improved Brix (above 10 at Alicedale 7 and up to 13 at NGB). Saint André, Nova and Nova SL were completely seedless this season (Saint André had low seed numbers in 2018) and fruit size varied between count 2 and count 1XX at NGB, and count 1 to 1XXX at Alicedale. Samba produced an average third (X639 rootstock) crop on the large fast-growing thorn-less 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/1XXX. Sirio produced erratic fruit sizes last season from medium to large coarse fruit (count 2 to 1XXX), this season the fruit size peaked at count 1XXX (very large fruit) with average to good internal quality; lower juice of 50%, Brix 11.3 and acids below 1.0% and better colour development on the trees (T1 to T3/4).

The Tambor fruit matured late this season after the last evaluation was completed. We will make provision to evaluate the fruit next season.

Additional selections (second crop)

Nadorcott (control) and Nadorcott ARCCIT 9 cropped the best yield on the young trees, followed by Leanri with large fruit size and good colour development. RHM cropped sufficient fruit to comply with four evaluations and Meirav 119 one evaluation this season. The acid levels from the RHM fruit were low early in the season (ranged from 0.6% to 0.5% - final evaluation), the external colour was delayed and fruit size varied from count 2 up to 1XXX (young trees). Meirav 119 developed very good colour on the young trees (from T1 to T3); acids were also fairly low early in the season for this hot dry production area. The yield on Dina (Edit x Nova) improved and three evaluations were possible with good juice and Brix levels; acids were on the low side, but with the early colour development Dina can be harvested as one of the earlier mandarin selections. Dina developed trees that were very upright in shape and pruning will be crucial to develop proper bearing branches. IRM 2 cropped minimal fruit this season and evaluations will continue next year.

Additional selections (first crop)

Goldup and Taylor Lee bore limited fruit to evaluate due to the young topworked trees. Goldup matures very early in the year with good external colour development.

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, TDEs and other early selections included in the trial, but ethylene reacted slowly or not at all for Tango and Nadorcott (W. Murcott selections). 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 fourth 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 were Page (good colour development and low seeded fruit), Saint André (bigger fruit size and later maturing) and Samba with good internal quality fruit (early maturing), good colour development and crop on the trees in combination with X639. Seed numbers on these selections were very low to completely seedless in the combination trial block with cross pollinating cultivars included. Furr (control for Leanri) developed the highest seed numbers per fruit, a typical characteristic of the selection with good colour development and internal quality.

This was the second evaluation (trees four years old – topworked end of 2015) of Nadorcott, Nadorcott ARCCIT 9, Dina (Edit x Nova), IRM 2, Leanri, Meirav 119 and RHM. The Nadorcott selections performed well with large fruit and good internal quality. RHM, Dina and Leanri matures early in the season with fairly low acid levels; RHM is experiencing delayed colour development. IRM 2 cropped a very light yield possibly due to alternate bearing patterns, future evaluations will confirm and fairly ribbed fruit.

Goldup and Taylor Lee were evaluated for the first time this year. The Goldup fruit seems fairly soft and post-harvest damage will be investigated. Taylor Lee performed well in terms of internal quality, colour development and low seed numbers, but large to extra-large fruit size.

Table 5.4.7.3. Internal fruit quality data for Mandarin hybrid selections at Alicedale (Tshipise) and NGB (Weipe) during the 2019 season.

Alicedale										
Cultivar	**Rootstock**	**Date harvested**	**Fruit size (mm)**	**Count**	**Juice (%)**	**Brix º**	**Acid (%)**	**Ratio**	**Avg seed**	**Fruit external colour**
Dina	X639	16/04/2019	60 - 79	2 -1XXX	52,6	11,6	0,65	17,8	0,0	T3 - T5
Dina	X639	09/05/2019	64 - 75	1 - 1XX	54,9	11,9	0,65	18,3	0,0	T2 - T4
Dina	X639	29/05/2019	60 - 73	2 - 1XX	55,4	11,2	0,85	13,2	0,0	T2 - T4
Etna	X639	16/04/2019	65 - 86	1 - 1XXX	52,3	10,1	0,65	15,5	0,0	T5 - T7
Etna	X639	09/05/2019	70 - 92	1X - 1XXX	49,6	10,0	0,65	15,4	0,0	T2 - T4
Etna	X639	29/05/2019	70 - 85	1X - 1XXX	47,1	9,5	0,75	12,7	0,0	T1 - T3
Furr	X639	29/05/2019	68 - 95	1X - 1XXX	58,4	11,8	1,05	11,2	0,8	T1 - T4
Gold Nugget	X639	16/04/2019	67 - 77	1X - 1XXX	51,1	10,2	1,15	8,9	0,0	T6 - T8

Gold Nugget	X639	09/05/2019	71 - 78	1X - 1XXX	53,3	11,1	1,00	11,1	0,0	T3 - T5
Gold Nugget	X639	29/05/2019	65 - 79	1 - 1XXX	50,5	11,9	1,05	11,3	0,0	T1 - T3
Goldup	X639	09/05/2019	57 - 72	3 - 1XX	54,2	9,1	0,45	20,2	0,0	T1 - T6
Goldup	X639	29/05/2019	58 - 70	3 - 1X	52,5	9,1	0,50	18,2	0,0	T1 - T2
IRM 2	X639	29/05/2019	65 - 75	1 - 1XX	49,1	10,7	0,50	21,4	0,0	T2 - T4
Leanri	X639	16/04/2019	74 - 82	1XX - 1XXX	50,0	10,7	0,75	14,3	0,0	T3 - T5
Leanri	X639	09/05/2019	76 - 85	1XX - 1XXX	58,0	11,7	0,90	13,0	0,6	T2 - T5
Leanri	X639	29/05/2019	76 - 89	1XX - 1XXX	51,7	12,0	0,95	12,6	0,2	T1 - T4
Meirav 119	X639	29/05/2019	64 - 79	1 - 1XXX	51,6	11,1	0,60	18,5	0,0	T1 - T3
ARCCIT 9	X639	29/05/2019	63 - 75	2 - 1XX	49,0	9,7	0,60	16,2	0,0	T2 - T4
Nova	X639	26/03/2019	67 - 75	1 - 1XX	52,6	10,4	0,65	16,0	0,0	T7 - T8
Nova	X639	16/04/2019	61 - 79	2 - 1XXX	51,5	11,1	0,60	18,5	0,0	T2 - T4
Nova	X639	09/05/2019	71 - 87	1XX - 1XXX	52,6	11,5	0,65	17,7	0,0	T1 - T4
Nova	X639	29/05/2019	72 - 84	1XX - 1XXX	43,4	10,1	0,65	15,5	0,0	T1 - T3
Nova ARC	X639	26/03/2019	63 - 68	2 - 1X	56,2	12,5	0,90	13,9	0,0	T6 - T7
Nova ARC	X639	29/05/2019	60 - 83	2 - 1XX	59,5	12,0	0,80	15,0	0,0	T1 - T3
Nova ARC	X639	16/04/2019	59 - 72	2 - 1XX	50,0	12,4	0,80	15,5	0,0	T2 - T4
Nova ARC	X639	09/05/2019	66 - 77	1 - 1XX	51,7	12,1	0,75	16,1	0,0	T1 - T3
Page	X639	16/04/2019	71 - 83	1X - 1XXX	65,0	10,1	0,60	16,8	0,0	T3 - T5
Page	X639	11/05/2019	70 - 85	1X - 1XXX	52,3	10,8	0,55	19,6	0,0	T1 - T3
RHM	X639	26/03/2019	56 - 64	2 - 1	64,8	9,7	0,60	16,2	0,0	T7 - T8
RHM	X639	16/04/2019	63 - 79	2 - 1XXX	57,5	9,7	0,50	19,4	0,0	T5 - T7
RHM	X639	09/05/2019	74 - 90	1XX - 1XXX	51,9	9,2	0,50	18,4	0,0	T4 - T6
RHM	X639	29/05/2019	66 - 83	1 - 1XXX	48,3	10,4	0,55	18,9	0,0	T1 - T3
Saint Andre	X639	26/03/2019	67 - 78	1 - 1XXX	53,8	10,2	0,55	18,5	0,0	T7 - T8
Saint Andre	X639	16/04/2019	73 - 78	1XX - 1XXX	53,2	10,9	0,65	16,8	0,0	T2 - T4
Saint Andre	X639	09/05/2019	73 - 83	1XX - 1XXX	53,1	10,4	0,50	20,8	0,0	T1 - T3
Samba	X639	26/03/2019	62 - 68	2 - 1X	61,0	10,2	0,80	12,8	0,0	T7 - T8
Samba	X639	16/04/2019	66 - 77	1 - 1XX	54,2	10,3	0,70	14,7	0,0	T1 - T4
Samba	X639	09/05/2019	72 - 90	1XX - 1XXX	49,5	10,1	0,65	15,5	0,0	T1 - T3
Samba	X639	29/05/2019	67 - 83	1 - 1XXX	48,8	9,9	0,65	15,2	0,0	T1 - T3
Sirio	X639	16/04/2019	78 - 91	1XXX	51,8	11,1	0,95	11,7	0,0	T4 - T6
Sirio	X639	09/05/2019	74 - 95	1XX - 1XXX	49,9	11,0	0,90	12,2	0,0	T1 - T4
Sirio	X639	29/05/2019	77 - 90	1XX - 1XXX	49,0	11,8	0,90	13,1	0,0	T1 - T3
Tahoe Gold	X639	16/04/2019	68 - 75	1X - 1XXX	58,0	11,7	1,45	8,1	0,0	T4 - T7
Tahoe Gold	X639	09/05/2019	71 - 93	1X - 1XXX	58,8	9,8	0,95	10,3	0,0	T2 - T4
Tahoe Gold	X639	29/05/2019	65 - 86	1 - 1XXX	56,7	9,8	1,05	9,3	0,0	T1 - T3
Tango	X639	16/04/2019	62 - 73	2 - 1XX	58,3	10,5	0,95	11,1	0,0	T3 - T5
Tango	X639	09/05/2019	66 - 75	1 - 1XX	55,6	11,1	0,70	15,9	0,0	T3 - T6
Tango	X639	29/05/2019	63 - 72	2 - 1XX	54,0	11,2	0,75	14,9	0,0	T1 - T4
Taylor lee	X639	29/05/2019	75 - 92	1XX - 1XXX	56,0	10,3	0,90	11,4	1,7	T1 - T4

NGB										
Cultivar	Rootstock	Date harvested	Fruit size (mm)	Count	Juice (%)	Brix º	Acid (%)	Ratio	Avg seed	Fruit external colour
Etna	X639	16/04/2019	60 - 75	2 - 1XX	58,0	10,2	0,70	14,6	0,0	T1 - T5
Etna	X639	09/05/2019	60 - 69	2 - 1X	60,0	11,0	0,65	16,9	0,0	T2 - T4
Etna	X639	28/05/2019	69 - 72	1X - 1XX	54,2	11,1	0,75	14,8	0,0	T1 - T3
Furr	X639	28/05/2019	71 - 84	1XX - 1XXX	57,1	12,1	0,80	15,1	2,1	T1 - T3
Furr	X639	18/06/2019	75 - 86	1XX - 1XXX	62,0	12,4	0,80	15,5	1,5	T1 - T3
Furr	X639	11/07/2019	70 - 83	1X - 1XXX	53,3	12,7	0,70	18,1	2,3	T1 - T4
Gold Nugget	X639	16/04/2019	64 - 84	1 - 1XXX	63,8	11,0	0,95	11,6	0,0	T5 - T7
Gold Nugget	X639	09/05/2019	62 - 81	2 - 1XXX	52,0	12,4	0,80	15,5	0,0	T2 - T6
Gold Nugget	X639	28/05/2019	65 - 76	1 - 1XX	47,7	13,1	0,90	14,6	0,0	T1 - T4
Gold Nugget	X639	18/06/2019	68 - 74	1X - 1XX	55,2	11,9	0,95	12,5	0,0	T1 - T3
Gold Nugget	X639	11/07/2019	65 - 78	1 - 1XXX	48,2	12,9	0,85	15,2	0,0	T1 - T4
Meirav	X639	28/05/2019	62 - 75	2 - 1XX	48,6	12,7	0,65	19,5	0,0	T1 - T3
Meirav	X639	18/06/2019	65 - 74	1 - 1XX	54,9	14,3	0,75	19,1	0,0	T1 - T3
Nova	X639	26/03/2019	62 - 68	2 - 1X	50,7	12,1	0,85	14,2	0,0	T7 - T8
Nova	X639	16/04/2019	66 - 74	1 - 1XX	47,3	11,3	0,75	15,1	0,0	T3 - T6
Nova	X639	09/05/2019	71 - 77	1X - 1XX	56,7	11,5	0,60	19,2	0,0	T3 - T5
Nova	X639	28/05/2019	70 - 79	1X - 1XXX	47,3	11,6	0,65	17,8	0,0	T1 - T3
Nova	X639	18/06/2019	70 - 79	1X - 1XXX	53,7	9,7	0,75	12,9	0,0	T1 - T3
Saint Andre	X639	26/03/2019	61 - 70	2 - 1X	57,9	11,5	0,80	14,4	0,0	T7 - T8
Saint Andre	X639	16/04/2019	67 - 77	1 - 1XX	55,6	13,1	0,60	21,8	0,0	T4 - T5
Saint Andre	X639	09/05/2019	65 - 75	1 - 1XX	56,7	12,0	0,60	20,0	0,0	T3 - T5
Saint Andre	X639	18/06/2019	65 - 71	1 - 1X	50,6	12,5	0,70	17,9	0,0	T1 - T3
Tahoe Gold	X639	16/04/2019	61 - 78	2 - 1XXX	66,2	11,1	1,55	7,2	0,0	T6 - T8
Tahoe Gold	X639	09/05/2019	69 - 84	1X - 1XXX	63,7	11,4	1,20	9,5	0,0	T4 - T6
Tahoe Gold	X639	11/07/2019	62 - 73	2 - 1XX	62,6	12,9	1,05	12,3	0,0	T1 - T3
Tango	X639	16/04/2019	59 - 70	2 - 1X	54,7	10,6	0,95	11,2	0,0	T4 - T6
Tango	X639	09/05/2019	60 - 82	2 - 1XX	56,4	11,6	0,60	19,3	0,0	T3 - T5
Tango	X639	18/06/2019	64 - 75	1 - 1XX	63,0	12,2	0,95	12,8	0,0	T1 - T4
Tango	X639	11/07/2019	60 - 65	2 - 1	58,4	12,6	0,80	15,8	0,0	T1 - T3
Tanor Late	X639	28/05/2019	70 - 80	1X - 1XXX	52,1	11,2	0,65	17,2	0,0	T1 - T3

5.4.8 **PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the intermediate production areas (Karino and Ngonini)**

Project 963C by J. Joubert (CRI)

Summary

The quality of the Mandarin Hybrid fruit was similar in the two different production areas (Nelspruit and eSwatini), due to the similar climatic region (intermediate areas). 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. Dina and Meirav 63 also indicated to be fairly early maturing selections, followed by Samba with good colour development. IRM 2 and Shani SL mature next towards the middle of the mandarin season with less ribbing

on the fruit compared to IRM 1 at the other trial sites. All the fruit evaluated this season had very low seed numbers due to cross pollination impact from the seeded cultivars close by, except for Sugar Belle, maturing ultra-late in the mandarin season.

Opsomming

Die kwaliteit van die Mandaryn Hibried vrugte het ooreengestem tussen die twee produksie areas (Nelspruit en eSwatini), a.g.v. die klimaatsone (intermediêre areas). 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. Dina en Meirav 63 het ook onder die vroeë seleksies ingepas gevolg deur Samba met goeie kleur ontwikkeling. 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 kruisbestuiwings impak van aangrensende variteite met saad, behalwe vir Sugar Belle, wat ultra-laat ryp word in die mandaryn seisoen.

Objectives

- To select Mandarin Hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new Mandarin Hybrid cultivars and to determine the climatic suitability of these cultivars in 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 eSwatini.

Table 5.4.8.1. List of Mandarin Hybrid selections evaluated at Karino-koöp (Nelspruit) during the 2019 season.

Selection	Rootstock	Planted
Dina (Edit x Nova)	CC	2011
Gold Nugget	CC	2014
Irradiated I22	CC	2011
IRM 1	CC	2014
IRM 2	CC	2011
Meirav 63	CC	2011
Meirav 119	CC	2014
Michal 6/47	CC	2014
Michal 89/64	CC	2014
Mor 2/15/26	CC	2014
Nadorcott ARC	CC	2011
Nadorcott ARCCIT9 LS	CC	2011
Page	CC	2017
Shani SL	CC	2011
Sugar Belle	CC	2016
Shasta Gold	CC	2014
Tango	CC	2014
Tahoe Gold	CC	2014
Valley Gold	CC	2011
Winola	CC	2014

Table 5.4.8.2. List of Mandarin Hybrid selections evaluated at Ngonini Estate (eSwatini) during the 2019 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
Tanor Late	C35	2015
Valley Gold	C35	2015

Results and discussion

The trees at Karino-koöp were evaluated for the fifth time this season; this is 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 bore 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.

<u>IRM 1 & IRM 2</u>
The tree shape of the IRM selections was very upright (V-shaped) with no thorns and aggressive growth. IRM 1 and 2 produced an alternative crop on the trees, with an on-year this season, and the fruit size peaked on IRM 2 from medium to large (count 1 to 1XX). The seed numbers on IRM 2 decreased, even more, this season from 2.7 seeds to 0.2 seeds per fruit, peels easily with some ribbing on the fruit (typical Murcott characteristic). Juice levels on IRM 2 decreased compared to 2018 and averaged above 56%, Brix was very good (up to 14.7) and acids were above 1.2%. 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

<u>Nadorcott ARC & ARCCIT9 LS</u>
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 (up to 59.7%), Brix averaging 13.2 and higher acids (avg. 1.2%). Nadorcott ARCCIT9 LS produced a better crop on the trees compared to the ARC selection and the fruit size was bigger this season inspite of a good crop load; varied from count 2-1XX. Both selections evaluated were completely seedless at the Karino trial site. Maturity seems to be two weeks earlier on the ARCCIT9 LS selection, but the 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.

<u>Additional selection (Karino)</u>
The internal quality of Dina (Edit x Nova) was good with high juice levels above 54%, no granulation problems in the fruit compared to Nova. Brix (above 13 – first and second evaluation) and acceptable acids (0.9%), indicating the early to mid-maturing characteristics of the selection in the intermediate productions areas, with completely seedless fruit. The fruit size peaked from count 1 to count 1XX.

Irradiated I22 bore a fair crop on the tree this season and three evaluations were possible. The fruit size was between count 1 and count 1XX (large fruit due to lighter crop); high Brix 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 59% at peak), Brix of 12.7 and good acids (average 0.9%). The seed content decreased back from 1.3 seeds to seedless fruit evaluated at the Karino trial site (cross pollination).

Shani SL bore a fair crop on the trees with medium to large fruit size (count 1 to count 1X) and deep orange rind colour (T1-T3). Internal quality was good early in the season with good Brix (16.2) and high acids (average 1.4%) levels, but low juice (50% first evaluation). The fruit was completely seedless (0.1 seeds).

Meirav 119, Michal 6/47 & 89/64, Tango, Tahoe Gold and Winola was included in the Karino trial site in 2014 and bore their second crop on the trees this season. Fruit size peaked from medium to large on Meirav 119 and Michal 89/64 (count 2 to 1XX), but smaller fruit was cropped on Michal 6/47 (between count 3 and 1X) with low-seeded to seedless fruit on all three selections. Tango cropped completely seedless fruit and Tahoe as well as Winola developed juice levels, ranging from 55% to 64%.

Sugar Belle was planted at the Karino trial site in 2016 and bore its first crop this season. The fruit size peaked from count 2 to count 1XX and colour development was favourable between T1 and T3 early in the season. Internal quality was very good with high juice and Brix levels, but exceptionally high acids, 1.85% with the fourth evaluation end of July.

<u>Gold Nugget</u>
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 cultivar, 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 peaked between medium and large/x-large (count 1-1XXX) and the fruit on all the trees were completely seedless. The internal quality of the fruit was good and developed fair juice (average 50%), higher Brix (12.3) and acid levels above 1.1% average and an external colour between T1 and T4. Based on the internal quality results in Table 5.4.8.3, estimated maturity will be the middle to end of June.

<u>Additional selection (Ngonini)</u>
Etna cropped better fruit numbers (second crop) this year with average to good (juice up to 63.3%) internal quality and improved colour development (T1-3). Nova was included as control cultivar for the Nona SL (ARC) and Saint André selection. Saint André produced a better crop on the trees with similar external colour development and internal quality, but bigger fruit size (between count 2 and 1XXX).

Page, Samba and Orri was included this season at the trial site topworked in 2015 on C35 rootstock. Page matured before Samba; juice and Brix were higher, but similar acids on the lower side for the cooler region. Orri being the later maturing selection of the three and low seed numbers due to cross-pollination (seedless fruit up to 3.0 seeds per fruit). Sugar Belle bore a light first crop on the trees with good colour development and high acid levels; high juice and Brix (62% and 14).

Conclusion

This was the fourth evaluation of Dina (Edit x Nova), Irradiated I22, Meirav 63 and Shani SL; Dina cropped large fruit size on the trees with good colour development (precocious bearing pattern). IRM 2, Nadorcott ARC and Nadorcott ARCCIT9 LS was evaluated for the fifth time in the old trial block; where the two Nadorcott selections performed similarly, except for a better crop on ARCCIT 9.

Meirav 119, Michal 6/47 & 89/64, Tango, Tahoe Gold and Winola at the Karino site were evaluated for the second time on the young tree, 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. There was a good external colour development on the Nadorcott, Meirav 63&119, Michal 6/47 & 89/64 and Shani SL selections (deep orange).

Gold Nugget and Sugar Belle bore fruit to evaluate; both cultivars had a good colour development early in the season, but Sugar Bell will be an ultra-late maturing option due to very high acids.

The Ngonini site in eSwatini cropped fruit for the second time and more information will become available with trees becoming more mature in future. Saint André seems to be an improved Nova selection that matured similar for 2019, 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 (eSwatini) during the 2019 season.

Karino										
Cultivar	**Rootstock**	**Date harvested**	**Fruit size (mm)**	**Count**	**Juice (%)**	**Brix º**	**Acid (%)**	**Ratio**	**Avg seed**	**Fruit external colour**
Dina	CC	17/05/2019	64 - 70	1 - 1X	58,3	13,0	0,85	15,3	0,0	T3 - 5
Dina	CC	11/06/2019	65 - 78	1 - 1XX	53,5	13,0	0,95	13,7	0,0	T1 - 5
Gold Nugget	CC	11/06/2019	69 - 74	1X - 1XX	50,3	12,2	1,30	9,4	0,0	T2 - 4
Gold Nugget	CC	02/07/2019	69 - 74	1X - 1XX	50,0	12,4	1,10	11,3	0,0	T1 - 4
I22	CC	11/06/2019	65 - 73	1 - 1XX	64,0	12,9	0,85	15,2	0,0	T3 - 5
I22	CC	02/07/2019	65 - 74	1 - 1XX	51,8	13,1	0,75	17,5	0,0	T1 - 4
I22	CC	23/07/2019	70 - 76	1X - 1XX	54,0	14,6	0,90	16,2	0,0	T1 - 3
IRM 1	CC	02/07/2019	68 - 74	1X - 1XX	59,6	14,2	1,00	14,2	0,0	T1 - 3
IRM 1	CC	23/07/2019	68 - 79	1X - 1XXX	62,8	14,5	1,10	13,2	1,4	T1 - 3
IRM 2	CC	17/05/2019	65 - 75	1 - 1XX	51,8	12,7	1,20	10,6	0,2	T3 - 5
IRM 2	CC	11/06/2019	68 - 77	1X - 1XX	56,8	13,8	1,30	10,6	0,0	T1 - 3
IRM 2	CC	02/07/2019	68 - 74	1 - 1XX	53,8	14,7	1,20	12,3	0,0	T1 - 3
IRM 2	CC	23/07/0219	64 - 74	1 - 1XX	60,3	13,3	1,40	9,5	0,0	T1 - 3
Sugar Belle	CC	17/05/2019	59 - 73	2 - 1XX	63,0	11,9	2,60	4,6	2,2	T2 - 4
Sugar Belle	CC	11/06/2019	61 - 70	2 - 1X	56,5	13,8	2,10	6,6	0,6	T1 - 3
Sugar Belle	CC	02/07/2019	62 - 69	2 - 1X	56,3	14,6	2,15	6,8	0,0	T1 - 3
Sugar Belle	CC	23/07/2019	54 - 74	4 - 1XX	58,6	12,8	1,85	6,9	0,9	T1 - 3
Sugar Belle	CC	08/08/2019	62 - 69	2 - 1X	58,7	16,2	1,90	8,5	0,0	T1 - 2
Meirav 63	CC	17/05/2019	64 - 73	1 - 1XX	61,2	11,5	0,80	14,4	0,0	T2 - 4
Meirav 63	CC	11/06/2019	62 - 75	2 - 1XX	59,1	13,3	0,95	14,0	0,0	T1 - 3
Meirav 63	CC	02/07/2019	66 - 75	1 - 1XX	54,3	13,4	0,80	16,8	0,0	T1 - 3
Meirav 119	CC	17/05/2019	62 - 77	2 - 1XX	60,0	11,1	1,05	10,6	0,7	T3 - 6
Meirav 119	CC	11/06/2019	65 - 72	1 - 1XX	61,9	13,6	1,00	13,6	0,0	T1 - 3
Meirav 119	CC	02/07/2019	60 - 72	2 - 1XX	51,1	14,3	0,85	16,8	0,0	T1 - 3
Michal 6/47	CC	17/05/2019	57 - 65	3 - 1	57,1	13,4	0,65	20,6	0,0	T1 - 4
Michal 6/47	CC	11/06/2019	66 - 69	1 - 1X	50,0	14,4	0,90	16,0	0,0	T1 - 3
Michal 89/64	CC	17/05/2019	65 - 71	1 - 1X	55,3	11,8	0,50	23,6	0,3	T2 - 4
Michal 89/64	CC	11/06/2019	63 - 72	2 - 1XX	52,1	12,4	0,70	17,7	0,8	T1 - 3
Michal 89/64	CC	02/07/2019	68 - 71	1X -	30,8	12,7	0,65	19,5	0,0	T1 - 3
Nadorcott ARC	CC	17/05/2019	65 - 71	1 - 1X	44,4	12,4	1,35	9,2	0,0	T3- 6
Nadorcott ARC	CC	11/06/2019	62 - 70	2 - 1X	56,4	13,5	0,75	18,0	0,0	T1 -4
ARCCIT 9 LS	CC	17/05/2019	63 - 75	2 - 1XX	57,5	10,8	1,50	7,2	0,0	T3 - 5
ARCCIT 9 LS	CC	11/06/2019	64 - 70	1 - 1X	57,0	13,3	0,90	14,8	0,0	T2 - 4
ARCCIT 9 LS	CC	23/07/2019	64 - 70	1 - 1X	59,7	15,8	1,30	12,2	0,0	T1 - 3
Shani SL	CC	11/06/2019	65 - 70	1 - 1X	49,7	14,6	1,45	10,1	0,0	T1 - 4

Shani SL	CC	02/07/2019	64 - 70	1 - 1X	46,2	16,2	1,40	11,6	0,0	T1 - 3
Shasta Gold	CC	02/07/2019	69 - 75	1X - 1XX	50,0	13,6	1,40	9,7	0,0	T1 - 3
Shasta Gold	CC	23/0/2019	75 - 80	1XX - 1XXX	49,6	13,6	1,35	10,1	0,0	T1 - 3
Tango	CC	17/05/2019	65 - 78	1 - 1XXX	53,7	10,9	0,85	12,8	0,0	T4 - 6
Tango	CC	11/06/2019	72 - 79	1XX - 1XXX	51,1	11,0	0,55	20,0	0,0	T2 - 4
Tahoe	CC	11/06/2019	65 - 75	1 - 1XX	59,5	12,7	0,50	25,4	0,0	T1 - 3
Winola	CC	17/05/2019	64 - 67	1	64,4	12,2	1,40	8,7	0,0	T1 - 3
Winola	CC	11/06/2019	64 - 72	1 - 1XX	57,4	13,0	1,80	7,2	0,0	T1 - 3
Winola	CC	02/07/2019	72 - 84	1XX - 1XXX	55,2	12,1	1,40	8,6	0,0	T1 - 3
Winola	CC	23/07/2019	66 - 72	1 - 1XX	55,3	12,7	1,50	8,5	0,0	T1 - 3

Ngonini										
Cultivar	**Rootstock**	**Date harvested**	**Fruit size (mm)**	**Count**	**Juice (%)**	**Brix º**	**Acid (%)**	**Ratio**	**Avg seed**	**Fruit external colour**
Etna	C35	04/04/2019	59 - 65	2 - 1	63,3	10,5	0,80	13,1	0,5	T3 - 5
Etna	C35	25/04/2019	59 - 69	2 - 1X	53,4	9,5	0,80	11,9	0,8	T1 - 3
Etna	C35	14/05/2019	69 - 75	1X - 1XX	60,3	10,4	0,70	14,9	0,8	T1 - 3
Sugar Belle	C35	25/05/2019	62 - 74	2 - 1XX	54,3	11,1	2,35	4,7	9,3	T1 - 5
Sugar Belle	C35	27/06/2019	63 - 72	2 - 1XX	63,4	14,2	2,00	7,1	5,8	T1 - 3
Sugar Belle	C35	18/07/2019	63 - 75	2 - 1XX	63,5	14,2	2,00	7,1	1,2	T1 - 3
Nova	C35	04/04/2019	58 - 73	3 - 1XX	60,0	10,3	0,70	14,7	3,3	T5 - 8
Nova	C35	25/04/2019	69 - 74	1X - 1XX	55,3	10,9	0,85	12,8	2,0	T1 - 4
Nova	C35	14/05/2019	66 - 75	1 - 1XX	57,5	11,3	0,55	20,5	4,8	T1 - 3
Orri	C35	24/24/2019	68 - 86	1X - 1XXX	52,1	11,8	1,05	11,2	3,0	T3 - 5
Orri	C35	05/06/2019	70 - 84	1X - 1XXX	58,8	12,1	0,90	13,4	0,8	T1 - 3
Orri	C35	27/06/2019	70 - 79	1X - 1XXX	55,3	12,4	1,05	11,8	1,1	T1 - 3
Orri	C35	18/07/2019	70 - 75	1X - 1XX	58,8	14,8	1,05	14,1	0,0	T1 - 3
Page	C35	04/04/2019	56 -68	3 - 1X	60,0	10,2	0,70	14,6	1,5	T3 - 5
Page	C35	25/04/2019	63 - 75	2 - 1XX	55,2	11,3	0,85	13,3	2,0	T1 - 2
Page	C35	14/05/2019	61 - 75	2 - 1XX	62,3	12,4	0,65	19,1	0,8	T1 - 2
Page	C35	05/06/2019	64 - 72	1 - 1XX	59,7	13,0	0,70	18,6	0,9	T1 - 2
Saint Andre	C35	04/04/2019	62 - 75	2 - 1XX	64,3	10,3	0,65	15,8	1,7	T5 - 8
Saint Andre	C35	14/05/2019	62 - 79	2 - 1XXX	58,5	11,4	0,60	19,0	1,9	T1 - 3
Saint Andre	C35	05/06/2019	66 - 79	1 - 1XXX	51,5	11,1	0,60	18,5	2,3	T1 - 3
Samba	C35	04/04/2019	62 - 68	2 - 1X	59,2	10,0	0,75	13,3	1,2	T3 - 6
Samba	C35	25/04/2019	69 - 83	1X - 1XXX	54,6	9,7	0,80	12,1	2,0	T1 - 3
Samba	C35	14/05/2019	66 - 80	1 - 1XXX	58,8	9,4	0,65	14,5	1,8	T1 - 3
Samba	C35	05/06/2019	71 - 79	1X - 1XXX	57,2	11,8	0,70	16,9	0,8	T1 - 2
Tanor Late	C35	27/06/2019	75 - 81	1XX - 1XXX	56,7	11,3	0,95	11,9	0,0	T1 - 3
Tanor Late	C35	18/07/2019	74 - 85	1XX - 1XXX	54,8	10,3	0,80	12,9	0,0	T1 - 3
Valley Gold	C35	05/06/2019	70 - 76	1X - 1XX	57,8	12,9	1,00	12,9	2,4	T1 - 4
Valley Gold	C35	27/06/2019	68 - 74	1X - 1XX	54,7	13,5	1,20	11,3	1,1	T1 - 3
Valley Gold	C35	18/07/2019	68 - 75	1X - 1XX	59,1	13,9	0,95	14,6	0,0	T1 - 3

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 good internal quality (high Brix) for the trial, followed by Fukumoto 2 with medium to large fruit size for this season and improved colour development. Newhall matures next with good juice levels and earlier 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 12). Hutton grouped well with Dream and Clarke this season, with good Brix content.

Opsomming

In die Nelspruit produksie area word M7 eerste ryp met goeie interne kwaliteit (hoë Brix) vir hierdie proef, gevolg deur Fukumoto 2 met medium tot groot vruggrootte vir hierdie seisoen en verbeterde kleur ontwikkeling. Newhall word volgende ryp met goeie sap vlakke en vroeer eksterne kleur op die vrugte. Clark, Fischer en Dream volg, om die middel van die nawel soetlemoen reeks te vul, met medium tot groot vruggrootte en hoë Brix vlakke (gemiddeld 12). Hutton se rypwordings inligting plaas die kultivar saam met Dream en Clarke met goeie Brix inhoud.

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.

When the ratio between sugar and acid is 10:1, the navel fruit is considered to be at peak maturity. A ratio of 9:1 is considered to be the build-up towards peak maturity of 10:1. After reaching the peak, the ratio increases to 11:1, after which the fruit are 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 2019 season.

Selection	Rootstock	Planted
Clarke	CC	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 fifth 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) fruit size on the trees at Karino and the external colour peaked at T1-4. The navel-end was fairly open and the fruit shape oblong; rind texture was fairly coarse. Clarke worsened on the juice levels (47%), better Brix 13 and acids 0.9% from the final 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 better this season compared to Clarke, averaging 52%, good Brix of up to 12.6 and similar acids (0.9%). Dream fruit developed a fairly smooth rind, small navel-end and fairly round fruit shape. The external colour development improved (older trees) and peaked between T1 and 4 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 of Fischer navel decreased, even more, averaging 0.7% for the season. Juice and Brix improved up to 52% (lower) and 12 (higher) respectively. Externally the fruit colour development peaked from T1 to T4. Fruit size was medium to large for navel production, medium fruit size (count 64 to 48). 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 56). Juice levels were higher (up to 56.4%), higher Brix of 11.5 and still very low acids (0.5%) with a delayed external colour of T2 to 4. 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 was erratic again and peaked from medium to large/extra-large fruit (count 72 to count 48). Juice levels decreased compared to the 2018 season and averaged at 50%, 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 T4 with the final evaluation. To estimate maturity was difficult due to the lower 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 (T1 to T4). There were chimeras and mutations on a number of the fruit in the trial block. The juice levels (up to 49%) was average for the season compared to the other navel selections at the trial site. Fruit size on CC was smaller (count 72 to count 64) compared to SC (count 88 to count 48) due to the crop load on the trees. Brix and acid content on both rootstocks were good to very good, up to 12.6 and 0.8% 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 to large fruit (count 72 to 56) on the trees with lower acids (0.7%) considering the Swingle rootstock combination. In general, Swingle increases acid levels in the fruit, as well as delaying external colour development. Brix average decreased to 12.2 and juice levels peaked at 53%. The Newhall fruit improved on external colour (T2 to 4) when the final evaluation was completed. Maturity seems to be the middle of April to end of April.

Conclusion

This was the fifth evaluation of all the navel selections at this new trial site in Karino, so information becomes available and future evaluations will improve these cultivars recommendations. 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 Clarke. Acids remained low from the beginning of the season up to peak maturity with the exception of Clarke. The external colour development improved on all the selections the season, creating a more ideal situation with better internal quality and more specifically high Brix 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 2019 season.

Karino									
Cultivar	**Rootstock**	**Date harvested**	**Fruit size (mm)**	**Count**	**Juice (%)**	**Brix º**	**Acid (%)**	**Ratio**	**Fruit external colour**
Clarke	CC	11/06/2019	76 - 88	72-48	46,8	12,9	0,90	14,3	T1 - 4
Dream	SC	17/05/2019	72 - 84	88-56	52,2	11,7	0,90	13,0	T3 - 6
Dream	SC	11/06/2019	79 - 90	64-40	51,8	12,6	0,85	14,8	T1 - 4
Fisher	SC	17/05/2019	80 - 89	64-48	51,4	11,5	0,70	16,4	T3 - 5
Fisher	SC	11/06/2019	80 - 87	64-48	51,6	11,7	0,60	19,5	T1 - 4
Fukumoto	SC	23/04/2019	72 - 79	88-64	43,9	11,3	0,70	16,1	T3 - 6
Fukumoto	SC	17/05/2019	74 - 85	72-56	56,4	11,5	0,55	20,9	T2 - 4
Hutton	CC	11/06/2019	75 - 87	72-48	50,3	12,7	0,90	14,1	T2 - 4
M7	CC	23/04/2019	75 - 80	72-64	46,3	12,6	0,80	15,8	T1 - 4
M7	SC	23/04/2019	71 - 89	88-48	47,9	12,2	0,80	15,3	T1 - 4
Newhall	CC	17/05/2019	77 - 85	72-56	52,8	12,2	0,70	17,4	T2 - 4

5.4.10 **PROGRESS REPORT: Evaluation of Mandarin hybrid selections in the cool-inland production areas (Orighstad and Burgersfort)**
Project 990A by J. Joubert (CRI)

Summary

Nova, Nova SL and Saint André mature first according to the results of the 2019 season for the cool inland production areas, and all three selections developed large to extra-large fruit calibre and good internal quality. Dina, Samba and RHM with high juice levels and Meirav 119, fit in before Leanri with large fruit size, which follows. RHM developed the highest seed count per fruit for this trial in the open blocks followed by Page, developing the second-highest seed count per fruit. The mid-maturing mandarins are represented by ARCCIT 9 LS Nadorcott and ARC Nadorcott, which developed good Brix levels and higher acids than the other selections (up to acid of 0.9%) season. Picking periods should not be longer than 3 - 4 weeks to maintain good internal quality and to avoid rind disorders.

Opsomming

Nova, Nova SL and Saint André word die vroegste ryp volgens resultate van die 2019 seisoen vir hierdie koel binnelandse produksie area, met al drie seleksies groot tot ekstra-groot vrugt kaliber en goeie interne kwaliteit. Dina, Samba en RHM, met hoë sap vlakke en Meirav 119, pas in voor Leanri, wat daarna volg met die groot vruggrootte. RHM het die hoogste saadtellings per vrug ontwikkel vir hierdie proef in die oop blokke. Volgende is Page, met die tweede hoogste saad telling per vrug. Die middel van die mandaryne word verteenwoordig deur ARCCIT 9 LS Nadorcott en ARC Nadorcott, met goeie Brix vlakke en hoër sure in vergelyking met die

ander seleksies (tot suur van 0.9%). 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 cool, inland production regions.

Materials and methods

Field evaluations and laboratory analyses were conducted on Mandarin Hybrid selections from Mountain Haven (Orighstad) and Waterval (Burgersfort).

Table 5.4.10.1. List of Mandarin Hybrid selections evaluated at Mountain Haven (Orighstad) during the 2019 season.

Selection	**Rootstock**	**Planted**
Dina	CC	2017
Furr	CC	2017
Leanri	CC	2017
Meirav 119	CC	2017
ARCCIT 9 LS	CC	2017
Nadorcott	CC	2017
Nova ARC	CC	2017
Nova	CC	2017
Page	CC	2017
RHM	CC	2017
Saint André	CC	2017
Samba	CC	2017

Table 5.4.10.2. List of Mandarin Hybrid selections evaluated at Waterval (Burgersfort) during the 2019 season.

Selection	**Rootstock**	**Planted**
Dina	CC	2017
Furr	CC	2017
Leanri	CC	2017
Meirav 119	CC	2017
ARCCIT 9 LS	CC	2017
Nadorcott ARC	CC	2017
Nova	CC	2017
Page	CC	2017
RHM	CC	2017
Saint André	CC	2017
Samba	CC	2017

Results and discussion

The trees at Mountain Haven and Waterval bore their second crop for this season with improved fruit numbers and more mature tree internal quality and fruit size characteristics. Mountain Haven was planted under net completely; Waterval consists of two adjacent trial blocks, one side under net and the other side open duplicating the same selections.

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.

<u>Dina</u>
The internal quality of Dina (Edit x Nova) was good this season at Mountain Haven, with high juice levels (average 57%) and no granulation problems in the fruit compared to Nova. Juice levels at Waterval under the net was better (55.3%) compared to low juices (average 48.2%) in the open plantings. Brix (average 10.5) and similar acids (0.9%), indicating the early-mid maturing characteristics of the selection in the cool inland production areas, with seedless fruit this season. The fruit size peaked from count 1 to count 1XX and one evaluation 1XXX.

<u>Furr (Clemcott)</u>
Furr developed large to extra-large fruit size (count 1 – 1XXX) on the trees at both trial sites (average bigger size counts at Waterval, Burgersfort), one of the cultivar's characteristics as well as a good crop on the trees. The external colour development on the fruit was good for the cool areas, as expected (T1-3). Internally the fruit quality was very good, developing high juice (up to 60%) and Brix (up to 13.0) levels with good acids. Another quality of the fruit is the high seed count (high self-and cross pollination). Maturity seems to be middle to the end of May to the middle of June for the cool production areas, according to the information in Table 5.4.10.3.

<u>Leanri</u>
Leanri developed a fairly large to extra-large fruit size between count 2/1 and 1XXX, smaller compared to the hot production areas. 2019 was the second crop on the trees and internal quality was good (Mountain Haven) to very good (Waterval) on the Carrizo rootstocks (average juice 51%, Brix 10, acid 0.9%). Seed numbers were fairly low; completely seedless up to 4.5 seeds per fruit.

<u>Meirav 119</u>
Meirav 119 developed a deep orange rind colour (T1 to 3 with peak maturity). Internal quality improved; juice content was better at Waterval compared to Mountain Haven, average to good (average above 46.4% up to51.5%), Brix of 10 and acids above 0.8%. The fruit evaluated was seedless at Mountain Haven and low seeded at Waterval (0.5 seeds per fruit).

<u>Nadorcott ARC & ARCCIT 9 LS</u>
The crop on both selections was good this season to evaluate. The fruit shape was very 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 ranged from count 1 and 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 3.0 seeds per fruit only with one evaluation) this season. Maturity seems to be two weeks earlier on the ARCCIT 9 LS selection, according to Table 5.4.10.3, but the information was limited due to the first crop on the trees being evaluated (beginning to middle of June).

<u>Nova, Nova SL, Saint André</u>
Nova was included at Waterval as early maturing control, and as a control for Nova SL and Saint André in the trial at Mountain Haven, the fruit peels fairly difficult and low numbers of seed was discovered in the fruit with some of the evaluation. External colour was later on Nova and Saint André, and the fruit size varied between count 2/1 and count 1XX/XXX (medium/large to extra-large fruit). Nova SL (ARC) produced a course rind texture on the fruit compared to the other two selections. The acid levels at Mountain Haven in the fruit was similar compared to higher acids under the net structures at Waterval than the open block, and the external colour development better (T1 to 2).

RHM

RHM cropped fruit for the second time this season with average seed numbers at Waterval under net (from 1.4 up to 6.8 seeds per fruit), but high numbers in the open block as well as Mountain Haven`s net block (avg. 18 seeds per fruit) due to crosspollination. There was a delayed colour development (T4 to 6/7) from the first evaluation with low acids (avg. 0.60%), indicating peak maturity Brix: acid ratio over 12. Future evaluations will determine optimum quality of the fruit evaluated.

Samba

Samba on Carrizo rootstock produced a good second crop with good internal quality on the larger fast growing thornless trees at both trial sites. Colour development was better early in the season (deep orange) at Mountain Haven with higher acids (0.80%) compared to Waterval, with a smooth rind texture on the fruit. Fruit was completely seedless this season, except for one evaluation under net at Waterval, in the combined trial blocks (future evaluations will confirm low seed numbers) and peaked from medium to large fruit size at Mountain Haven (average count 2 to 1X, cooler area) and count 1 to 1XXX at Waterval (large to extra-large fruit size). Internal quality was good with higher juice at Orighstad (avg. 56%) compared to Burgersfort (avg. 52%), high Brix levels (from 9 to 11.4), and lower acids (avg. 0.6 to 0.8%). Based on the internal quality results in Table 5.4.4.5, the estimated maturity will be middle to the end of April.

Additional selections

Page matured before Samba; juice and Brix was higher at Waterval and the opposite at Mahela with delayed colour development, but similar acids on the lower side for the cooler regions, except for Mountain Haven where Samba developed good acids throughout the season.

Conclusion

This was the first evaluation of all the sections at the two trial sites, with Mountain Haven cooler than Burgersfort. Trees are still young and fruit quality will improve with future evaluations.

The highest seed numbers were on Page, RHM, Saint André, followed then by Leanri with lower numbers in the open trial block at Waterval (cross-pollination). All the other selections developed very low seed numbers in the fruit. Dina, Meirav 119, Leanri, Samba and the Nadorcott selections performed well with deep orange colour development, as well as the Nova selections and Saint André. RHM continued with delayed external colour development on the fruit and lower acids and large fruit size at Waterval.

Table 5.4.10.3. Internal fruit quality data for Mandarin hybrid selections at Mountain Haven (Orighstad) and Waterval (Burgersfort) during the 2019 season.

Mountain Haven (Orighstad)										
Cultivar	**Rootstock**	**Date harvested**	**Fruit size (mm)**	**Count**	**Juice (%)**	**Brix º**	**Acid (%)**	**Ratio**	**Avg seed**	**Fruit external colour**
Dina	CC	21/05/2019	71 - 82	1X - 1XXX	59,5	10,4	0,90	11,6	0,0	T2 - 5
Dina	CC	13/06/2019	69 - 75	1X - 1XX	54,5	10,6	0,90	11,8	0,0	T1 - 3
Furr	CC	21/05/2019	66 - 80	1 - 1XXX	57,4	11,2	0,85	13,2	1,3	T3 - 5
Furr	CC	03/07/2019	65 - 79	1 - 1XXX	58,9	12,6	1,05	12,0	5,6	T1 - 3
Leanri	CC	09/04/2019	65 - 72	1 - 1XX	48,1	8,5	0,90	9,4	0,0	T7 -8
Leanri	CC	29/04/2019	60 - 74	2 - 1XX	43,5	8,6	0,75	11,5	0,0	T6 - 8
Leanri	CC	21/05/2019	66 - 75	1 - 1XX	53,3	10,9	0,80	13,6	0,0	T4 - 6
Leanri	CC	12/06/2019	65 - 70	1 - 1X	52,5	11,6	0,80	14,5	0,0	T1 - 4
Leanri	CC	03/07/2019	66 - 77	1 - 1XX	51,4	10,7	0,65	16,5	0,0	T1 - 3
Meirav 119	CC	21/05/2019	70 - 76	1X - 1XX	44,4	9,2	0,70	13,1	0,0	T2 - 6
Meirav 119	CC	12/06/2019	68 -79	1X - 1XXX	49,4	10,2	0,80	12,8	0,0	T2 - 4
Meirav 119	CC	03/07/2019	67 - 75	1 - 1XX	45,5	11,7	1,05	11,1	0,0	T1 - 4

ARCCIT 9 LS	CC	12/06/2019	67 - 74	1 - 1XX	43,6	10,4	0,75	13,9	0,0	T4 - 6
ARCCIT 9 LS	CC	03/07/2019	64 - 73	1 - 1XX	60,0	11,0	0,80	13,8	0,0	T1 - 4
Nadorcott	CC	12/06/2019	65 - 73	1 - 1XX	55,3	10,4	0,90	11,6	0,0	T2 - 5
Nadorcott	CC	03/07/2019	71 - 77	1X - 1XX	52,5	10,7	0,90	11,9	1,5	T1 - 3
Nova ARC	CC	09/04/2019	64 - 75	1 - 1XX	60,2	9,5	0,80	11,9	0,2	T7 -8
Nova ARC	CC	30/04/2019	75 - 86	1XX - 1XXX	51,3	11,7	0,85	13,8	0,0	T2 - 5
Nova ARC	CC	21/05/2019	67 - 74	1 - 1XX	54,0	11,1	0,65	17,1	0,0	T1 - 3
Nova ARC	CC	12/06/2019	74 - 79	1XX - 1XXX	56,0	8,0	0,50	16,0	1,7	T1 - 3
Nova ARC	CC	03/07/2019	73 - 78	1XX - 1XXX	42,1	9,8	0,80	12,3	0,0	T1 - 3
Nova	CC	09/04/2019	65 - 76	1 - 1XX	50,0	7,7	0,75	10,3	0,0	T7 - 8
Nova	CC	30/04/2019	58 - 78	3 - 1XXX	48,4	8,2	0,60	13,7	0,0	T5 - 7
Nova	CC	21/05/2019	65 - 79	1 - 1XXX	65,2	8,2	0,55	14,9	0,0	T3 - 5
Nova	CC	12/06/2019	70 - 81	1X - 1XXX	46,1	11,2	0,90	12,4	0,0	T1 - 3
Page	CC	09/04/2019	65 - 79	1 - 1XXX	45,8	7,9	0,75	10,5	0,0	T8
Page	CC	30/04/2019	60 - 69	2 - 1X	54,5	10,1	0,75	13,5	0,0	T3 - 6
Page	CC	21/05/2019	62 - 74	2 - 1XX	60,0	9,9	0,60	16,5	0,0	T1 - 3
Page	CC	12/06/2019	69 - 85	1X - 1XXX	54,7	10,3	0,85	12,1	0,0	T1 - 3
Page	CC	03/07/2019	67 - 79	1 - 1XXX	57,1	11,8	0,90	13,1	0,0	T1 - 3
RHM	CC	30/04/2019	58 - 72	3 - 1XX	53,7	9,3	0,60	15,5	0,0	T5 - 7
RHM	CC	03/07/2019	60 - 65	2 - 1	59,7	11,6	0,50	23,2	0,0	T1 - 3
Saint André	CC	09 /04/2019	57 - 66	3 - 1	57,7	10,0	0,75	13,3	0,0	T6 - 8
Saint André	CC	29/04/2019	61 - 82	2 - 1XXX	42,5	9,0	0,75	12,0	0,0	T5 - 6
Saint André	CC	21/05/2019	68 - 82	1X - 1XXX	46,8	9,4	0,65	14,5	0,0	T2 - 4
Saint André	CC	12/06/2016	70 - 75	1X - 1XX	38,2	9,6	0,60	16,0	0,0	T1 - 3
Samba	CC	09/04/2019	56 -64	3 - 1	55,4	9,1	0,90	10,1	0,0	T7 - 8
Samba	CC	30/04/2019	63 - 69	2 - 1X	54,3	9,8	0,80	12,3	0,0	T4 - 6
Samba	CC	21/05/2019	61 - 69	2 - 1X	58,6	10,1	0,80	12,6	0,0	T1 - 4
Samba	CC	12/06/2019	61 - 67	2 - 1	55,6	10,8	0,85	12,7	0,0	T1 - 3
Samba	CC	03/07/2019	63 - 65	2 - 1	56,0	11,4	0,85	13,4	0,0	T1 - 3

Waterval (Burgersfort)											
Cultivar	**Rootstock**	**Net/Open**	**Date harvested**	**Fruit size (mm)**	**Count**	**Juice (%)**	**Brix º**	**Acid (%)**	**Ratio**	**Avg seed**	**Fruit external colour**
Dina	CC	Open	21/05/2019	71 - 77	1X - 1XX	49,5	10,2	0,85	12,0	0,2	T4 - 6
Dina	CC	Open	12/06/2019	54 - 83	1 - 1XXX	46,3	12,7	1,20	10,6	0,0	T1 - 4
Dina	CC	Open	03/07/2019	70 - 76	1X - 1XX	48,7	11,7	1,15	10,2	0,6	T1 - 3
Dina	CC	Net	12/06/2019	66 - 78	1 - 1XXX	56,5	11,5	1,05	11,0	0,0	T1 - 3
Dina	CC	Net	03/07/2019	70 - 75	1X - 1XX	54,0	11,5	1,05	11,0	0,0	T1 - 3
Furr	CC	Open	21/05/2019	77 - 89	1XX - 1XXX	58,5	10,3	0,85	12,1	9,8	T3 - 6
Furr	CC	Open	12/06/2019	80 - 84	1XXX	56,4	10,9	0,80	13,6	11,3	T2 - 4
Furr	CC	Net	12/06/2019	75 - 88	1XX - 1XXX	57,7	12,3	1,10	11,2	4,8	T1 - 4
Furr	CC	Net	07/07/2019	72 - 92	1XX - 1XXX	60,3	13,0	0,75	17,3	8,3	T1 - 3
Leanri	CC	Open	09/04/2019	68 - 77	1X - 1XX	58,8	9,6	1,00	9,6	2,5	T7 - 8
Leanri	CC	Open	21/05/2019	75 - 86	1XX - 1XXX	60,9	11,7	0,85	13,8	4,5	T2 - 4

Leanri	CC	Open	12/06/2019	71 - 82	1X - 1XXX	56,9	10,5	0,90	11,7	1,0	T1 - 4
Leanri	CC	Open	03/07/2019	79 - 88	1XXX	55,6	11,6	0,80	14,5	0,0	T1 - 3
Leanri	CC	Net	29/04/2019	76 - 85	1XX - 1XXX	52,9	8,0	0,80	10,0	1,2	T4 - 6
Leanri	CC	Net	21/05/2019	75 - 85	1XX - 1XXX	63,8	9,3	0,85	10,9	0,0	T2 - 5
Leanri	CC	Net	12/06/2019	71 - 80	1X - 1XXX	41,8	10,2	0,90	11,3	0,0	T2 - 4
Leanri	CC	Net	03/07/2019	79 - 89	1XXX	46,8	9,3	0,85	10,9	0,0	T1 - 3
Meirav 119	CC	Open	21/05/2019	66 - 83	1 - 1XXX	53,4	9,0	0,80	11,3	0,0	T3 - 5
Meirav 119	CC	Open	12/06/2019	69 - 80	1X - 1XXX	50,9	10,0	0,70	14,3	1,5	T1 - 3
Meirav 119	CC	Open	03/07/2019	70 - 78	1X - 1XXX	51,3	10,8	0,85	12,7	0,5	T1 - 3
Meirav 119	CC	Net	12/06/2019	68 - 79	1X - 1XXX	50,2	10,9	0,70	15,6	0,0	T1 - 3
ARCCIT 9 LS	CC	Open	12/06/2019	72 - 76	1XX	48,5	10,7	0,80	13,4	0,0	T1 - 5
ARCCIT 9 LS	CC	Open	03/07/2019	68 - 75	1X- 1XX	53,1	10,9	0,75	14,5	0,0	T1 - 4
ARCCIT 9 LS	CC	Net	12/06/2019	70 - 79	1X - 1XXX	54,8	10,6	0,60	17,7	0,0	T3 - 5
ARCCIT 9 LS	CC	Net	03/07/2019	64 - 75	1 - 1XX	54,2	10,5	0,65	16,2	0,0	T1 - 4
Nadorcott ARC	CC	Open	12/06/2019	69 - 74	1X - 1XX	53,1	11,0	0,60	18,3	3,0	T3 - 4
Nadorcott ARC	CC	Open	03/07/2019	68 - 72	1X - 1XX	50,3	10,0	0,85	11,8	1,4	T1 - 3
Nadorcott ARC	CC	Net	12/06/2019	70 - 77	1X - 1XX	51,6	11,0	0,85	12,9	0,0	T3 - 5
Nadorcott ARC	CC	Net	03/07/2019	70 - 77	1X -1XX	52,9	12,6	0,95	13,3	0,0	T1 - 3
Nova	CC	Open	29/04/2019	70 - 80	1X - 1XXX	54,1	8,3	0,56	14,8	0,2	T4 - 6
Nova	CC	Open	21/05/2019	68 - 85	1X - 1XXX	43,0	8,4	0,55	15,3	2,8	T3 - 5
Nova	CC	Net	09/04/2019	64 - 70	1 - 1XX	56,3	8,4	0,80	10,5	0,0	T7 - 8
Nova	CC	Net	29/04/2019	67 - 84	1 - 1XXX	50,9	8,1	0,65	12,5	0,0	T3 - 6
Nova	CC	Net	21/05/2019	70 - 84	1X - 1XXX	54,6	9,4	0,60	15,7	0,0	T2 - 4
Page	CC	Open	09/04/2019	61 - 69	2 - 1X	53,3	8,7	0,80	10,9	1,5	T7 - 8
Page	CC	Open	29/04/2019	64 - 78	1 - 1XXX	64,9	8,3	0,65	12,8	8,2	T4 - 6
Page	CC	Open	21/05/2019	69 - 79	1X - 1XXX	53,2	8,5	0,65	13,1	3,5	T2 - 4
Page	CC	Open	12/06/2019	71 - 80	1X - 1XXX	47,7	8,9	0,75	11,9	0,5	T1 - 3
Page	CC	Open	03/07/2019	72 - 76	1XX	46,8	9,7	0,80	12,1	2,2	T1 - 3
Page	CC	Net	09/04/2019	60 - 74	2 - 1XX	64,6	9,2	0,85	10,8	0,0	T7 - 8
Page	CC	Net	29/04/2019	60 - 88	2 - 1XXX	52,1	8,1	0,65	12,5	4,7	T3 - 5
Page	CC	Net	21/05/2019	68 - 78	1X - 1XXX	54,7	9,2	0,8	11,5	0,0	T2 - 4
Page	CC	Net	12/06/2019	65 - 72	1 - 1XX	50,0	10,4	0,75	13,9	0,0	T1 - 3
RHM	CC	Open	09/04/2019	61 - 68	2 - 1X	57,4	9,1	0,60	15,2	6,7	T7 - 8
RHM	CC	Open	29/04/2019	67 - 78	1 - 1XXX	52,1	7,6	0,50	15,2	11,5	T4 - 6
RHM	CC	Open	21/05/2019	67 - 77	1 - 1XX	54,8	9,6	0,70	13,7	16,2	T3 - 6
RHM	CC	Open	12/06/2019	64 - 78	1 - 1XXX	47,6	11,0	0,55	20,0	2,2	T2 - 4
RHM	CC	Open	03/07/2019	69 - 75	1X - 1XX	55,9	12,1	0,70	17,3	6,3	T1 - 3
RHM	CC	Net	09/04/2019	58 - 65	3 - 1	58,8	8,8	0,70	12,6	3,8	T7 - 8
RHM	CC	Net	29/04/2019	67 - 75	1 - 1XX	49,5	9,0	0,50	18,0	6,8	T4 - 6
RHM	CC	Net	21/05/2019	69 - 78	1X - 1XXX	56,9	9,1	0,60	15,2	6,0	T3 - 6
RHM	CC	Net	12/06/2019	64 - 70	1 - 1X	55,1	10,0	0,55	18,2	0,0	T1 - 4
RHM	CC	Net	03/07/2019	67 - 74	1 - 1XX	58,6	11,2	0,70	16,0	1,4	T1 - 3

Saint Andre	CC	Open	09/04/2019	64 - 71	1 - 1X	49,3	8,5	0,70	12,1	2,8	T7 - 8
Saint Andre	CC	Open	29/04/2019	76 - 88	1XX - 1XXX	52,4	9,2	0,65	14,2	13,2	T4 - 6
Saint Andre	CC	Open	21/05/2019	70 - 85	1X - 1XXX	51,5	9,3	0,60	15,5	2,8	T3 - 5
Saint Andre	CC	Net	29/04/2019	84 -90	1XXX	45,2	6,3	0,60	10,5	1,7	T4 - 6
Samba	CC	Net	09/04/2019	62 -68	2 - 1X	56,1	9,7	0,70	13,9	0,0	T7 - 8
Samba	CC	Net	29/04/2019	69 - 84	1X -1XXX	51,3	9,2	0,55	16,7	1,8	T4 - 6
Samba	CC	Net	21/05/2019	64 - 80	1 - 1XXX	47,1	8,6	0,60	14,3	0,0	T1 - 3

5.4.11 PROGRESS REPORT: Evaluation of Valencia selections in the intermediate production areas (Nelspruit)

Project 963A by J. Joubert (CRI)

Summary

The Valencia season starts with mid-maturing selections and proceeds to the late maturing selections suitable for this intermediate production area. Recommendations have therefore, been made accordingly. Midknight was first to mature in the middle of the Valencia season as a control for the trial; fruit size peaked at count 88. McClean SL followed, producing the best crop on the trees (217 kg per tree) with good internal quality and soft fibre. Jassie followed after McClean SL with good production (180 kg per tree) and compact tree canopy. Kobus du Toit Late followed slightly after Jassie and fits in before Skilderkrans. Skilderkrans follows next with completely seedless fruit and very good juice levels on Sunki 812 (60%). Valencia Late is currently one of the latest maturing Valencia selections that are being planted commercially, developing small to medium fruit size, smooth rind texture and good yield.

Opsomming

Die Valencia seisoen begin met mid-rypwordende seleksies en duur voort met die laat rypwordende seleksies in die intermediere produksie areas en aanbevelings is daarvolgens gebaseer. Midknight sal eerste ryp word in die middel van die Valencia seisoen as kontrole vir die proef; vruggrootte piek by telling 88. McClean SL kan nou volg, wat die beste oes op die bome produseer het (217 kg per boom) met goeie interne kwaliteit en sagte vesel. Jassie volg na McClean SL met goeie produksie (180 kg per boom) en kompakte boom volume. Kobus du Toit Laat volg net na Jassie en pas in voor Skilderkrans. Skilderkrans volg dan met totaal saadlose vrugte en baie goeie sap vlakke op Sunki 812 (60%). Val Late is huidiglik een van die laatste rypwordende Valencia seleksie wat kommersieel aangeplant word, met klein tot medium vruggrootte, gladde skil tekstuurvertraagde en goeie produksie.

Objectives

Determine the suitability of seven Valencia selections on Swingle Citrumelo (SC) and Sunki 812 citrandarin (Sunki) rootstocks, in an intermediate citrus production area, by evaluating internal fruit quality, crop production and export fruit counts.

Materials and methods

Trees were planted at Crocodile Valley, Nelspruit in February 2012 and harvested for the first time in August 2019 to determine crop production, internal quality and fruit size distribution. Scion cultivars included Jassie, McClean SL, Kobus du Toit Late, Late Valencia (control), Skilderkrans, and Midknight (control) for this intermediate production area. SC and Sunki rootstocks were used to compare the performance of the different combinations. Trees were planted in a commercial Midknight Valencia orchard under optimal commercial production management and practices.

Table 5.4.11.1. List of Valencia selections evaluated at Crocodile Valley (Nelspruit) during the 2019 season.

Selection	Rootstock	Planted
Jassie	SC, Sunki 812	2012
Kobus du Toit Late	SC, Sunki 812	2012
McClean SL	SC, Sunki 812	2012
Midknight	SC, Sunki 812	2012
Skilderkrans	SC, Sunki 812	2012
Val Late	SC, Sunki 812	2012

Results and discussion

Brix:acid ratio indicates harvest maturity and 'Midknight' on Sunki and SC were first to mature, followed by 'McClean SL' on both rootstocks, Late Valencia on Sunki and then 'Kobus du Toit Late'. 'Skilderkrans' with ratios between 8.5 (SC) and 8.6 (Sunki) was next in line. Jassie confirmed its status as a late maturing Valencia selection, reaching optimum just before 'Late Valencia' on SC which was the last cultivar to mature.

The highest juice content was obtained with 'Midknight' on Sunki (60.3%) and Skilderkrans also on Sunki (12.5) had the highest Brix levels. 'Jassie' and 'Kobus du Toit Late' had the highest seed counts, from 1 up to 4 seeds per fruit, with the rest <1. 'McClean SL' on SC cropped the best average yield (217 kg/tree), followed by Kobus du Toit Late on SC with 213 kg/tree and Jassie on SC producing 180 kg/tree. The lowest yield was 'Skilderkrans' on Sunki (93.6 kg/tree), bearing in mind it is a completely seedless variety and requires fruit set manipulation. All the combinations peaked at count 88 due to a heavy fruit set, except for 'Skilderkrans' and 'Kobus du Toit Late' on SC, and 'Late Valencia' on both rootstocks which peaked at count 105/125.

Conclusion

The 2019 season was the first harvest and evaluation of all the sections at Crocodile Valley's trial site. Trees are still young; we need to generate production data with future evaluations over numerous seasons to ensure good comparisons between the different scion:rootstock combinations.

Documentations of all aspects relevant to sustainable Valencia production need to be part of scion, rootstock combination evaluation. The first season`s results in this trial indicate clear interactions between rootstock and scion i.t.o. yield, fruit size, internal quality as well as canopy volume. Over the next few seasons, data from this project will give direction in identifying the optimum tree canopy size (reduce harvest time, pruning, spray volume) without compromising on yield or fruit quality.

Table 5.4.11.2. Internal fruit quality data for Valencia selections at Crocodile Valley (Nelspruit) during the 2019 season.

Selection	Root-stock	Date harvested	Juice (%)	Brix º	Acid (%)	Ratio	Avg. seed	Colour
Jassie	SC	08/07/2019	56,9	11,8	1,65	7,15	1,3	T1 - 3
Jassie	SC	06/08/2019	56,1	11,6	1,40	8,29	3,2	T1 - 3
Jassie	US812	08/07/2019	54,8	11,4	1,65	6,91	1,2	T1 - 4
Jassie	US812	06/08/2019	51,7	11,3	1,35	8,37	2,5	T1 - 4
K du Toit Late	SC	08/07/2019	59,6	11,0	1,60	6,88	1,0	T2 - 5
K du Toit Late	SC	06/08/2019	52,7	11,2	1,30	8,62	4,0	T1 - 4
K du Toit Late	US812	08/07/2019	58,8	10,4	1,40	7,43	2,2	T1 - 4
K du Toit Late	US812	06/08/2019	54,7	11,4	1,30	8,77	1,3	T1 - 3
McClean SL	SC	08/07/2019	57,9	11,1	1,20	9,25	0,0	T1 - 3
McClean SL	SC	06/08/2019	56,1	11,0	1,15	9,57	0,0	T1 - 3
McClean SL	US812	08/07/2019	56,6	11,4	1,45	7,86	0,5	T1 - 4
McClean SL	US812	06/08/2019	54,5	10,9	1,10	9,91	0,0	T1 - 4
Midknight	SC	08/07/2019	57,9	12,1	1,70	7,12	0,0	T1 - 4
Midknight	SC	07/08/2019	58,4	11,2	1,05	10,67	0,0	T1 - 3
Midknight	US812	08/07/2019	56,4	11,8	1,35	8,74	1,0	T2 - 4

Midknight	US812	07/08/2019	60,3	12,1	1,05	11,52	0,0	T1 - 3
Skilderkrans	SC	08/07/2019	56,6	11,3	1,65	6,85	0,0	T2 - 5
Skilderkrans	SC	07/08/2019	52,2	11,0	1,30	8,46	0,0	T1 - 3
Skilderkrans	US812	08/07/2019	60,0	12,4	1,60	7,75	0,0	T1 - 4
Skilderkrans	US812	07/08/2019	59,3	12,5	1,45	8,62	0,0	T1 - 3
Val Late	SC	08/07/2019	58,3	10,5	1,55	6,77	0,0	T1 - 4
Val Late	SC	06/08/2019	52,9	11,3	1,40	8,07	0,0	T1 - 4
Val Late	US812	08/07/2019	58,0	11,1	1,50	7,40	1,0	T1 - 4
Val Late	US812	07/08/2019	57,8	11,1	1,25	8,88	0,0	T1 - 4

Table 5.4.11.3. Fruit size distribution for Valencia selections at Crocodile Valley (Nelspruit) during the 2019 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Jassie	Sunki 812	> 48	0,11	Jassie	SC	> 48	0,00
Jassie	Sunki 812	48	1,51	Jassie	SC	48	0,06
Jassie	Sunki 812	56	4,41	Jassie	SC	56	0,99
Jassie	Sunki 812	72	12,51	Jassie	SC	72	7,91
Jassie	Sunki 812	88	58,95	Jassie	SC	88	67,20
Jassie	Sunki 812	105/125	22,52	Jassie	SC	105/125	23,84
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
McClean SL	Sunki 812	> 48	0,17	McClean SL	SC	> 48	0,00
McClean SL	Sunki 812	48	0,71	McClean SL	SC	48	0,36
McClean SL	Sunki 812	56	3,10	McClean SL	SC	56	2,56
McClean SL	Sunki 812	72	9,84	McClean SL	SC	72	8,27
McClean SL	Sunki 812	88	53,23	McClean SL	SC	88	50,27
McClean SL	Sunki 812	105/125	32,96	McClean SL	SC	105/125	38,53
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
K du Toit	Sunki 812	> 48	0,00	K du Toit	SC	> 48	0,00
K du Toit	Sunki 812	48	0,70	K du Toit	SC	48	0,02
K du Toit	Sunki 812	56	4,06	K du Toit	SC	56	0,36
K du Toit	Sunki 812	72	15,31	K du Toit	SC	72	2,16
K du Toit	Sunki 812	88	41,81	K du Toit	SC	88	45,98
K du Toit	Sunki 812	105/125	38,11	K du Toit	SC	105/125	51,48
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Val Late	Sunki 812	> 48	0,00	Val Late	SC	> 48	0,00
Val Late	Sunki 812	48	0,79	Val Late	SC	48	0,06
Val Late	Sunki 812	56	5,33	Val Late	SC	56	0,58
Val Late	Sunki 812	72	18,33	Val Late	SC	72	3,41
Val Late	Sunki 812	88	33,15	Val Late	SC	88	20,27
Val Late	Sunki 812	105/125	42,40	Val Late	SC	105/125	75,67
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Skilderkrans	Sunki 812	> 48	0,28	Skilderkrans	SC	> 48	0,29
Skilderkrans	Sunki 812	48	3,49	Skilderkrans	SC	48	1,48
Skilderkrans	Sunki 812	56	15,27	Skilderkrans	SC	56	5,86
Skilderkrans	Sunki 812	72	30,14	Skilderkrans	SC	72	12,83
Skilderkrans	Sunki 812	88	30,54	Skilderkrans	SC	88	29,29
Skilderkrans	Sunki 812	105/125	20,28	Skilderkrans	SC	105/125	50,26
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Midknight	Sunki 812	> 48	0,13	Midknight	SC	> 48	1,55
Midknight	Sunki 812	48	2,35	Midknight	SC	48	6,56
Midknight	Sunki 812	56	5,96	Midknight	SC	56	15,31

Midknight	Sunki 812	72	15,42	Midknight	SC	72	30,44
Midknight	Sunki 812	88	38,28	Midknight	SC	88	31,72
Midknight	Sunki 812	105/125	37,86	Midknight	SC	105/125	14,41

Table 5.4.11.4. Production per tree for Valencia selections at Crocodile Valley (Nelspruit) during the 2019 season.

Cultivar	Rootstock	Kg/tree (2019)	Tree Volume m3
Jassie	Sunki 812	123,1	18,9
Jassie	SC	180,4	22,1
McClean SL	Sunki 812	149,1	19,5
McClean SL	SC	217,0	31,0
K du Toit	Sunki 812	120,9	19,9
K du Toit	SC	212,7	23,0
Val Late	Sunki 812	107,0	16,7
Val Late	SC	148,6	22,7
Skilderkrans	Sunki 812	93,6	14,8
Skilderkrans	SC	139,5	25,8
Midknight	Sunki 812	116,6	18,1
Midknight	SC	116,6	19,1

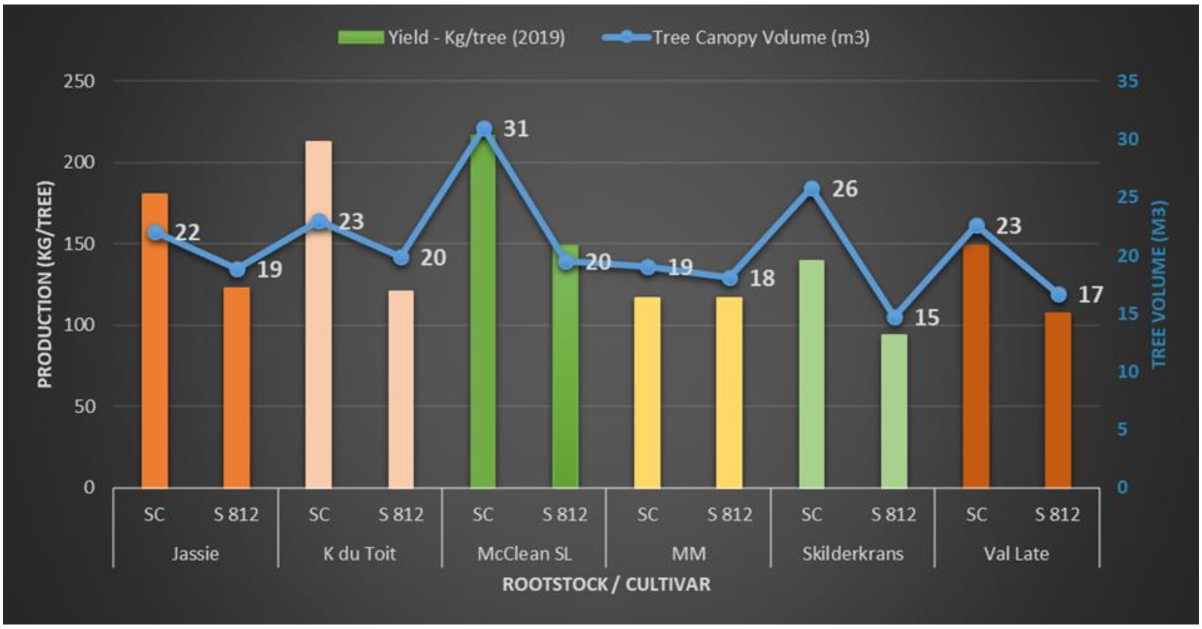

Figure 5.4.11.1. Average production per tree (kg/tree) versus tree canopy volume (m³) of seven Valencia cultivars grafted on Swingle citrange (SC) and Sunki citrandarin (S 812) in Nelspruit area.

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 trifoliate (US812). 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 up this season due to trees adjusting to the new pruning techniques to improve fruit size. Star Ruby outperformed Nelruby during the season. Both selections' fruit size ranged counts 64 – 40.

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 rootstock, Citrange 35 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 (USi 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 verbeter hierdie seisoen a.g.v. die bome wat aangepas het met die nuwe snoeipraktyke om vrug grootte te verbeter. Star Ruby het beter presteer hierdie seisoen as Nelruby in hierdie proef. Albei seleksies se vruggrootte het gepiek tussen grootte 64 – 40.

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. Die verdwergde onderstam, Citrange 35 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 were budded onto the following rootstocks at Cedarberg nursery in 2010: Benton Citrange, Citrange 35, Carrizo Citrange, Rough Lemon, Sunki 812, Swingle Citrumelo, Terra Bella and X639. The trees were planted at Karsten in March 2012.

Table 5.4.12.1. The 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 fourth time this season with a fair to good crop on the trees.

Star Ruby

The lowest crop production for the 2019 season was in combination with BC yielding 73 kg/tree (2018; TC – 20.9 kg/tree) and the best on RL yielding 105.9 kg/tree (2018; RL – 66.5 kg/tree), selected for high pH soil conditions (Table 5.4.12.4). The second highest crop was produced on CC with 99.9 kg/tree, and the average yield for the Star Ruby trial was 90.8 KG (2018 – 38.8 kg). Internally fruit quality was good with Brix ranging from 8.8 up to 10.9 (average 9.6) and juice levels above 54% (Table 5.4.12.2).

The acid content remained fairly high this season at1.36% average (slightly higher than 2018), The higher Brix increased the Brix:acid ratio to 7.0 (higher compare to 2018 ratio 6). Fruit size was smaller compared to 2018 due to the bigger crop. Most of the fruit peaked at (count 64) followed by count 48 and count 40.

Nelruby

Nelruby on TB produced the best juice content (66.7%), but the lowest Brix:acid ratio of 6.99. SC produced the highest Brix:acid ratio 8.55 followed by Sunki 812 and BC all above 8 ratios, (Table 5.4.12.2). Sunki 812 had the highest Brix 10.8 followed by BC and C35 both dwarfing rootstocks. The external colour development on all 6 rootstock combinations peaking at T1 to T3/4. Most of the combinations peaked at count 64 (2018 peaked at count 36), followed by counts 48 and 40. The best crop on the Nelruby trees was in combination with SC (114.6 kg/tree), followed by TB (92.2 kg/tree) and BC (86.8 kg/tree).

Conclusions

The seed content in the Star Ruby fruit remained significantly lower in comparison with the Nelruby fruit. Fruit size distribution from Star Ruby and Nelruby was the same (counts 36, 48 and 40). The smaller fruit size was due to the bigger crop on the trees. Star Ruby cropped a better yield on the trees (average 90.8 kg/tree versus 86.2 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 were evaluated on eight and six rootstocks, respectively, the most important combination of the above mentioned was Sunki 812 (Sunki mandarin x Beneke trifoliate). Sunki 812 was selected for replanting conditions, very specific high pH and calcareous soils. The first and second evaluations 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 1 June 2019.

Cultivar	Root-stock	Juice (%)	Brix º	Acid (%)	Ratio	Avg. seed	Colour
Nelruby	BC	54,4	10,4	1,29	8,06	0,8	T1-3
Nelruby	C35	59,6	10,2	1,31	7,79	1,0	T1-4
Nelruby	Sunki 812	51,1	10,8	1,29	8,37	1,7	T1-3
Nelruby	SC	56,6	10,0	1,17	8,55	1,5	T1-3
Nelruby	TB	66,7	9,3	1,33	6,99	0,8	T1-3
Nelruby	X639	52,6	9,4	1,33	7,09	2,5	T1-3
Star Ruby	BC	56,3	10,1	1,42	7,14	0,0	T1-3
Star Ruby	C35	55,8	9,6	1,39	6,93	0,0	T1-2
Star Ruby	CC	59,5	9,9	1,34	7,42	0,0	T1-2
Star Ruby	RL	59,1	8,8	1,23	7,15	0,0	T1-3
Star Ruby	Sunki 812	58,1	10,9	1,43	7,62	0,0	T1-3
Star Ruby	SC	55,2	9,4	1,34	7,01	0,0	T1-2
Star Ruby	TB	54,5	9,3	1,47	6,33	0,0	T1-3
Star Ruby	X639	57,8	9,4	1,27	7,40	0,0	T1-2

Table 5.4.12.3. Fruit size distribution at Karsten Boerdery during the 2019 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	BC	27	1,10	Nelruby	BC	27	0,34
Star Ruby	BC	32	4,60	Nelruby	BC	32	1,34
Star Ruby	BC	36	12,93	Nelruby	BC	36	6,83
Star Ruby	BC	40	26,37	Nelruby	BC	40	20,72
Star Ruby	BC	48	30,83	Nelruby	BC	48	27,60
Star Ruby	BC	64	24,18	Nelruby	BC	64	43,17
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Star Ruby	C35	27	1,68	Nelruby	C35	27	0,75
Star Ruby	C35	32	4,61	Nelruby	C35	32	1,98
Star Ruby	C35	36	15,34	Nelruby	C35	36	10,32
Star Ruby	C35	40	29,86	Nelruby	C35	40	23,58
Star Ruby	C35	48	24,69	Nelruby	C35	48	24,74
Star Ruby	C35	64	23,82	Nelruby	C35	64	38,62
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Star Ruby	CC	27	0,34	Nelruby	Sunki 812	27	0,41
Star Ruby	CC	32	1,69	Nelruby	Sunki 812	32	3,57
Star Ruby	CC	36	6,53	Nelruby	Sunki 812	36	13,88
Star Ruby	CC	40	19,10	Nelruby	Sunki 812	40	23,21
Star Ruby	CC	48	27,42	Nelruby	Sunki 812	48	27,52
Star Ruby	CC	64	44,92	Nelruby	Sunki 812	64	31,41
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**
Star Ruby	RL	27	1,05	Nelruby	SC	27	0,89
Star Ruby	RL	32	2,94	Nelruby	SC	32	4,64
Star Ruby	RL	36	17,94	Nelruby	SC	36	15,18
Star Ruby	RL	40	30,22	Nelruby	SC	40	29,13
Star Ruby	RL	48	25,71	Nelruby	SC	48	25,53
Star Ruby	RL	64	22,14	Nelruby	SC	64	24,64
Cultivar	**Rootstock**	**Size**	**% Fruit**	**Cultivar**	**Rootstock**	**Size**	**% Fruit**

Star Ruby	Sunki 812	27	1,46
Star Ruby	Sunki 812	32	3,56
Star Ruby	Sunki 812	36	10,43
Star Ruby	Sunki 812	40	25,30
Star Ruby	Sunki 812	48	26,60
Star Ruby	Sunki 812	64	32,66
Cultivar	**Rootstock**	**Size**	**% Fruit**
Star Ruby	SC	27	0,50
Star Ruby	SC	32	1,90
Star Ruby	SC	36	8,32
Star Ruby	SC	40	22,51
Star Ruby	SC	48	28,21
Star Ruby	SC	64	38,55
Cultivar	**Rootstock**	**Size**	**% Fruit**
Star Ruby	TB	27	0,31
Star Ruby	TB	32	1,35
Star Ruby	TB	36	9,16
Star Ruby	TB	40	24,25
Star Ruby	TB	48	29,14
Star Ruby	TB	64	35,80
Cultivar	**Rootstock**	**Size**	**% Fruit**
Star Ruby	X639	27	0,00
Star Ruby	X639	32	1,21
Star Ruby	X639	36	6,14
Star Ruby	X639	40	17,63
Star Ruby	X639	48	27,62
Star Ruby	X639	64	47,39

Nelruby	TB	27	0,16
Nelruby	TB	32	1,70
Nelruby	TB	36	7,67
Nelruby	TB	40	20,50
Nelruby	TB	48	29,55
Nelruby	TB	64	40,42
Cultivar	**Rootstock**	**Size**	**% Fruit**
Nelruby	X639	27	0,06
Nelruby	X639	32	0,97
Nelruby	X639	36	5,66
Nelruby	X639	40	17,37
Nelruby	X639	48	29,79
Nelruby	X639	64	46,14

Table 5.4.12.4. Production per tree of Star Ruby and Nelruby Grapefruit trees on different rootstocks at Karsten Boerdery (Kakamas) during the 2019 season.

Cultivar	Rootstock	2017 Kg/tree	2018 Kg/tree	Kg/tree (2019)
Nelruby	BC	75.3	28,1	**86,8**
Nelruby	C35	80.2	74,4	**73,4**
Nelruby	Sunki 812	51.8	31,7	**76,6**
Nelruby	SC	83.9	48,7	**114,6**
Nelruby	TB	62.3	7,9	**92,2**
Nelruby	X639	62.1	35,1	**74,1**
Star Ruby	BC	49.9	34,3	**73,0**
Star Ruby	C35	58.4	45,2	**86,8**
Star Ruby	CC	61.7	30,7	**99,9**
Star Ruby	RL	62.0	66,5	**105,9**
Star Ruby	Sunki 812	58.5	40,8	**95,9**
Star Ruby	SC	67.8	32,7	**89,0**
Star Ruby	TB	58.2	20,9	**96,4**
Star Ruby	X639	58.6	39,4	**80,0**

5.4.13 **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

2019 was the fourth 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, Ueno, Aoshima, Dobashi Beni, and the season was finished off with Imamura. 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 2019 seisoen was die bome se vierde drag. Die bome was in 2012 getopwerk na die volgende seleksies toe, wat ook dien as die volgorde van rypwording; Miho Wase, Ueno, Aoshima, Dobashi Beni en die seisoen was afgesluit met Imamura. 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 are considered over mature. This process is approximately three weeks long. Fruit harvested before and after this period would result in higher instances of quality and rind issues.

Table 5.4.13.1. List of Satsuma selections evaluated at Invercloy (Kirkwood) during 2019.

Selection	Rootstock	Topworked
Aoshima	Carrizo	2012
Miho Wase	Carrizo	2012
Ueno	Carrizo	2012
Dobashi Beni	Carrizo	2012
Imamura	Carrizo	2012

Results and discussion

<u>Aoshima</u>
Aoshima was the third selection to reach peak maturity. The fruit size count for Aoshima was count 1xx - 1xxx (big fruit). Aoshima juice percentage was just above 51% (low). In all 4 seasons so Aoshima had a low juice %. Brix was 8.5°, third lowest Brix for the season compared to some of the other selections. The selection

started with a low acid %. 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 and very pebbly.

Miho Wase

Miho Wase was the first selection to mature to peak maturity this year. Miho Wase are also used as the control. The selection had a big fruit size count of 1xx - 1xxx. Juice percentage for Miho Wase was lower with 50.9% this season compared to last season's 54.6% juice. Miho Wase had a Brix° of 8.3° and acid percentage of 0.64% with a 13 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 end of 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 higher this season around 52%, compared to last season's 50.5%. Ueno Brix° was 8.3° and the acid percentage was 0.72% at a ratio of 11.5. Ueno had no seeds and the colour of Ueno on the colour plate was a T7 towards peak maturity and a T6 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, and it was the last selection to reach peak maturity. Imamura normally reach peak maturity beginning to end of May in cool production regions. The juice percentage for Imamura was the highest with a juice percentage of 57.8%. Imamura Brix° was 10.5° the highest of all the selections and a very good acid of 1.13% at a ratio of 9.3. Seed count was seedless. The colour development was T5 on the colour plate, one of the best compared to the other selections, but still delayed. The 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 second last selection to reach peak maturity. Fruit size count was very good with count 1 – 1x. The juice percentage towards peak maturity, was 56.9%. Brix° was 7.8° and acid % was 0.91% towards peak maturity. The acid percentage was low to start off with, 0.91%. Internal colour is deep orange, rind is smooth and peelability is easy. Dobashi Beni was seedless. This selection had a delayed external colour development being T6 on the colour plate during at 11.2 ratio being over mature.

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. Imamura had the best juice percentage being 57.8 % followed by Dobashi Beni 56.9%. All the other selections had a juice percentage below 55%. Imamura had the highest Brix° at peak maturity being 10.5 and Dobashi Beni had the lowest Brix° being 8.2° of all the Satsuma selections. All the selections were seedless.

Table 5.4.13.2. Internal fruit quality data for Satsuma selections in the Addo and Kirkwood region of the Eastern Cape during the 2019 season.

Date	Cultivar	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2019-04-08	Aoshima	CC	1xx	51.5	7.8	0.87	9.0	0	T 7
2019-04-28	Aoshima	CC	1xxx	51.9	8.5	0.74	11.5	0	T 6
2019-03-25	Dobashi Beni	CC	1	51.9	7.7	1.24	6.2	0.1	T 8

2019-04-08	Dobashi Beni	CC	1x	56.9	7.8	0.91	8.6	0	T 7
2019-04-28	Dobashi Beni	CC	1x	50.7	8.2	0.73	11.2	0.0	T 6
2019-04-08	Imamura	CC	1xx	57.0	9.3	1.23	7.6	0	T 7
2019-04-28	Imamura	CC	1xx	57.8	10.5	1.13	9.3	0.0	T 5
2019-06-10	Imamura	CC	1xx	38.1	10.9	0.61	17.9	0.0	T 1
2019-03-25	Miho Wase	CC	1xxx	50.9	8.3	0.64	13.0	0	T 6
2019-04-08	Miho Wase	CC	1xx	51.1	9.3	0.58	16.0	0	T 5
2019-04-08	Ueno	CC	1xx	52.2	8.1	1.07	7.6	0	T 7
2019-04-28	Ueno	CC	1xxx	52.7	8.3	0.72	11.5	0.0	T 6

5.4.14 **PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (Sundays River Valley)**

Project 997B by W. Swiegers and Z. Zondi (CRI)

Summary

The mandarin trial is divided into two different trial sites. Two of the sites are in the Kirkwood region of the Sundays River Valley. In the Dunbrody trial site some of the selections have an interstock and it is the second harvest for those selections. The selections in Dunbrody trial site are as follows and this is also the order of ripening: Saint Andre, Samba, Tasty1, RHM, Edit x Nova, Tango, Leanri, Gold Nugget, Mor 26, IRM 2, IRM 1, Tanor Late and Shani SL. At the other site in the Kirkwood region, we evaluated the following selections in their order of ripening: Etna, Sirio and Tanor Late 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 nl. Kirkwood. By die Dunbrody proef perseel in die Kirkwood area is van die seleksies op 'n tussenstam getopwerk. Die volgorde van rypwording by die perseel was as volg: Saint Andre, Samba, Tasty1, RHM, Edit x Nova, Tango, Leanri, Gold Nugget, Mor 26, IRM 2, IRM 1, Tanor Late en Shani SL. By die ander perseel in Kirkwood is al die seleksies direk op die onderstam en was die volgende seleksies geevalueer in hulle orde van rypwording: Sirio, Etna en Tanor Late.

Objectives

- To select mandarin hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability, internal fruit quality (Brix, acidity and ratio), seedlessness and extended harvest period (both earlier and later maturity).
- To describe the characteristics of new mandarin hybrid cultivars and to determine the climatic suitability of these cultivars in cold production regions

Materials and methods

Field evaluations and laboratory analyses were conducted on mandarin hybrid selections from the Sundays River Valley. A range of new mandarin hybrids has been added to this area. The following varieties were evaluated: Edit x Nova, Saint Andre, Leanri, Gold Nugget, RHM, Samba, Tanor Late, Tango, IRM 1, IRM 2, Shani SL, Mor 26, Etna, Sirio, 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 5.4.14.1. List of Mandarin hybrid selections from Kirkwood region of the Sundays River Valley (Dunbrody) during the 2019 season.

Selection	Rootstock	Topwork
Saint Andre	Carrizo	2013
Edit x Nova	Carrizo with Midknight interstock	2015
Leanri	Carrizo with Midknight interstock	2015
Gold Nugget	Carrizo with Midknight interstock	2015
RHM	Carrizo with Midknight interstock	2015
Samba	Carrizo with Midknight interstock	2015
Tanor Late	Carrizo with Midknight interstock	2015
Tango	Carrizo	2013
Tasty 1	Carrizo with Midknight interstock	2015
IRM 1	CC	
IRM 2	CC	
Shani SL	CC	
Mor 26	CC	

Table 5.4.14.2 List of Mandarin hybrid selections from Kirkwood region of the Sundays River Valley (Invercloy) during the 2019 season.

Selection	Rootstock	Topwork
Etna	Carrizo	2012
Sirio	Carrizo	2012
Tanor Late	Carrizo	2012

Results and Discussion

RHM

RHM had a good fruit size count of 1x. Acids can below and drop quickly, the acid percentage towards peak maturity was 1.03% at ratio 10.5. The external colour development was T4 on the colour plate towards peak maturity. The selection has good Brix° above 10° and acid % just around 1.0% towards peak maturity. Juice percentage for RHM was very high just below 60%. This selection was seedless. The fruit is firm with a smooth rind and a deep orange internal colour.

Edit x Nova

Edit x Nova is an early to mid-maturing mandarin hybrid. It had a good fruit size count ranging from 1x - 1xxx. Edit x Nova hangs 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 good internal quality. The selection has good Brix° close to peak maturity of 12.6° and acid % just above 1.00%. This internal quality contributes to good fruit flavours. Juice percentage for Edit x Nova was lower this season at 50.6%. 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

Saint Andre was the first selection to reach peak maturity. Close to peak maturity the Saint Andre has a very good juice percentage peaking at 59.2%. Fruit size of Saint Andre was good, ranged from 2 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 10.5° towards the build up to peak maturity and 11.6° close to 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 T2 on the colour plate. Rind is slightly pebbly and flesh is deep orange. The 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 just above 50 %. At peak maturity, the Gold Nugget had a T1 colour on the colour plate. Brix° increased toward peak maturity to a high Brix of 12.5° at peak maturity. Acid percentage did decrease a bit, but the acid percentage was still good at 0.90% 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. Tango was T2 on the colour plate at peak maturity. Juice percentages (around 56.6%) at peak maturity. The fruit size for Tango was count 1. Tango was one of the selections with the highest Brix° and acid percentage this season at peak maturity. Brix° was 13.3° and acid percentage was 1.10% at peak maturity. The rind is shiny and smooth and the flesh has a deep orange colour.

Etna

Etna is an early maturing mandarin hybrid. Etna reached peak maturity 1st on this trial site. External colour development was delayed T6 on the colour plate at peak maturity. Etna will be able to degreen. Fruit size count is 1 – 1x. Juice percentage for Etna was very good, just above 60% at peak maturity. Etna had 0.2 – 0.9 seeds per fruit during all the evaluations. Etna was one of the selections with the lowest sugar and acid percentage at peak maturity. Brix° 9.2° and acid percentage was 0.77%. Etna has a deep orange internal colour.

Sirio

Sirio is also an early maturing mandarin hybrid. It was the second selection to reach peak maturity at this trial site. Fruit size count for Sirio was count 1 - 1xxx. Juice percentage was above 50% for the selection at peak maturity. Seed count was low 0.0 – 0.8 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. Peelability was not easy.

Tanor Late

Tanor Late is a late-maturing mandarin hybrid. Fruit size count for the selection was large with a 1xxx count on both sites and with an interstock. Tanor Late had a good juice percentage, above 55 % (peak maturity) directly on the rootstock and below 55% with an interstock and was seedless. Brix: Acid ratio towards peak maturity was good, the Brix: Acid ratio was better directly on the rootstock. The selection with the interstock is 3 years younger than the selection directly on the rootstock. Both selections are relatively young. 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 but there are small thorns on the bearing branches.

Samba

Samba is an early to mid-maturing mandarin hybrid selection. Samba on Carrizo rootstock with Midknight interstock produced a good 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. T5 on the colour plate towards peak maturity and T2 at peak maturity. Fruit were virtually seedless this season in the combined trial block and fruit size peaked (count 2 to 1) very good fruit size. Juice % towards peak maturity was very good above 55%. Brix was already at 10.2° towards peak maturity and acid 0.93%.

Leanri

Leanri is also an early to mid-maturing mandarin hybrid. It had a good fruit size count ranging from 1 - 1xx. Slightly smaller compared to last season's count of 1xxx. Leanri have a very good juice percentage. Juice % was just below 60% towards peak maturity. The sugars and acid percentage was one of the highest at peak maturity. The Brix° was 12.9° and acid % of 1.05% when the ratio was 12.3. This internal quality contributes to the good fruit flavour. The selection was virtually seedless during the evaluations with seed count of 0.0 – 0.3 seeds per fruit. 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 fruit size count was 1xxx (big fruit). Juice percentage was below 50% (very low). Brix 10.4 and acid % was 0.91% towards peak maturity. Colour development was delayed for Tasty 1 being T5 on the colour plate. The selection was seedless and had a good crop.

IRM 1&2

IRM 2 external colour development was better, more of darker orange colour, with less ribbing on the fruit compared to IRM 1. IRM 1&2 produced very good internal quality fruit close to 60% juice, Brix above 14 and acids above 1.0%. IRM 1&2 reached T1 on the colour plate before peak maturity. Seed counts for IRM 2 were very low and peaked at 2.0 compared to IRM 1 with 2.4 seeds per fruit in the trial block.

Shani SL

Shani SL fruit size was small to medium, fruit size (count 3 to count 2). Rind colour is deep orange colour (T1) before peak maturity. Internal quality was very good in the season with juice above (60%), Brix above (14) and acids around 1.3%. The fruit was completely seedless. Shani SL good internal quality contributes to the good flavour and eating quality.

Mor 26

The fruit size count was good, with count 1x, large fruit. The external colour development peaked between T2 and T1. Mor 26 internal quality was good with juice levels of up to 60% (peak maturity), Brix above 14.0% and fairly high acid levels (around 1.0%). The seed count peaked at 0.7 seeds in the fruit.

Conclusion

The following selections had the largest fruit size count with a 1xxx count: Edit x Nova, Tanor Late, Tasty 1 and Sirio. The selections with the highest juice percentage above 60% were IRM 2, Mor 26, Shani SL and Etna. The selections with the highest °Brix above 14 was IRM 1&2, Mor 26 and Shani SL. IRM 1&2 were the selections that had the most seeds per fruit. Most of the selections had a colour T1 on the colour plate.

Table 5.4.14.3. Internal fruit quality data for Mandarin hybrid selections from Kirkwood region of the Sundays River Valley (Dunbrody) during the 2019 season.

Date	Selection	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2019-04-28	Edit x Nova	CC with Valencia Interstock	1x	56.6	11.7	1.25	9.4	0.0	T 8
2019-05-20	Edit x Nova	CC with Valencia Interstock	1x	52.5	13.4	1.23	10.9	0.0	T 6
2019-06-10	Edit x Nova	CC with Valencia Interstock	1xxx	50.6	12.6	1.08	11.7	0.0	T 1
2019-06-10	Gold Nugget	CC with Valencia Interstock	1x	51.6	9.3	1.06	8.8	0.0	T 6
2019-07-01	Gold Nugget	CC with Valencia Interstock	1x	51.5	10.5	0.98	10.7	0.0	T 4

2019-07-29	Gold Nugget	CC with Valencia Interstock	1x	50.7	12.3	0.90	13.7	0.0	T 1
2019-07-01	IRM 1	CC	1x	56.2	14.6	1.56	9.4	2.4	T 1
2019-07-29	IRM 1	CC	1x	58.3	16.1	1.37	11.8	1.8	T 1
2019-06-10	IRM 2	CC	1x	59.2	11.1	1.40	7.9	0.8	T 3
2019-07-01	IRM 2	CC	1x	59.4	12.4	1.23	10.1	1.2	T 1
2019-07-29	IRM 2	CC	1x	63.0	14.0	1.21	11.6	2.0	T 1
2019-04-28	Leanri	CC with Valencia Interstock	1	59.9	10.6	1.08	9.8	0.3	T 6
2019-06-10	Leanri	CC with Valencia Interstock	1x	58.7	11.5	1.01	11.4	0.0	T 1
2019-07-01	Leanri	CC with Valencia Interstock	1xx	59.8	12.9	1.05	12.3	0.2	T 1
2019-06-10	Mor 26	CC	1x	57.1	12.6	1.45	8.7	0.0	T 3
2019-07-01	Mor 26	CC	1x	57.3	13.9	1.28	10.9	0.5	T 2
2019-07-29	Mor 26	CC	1x	60.6	15.7	1.19	13.2	0.7	T 1
2019-05-20	RHM	CC with Valencia Interstock	1x	59.6	10.8	1.03	10.5	0.0	T 4
2019-06-10	RHM	CC with Valencia Interstock	1	58.3	10.7	0.51	21.0	0.0	T 1
2019-04-08	Saint Andre	CC	2	51.8	10.5	1.15	9.1	0	T 7
2019-04-28	Saint Andre	CC	1	59.2	11.6	1.00	11.6	0.0	T 5
2019-05-20	Saint Andre	CC	1x	56.9	11.8	0.84	14.0	0.0	T 2
2019-04-28	Samba	CC with Valencia Interstock	2	57.4	10.2	0.94	10.9	0.2	T 5
2019-05-20	Samba	CC with Valencia Interstock	1	53.6	10.5	0.81	13.0	0.0	T 2
2019-07-01	Shani SL	CC	2	60.7	14.2	1.43	9.9	0.0	T 1
2019-08-21	Shani SL	CC	3	62.4	17.1	1.33	12.6	0.0	T 1
2019-05-20	Tango	CC	1	51.1	9.9	1.08	9.2	0.0	T 6
2019-06-10	Tango	CC	1	49.9	11.1	0.96	11.6	0.0	T 5
2019-07-01	Tango	CC	1	56.6	13.3	1.10	12.1	0.0	T 2
2019-07-29	Tango	CC	1	58.6	14.1	1.01	14.1	0.0	T 1
2019-07-01	Tanor Late	CC with Valencia Interstock	1xxx	52.6	10.0	0.95	10.5	0.0	T 5
2019-08-13	Tanor Late	CC with Valencia Interstock	1xxx	52.2	11.9	0.86	13.8	0.0	T 1
2019-05-20	Tasty 1	CC with Valencia Interstock	1xxx	44.5	10.4	0.91	11.4	0.0	T 5

2019-06-10	Tasty 1	CC with Valencia Interstock	1xxx	46.4	10.0	0.65	15.4	0.0	T 1

Table 5.4.14.4. Internal fruit quality data for Mandarin hybrid selections from Kirkwood region of the Sundays River Valley (Invercloy) during the 2019 season.

Date	Selection	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2019-04-08	Etna	CC	1	61.8	8.6	0.92	9.3	0.4	T 6
2019-04-28	Etna	CC	1x	61.2	9.2	0.77	11.9	0.9	T 6
2019-05-20	Etna	CC	1x	59.9	9.8	0.69	14.2	0.2	T 1
2019-04-28	Sirio	CC	1xx	52.4	9.4	0.88	10.7	0.3	T 5
2019-05-20	Sirio	CC	1xxx	50.9	9.9	0.80	12.4	0.0	T 1
2019-06-10	Sirio	CC	1	47.5	11.1	0.88	12.8	0.8	T 1
2019-07-01	Tanor Late	CC	1xxx	55.4	11.4	1.37	8.3	0.0	T 1
2019-07-29	Tanor Late	CC	1xxx	57.6	12,5	1.23	10.2	0.0	T 1
2019-08-13	Tanor Late	CC	1xxx	55.4	12.7	1.10	11.5	0.0	T 1

5.4.15 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 were planted in 2017. Both trial sites will form part of the Gamtoos River Valley. At Loerie the season started off with Sirio and was followed up by St Andre, Etna, Tasty 1, Nadorcott, Tanor Late, Tango and the season ended with Gold Nugget.

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 Sirio gevolg deur St Andre, Etna, Tasty 1, Nadorcott, Tanor Late, Tango, en die seisoen het geeindig met Gold Nugget.

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 cultivars were evaluated: Saint Andre, Etna, Sirio, Tanor Late, 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 5.4.15.1 List of experimental mandarin hybrid selections evaluated in the Loerie (N. Ferreira) region of the Gamtoos River Valley during the 2019 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
Tanor Late	Carrizo	2012
Nadorcott	Carrizo	2012

Results and discussion

Gold Nugget

The fruit size for Gold Nugget was count 1x – 1xx. Fruit for this selection is pebbly and peelability is easy. At peak maturity the external colour development was T5 on the colour plate. For this selection it is a yellow orange colour. There were thorns on the bearing branches, but they get smaller as the tree gets older. Gold Nugget is a seedless cultivar. At peak maturity the internal juice percentages were 53.5%. Internal quality for the selection is good with high sugars above 11 and good acids around 0.9%, this also contributes to the good flavour of Gold Nugget.

Tango

Tango had a very good fruit size (count 1) and was seedless, as it is a seedless variety. The selection had good external colour development T1 on the colour plate at peak maturity. Tango rind and internal colour is a deep orange. The rind also has a natural shine and peels very easily, and the rind oil doesn't bother. Tango juice percentage was below 55%. Tango's Brix and acid ratios were good, indicating that the fruit will have a good shelf life and give Tango a good flavour.

Tanor Late

Tanor Late is a late maturing mandarin hybrid. Fruit size count for the selection was extra-large with a 1xxx count. Tanor Late juice percentage, below 55 % (peak maturity). Tanor Late was seedless. Brix: Acid ratio towards peak maturity was good. The good internal quality contributes to better shelf life for the fruit and the good flavour of the fruit. External colour development was very good, being T1 on the colour plate towards peak maturity.

Saint Andre

Saint Andre was the second selection to reach peak maturity in this production region. The fruit size count was between 1 and 1x. The juice percentage this season was very good just below 60%. At peak maturity the colour was delayed T6 on the colour plate. The acids and sugars remained stable during the production season for Saint Andre. At peak maturity Brix 10.2 and Acid % were 0.82%. Internal quality was good and it contributes to the good flavour. The selection had a seed count peaking at 0.2 seeds per fruit, (virtually seedless) during the evaluations.

Etna

Etna is an early maturing mandarin hybrid. The fruit size count for the Etna this season was slightly smaller, count 1x – 1xx count, compared to last season 1xx to 1xxx. The juice percentage for Etna was good, just below 60 % juice at peak maturity. Compared with Sirio, Etna's juice % was higher. 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 T2 on the colour plate range at peak maturity. Etna's seed count peaked at 0.2 seeds per fruit, slightly lower than Sirio.

Sirio

Sirio also an early maturing mandarin hybrid. Sirio had an extra-large fruit size count of 1xx. Sirio developed a lower juice percentage compared to Etna. The juice percentage decreased towards peak maturity to below 55%. The Brix and Acid levels of Sirio were slightly higher than Etna at peak maturity. Sirio external colour development was slightly delayed, being T3 on the colour plate at peak maturity. Sirio seed count ranged between 0.0 – 0.5 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 T1 on the colour plate at 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. Towards peak maturity the Brix was 10.7 with acid % of 1.01%.

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. The selection developed good internal quality with juice levels (below 55%), Brix averaging 10.5 and acceptable acids (0.9%).

Conclusion

Half of the selections (Gold Nugget, Nadorcott, Tanor Late and Tango) had very good external colour development (T1) at peak maturity. The following selections had the largest fruit size (count 1xxx); Tanor Late and Tasty 1. Nadorcott, and Tango cropped the smallest fruit size (count 1). Etna and St Andre developed juice percentages below 60%. Tasty 1 had juice percentages below 50%. Nadorcott, Gold Nugget, Tango and Tanor Late had the highest Brix level above 11°. The selection with the highest seed count was Sirio.

Table 5.4.15.2. Internal fruit quality data for experimental mandarin hybrid selections from the Loerie (N. Ferreira) region of the Gamtoos River Valley region during the 2019 season.

Date	Selection	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2019-04-15	Etna	CC	1x	61.3	8.1	0.93	8.7	0	T 6
2019-05-06	Etna	CC	1xx	58.7	9.1	0.77	11.8	0.2	T 2
2019-05-27	Etna	CC	1	58.3	9.7	0.68	14.3	0.0	T 1
2019-06-10	Gold Nugget	CC	1x	50.8	11.1	0.93	11.9	0.0	T 5
2019-07-10	Gold Nugget	CC	1xx	53.5	12.3	0.93	13.2	0.0	T 1
2019-06-10	Nadorcott	CC	1	53.4	10.3	1.00	10.3	0.0	T 4
2019-07-10	Nadorcott	CC	1	54.4	11.2	0.83	13.5	0.0	T 1
2019-04-15	Saint Andre	CC	1	59.6	10.0	1.05	9.5	0.1	T 6
2019-05-06	Saint Andre	CC	1x	58.7	10.2	0.82	12.4	0.0	T 6
2019-05-27	Saint Andre	CC	1x	61.9	11.3	0.71	19.9	0.2	T 1
2019-04-15	Sirio	CC	1xx	55.9	8.9	1.13	7.9	0	T 6
2019-05-06	Sirio	CC	1xx	53.2	10.2	0.79	12.9	0.5	T 3
2019-06-10	Tango	CC	1	52.9	10.8	1.03	10.5	0.0	T 4
2019-07-10	Tango	CC	1	52.2	12.0	0.93	12.9	0.0	T 1
2019-07-10	Tanor Late	CC	1xxx	52.0	11.6	0.89	13.0	0.0	T 1
2019-07-25	Tanor Late	CC	1xxx	51.7	12.3	0.83	14.8	0.0	T 1
2019-04-15	Tasty 1	CC	1xx	49.4	10.0	1.63	6.1	0.2	T 7

2019-05-27	Tasty 1	CC	1x	52.9	10.7	1.01	10.6	0.0	T 5
2019-06-10	Tasty 1	CC	1xxx	46.6	11.9	0.85	14.0	0.0	T 1

5.4.16 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 cultivar block with a selection of all the new experimental cultivars from early-maturing to late maturing selections. Cross pollination was high in this block due to all the different selections present. The season started with Gold up, Tami 2/65 and then RHM followed, Lea, Edit x Nova, Furr, Etna, Sirio, Samba, Mor 26, IRM 2, Gold Nugget, IRM 1, Or 4, Tango, Nadorcott ARC and Nadorcott. At the Paarl site most of the new experimental selections were topworked. Cross pollination is also high in this site. During the season the order of ripening was as follows: Samba, Gold Nugget, Tanor Late and Tango finished off the season.

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 Gold up, Tami 2/65 gevolg deur RHM, Lea, Edit x Nova, Furr, Etna, Sirio, Samba, Mor 26, IRM 2, Gold Nugget, IRM 1, Or 4, Tango, Nadorcott ARC en Nadorcott. By die Paarl perseel is die meeste van die nuwe seleksies nou oorgewerk. Kruisbestuiwing is ook baie hoog in die perseel. Die volgorde van rypwording by die perseel was as volg Samba, Gold Nugget, Tanor Late en Tango het die seisoen afgesluit.

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 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, Nadorcott ARC, Nadorcott, Edit x Nova, Gold Up, Lea, Nova ARC, Nova, Tasty 1, Tanor Late 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 5.4.16.1. List of experimental mandarin hybrid selections evaluated in the Citrusdal region of the Western Cape during the 2019 season.

Selection	Rootstock	Topwork	Planted
Furr (Clemcott)	CC	2011	
Gold Nugget	CC	2010	

ARC Nadorcott	CC	2010	
Nadorcott	CC		2009
RHM	CC		2013
Tango	CC	2010	
Edit x Nova	CC	2010	
Gold up	CC	2015	
Lea	CC	2016	
Sirio	CC	2012	
Etna	CC	2012	
Samba	CC	2016	
IRM 1	CC		2009
IRM 2	CC	2010	
Mor 26	CC		Unknown
Or 4	CC		Unknown
Tami 2/65	CC	2010	

Table 5.4.16.2. List of experimental mandarin hybrid selections evaluated in the Paarl region (Babylonstoren & Lustigaan) of the Western Cape during the 2019 season.

Selection	**Rootstock**	**Topworked**
Samba	CC	2016
Tanor Late	CC	2016
Gold Nugget	CC	2008
Tango	CC	2012

Results and discussion

Tami 2/65

Tami 2/65 is an early maturing mandarin hybrid. Tami 2/65 was the second selection to reach peak maturity at the Citrusdal site. The fruit size for Tami 2/65 was good with a 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 T2 - T6 on the colour plate at peak maturity. The selection doesn’t have high acid and it tends to drops 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 good, just below 60% (peak maturity). The fruit size count was range from 1 - 1xxx. Furr peels easily and has 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 1.3. 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 mandarin hybrid. Etna reached peak maturity more to the mid-maturing range. The fruit size count for the Etna this season ranged from count 3 – 1, slightly smaller compared to last season’s count 1 – 1xx. Good crop could have contributed to the smaller fruit. Etna had a juice percentage below 55%. Internal quality at peak maturity for Etna was fair. Brix was around 10 and acid % around 0.8 %. Etna had a T1 – T4 on the colour plate at peak maturity. Etna have a deep orange internal colour. Etna were seedless, but it is not a seedless variety.

Sirio

Sirio is also an early experimental mandarin hybrid, but it also reached peak maturity more to the mid-maturing range the same as Etna. Fruit size count for Sirio was large to extra-large, it ranged between 1 - 1xxx. The crop was fair. Internal quality for Sirio was similar to that of Etna, although Sirio has better flavour. Sirio seed count was 0.0 – 0.4 seeds per fruit. Sirio had no external colour development problems, being fully coloured at peak maturity. Sirio has internally as well externally deep orange colour and peelability is not easy.

<u>Mor 26</u>
Size for the selection was very good with count 2 – 1x. The juice percentage for Mor 26 was very good, 61.5% juice percentage at peak maturity. Rind colour development was good to reach T1 - T2 on the colour plate at peak maturity. The seed count was 0.2 – 0.5 seeds per fruit. Internal quality was good at peak maturity Brix° above 12° and acid % were just above 1.0%. This good Brix: Acid ratio will contribute to good eating fruit with good flavour and shelf life.

<u>Or 4</u>
The size count for this selection was medium - large count 3 - 1x. The fruit is round to oblate. Juice percentage at peak maturity was very good at 61%; better than last season's 45%. Internal quality for Or is very good. Brix is high above 14° and the acids were 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 and average seed count was 0.3 seeds per fruit.

<u>Gold Nugget</u>
Tree manipulation is necessary to control the strong vegetative and upright growth habit. Gold Nugget developed good tasting fruit with a high Brix: acid ratio. The fruit peaked internally with Brix around 12.0°. 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 2 – 1xx in the Citrusdal trial site, and in the Paarl site slightly smaller fruit size count 3 – 1, at peak maturity and fully coloured (T1). The juice percentage for this selection at both sites was around 55%. Gold Nugget is seedless.

<u>Nadorcott & Nadorcott ARC</u>
Nadorcott ARC are induced Nadorcott selections to minimise the average seeds per fruit. Nadorcott ARC have the same growth habit and characteristics as the Nadorcott. Fruit size for Nadorcott ARC ranged between counts 3 – 1xx and for Nadorcott it ranged between 2 – 1x. The internal juice percentages were around 55% at peak maturity for both selections. The Nadorcott selections developed good Brix above 13° with acids around 1.00%, ensuring a good balance and eating quality as well as shelf life. 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, followed by Nadorcott.

<u>Samba</u>
Samba is an early – mid maturing experimental low seeded mandarin hybrid, with seed count that peaked at 1.0 seeds per fruit. Samba has a favourable fruit size count that range between counts 3 – 1. In the Paarl trial site, it was the first crop and the fruit size was slightly smaller count 4 – 2. At the Citrusdal trial site the crop was good for the second crop and at the Paarl site the crop was fair for the first crop. 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 has an exceptional deep orange external colour and a very deep internal colour. At peak maturity; Samba has high Brix and good acids and this give Samba its unique and excellent flavour. The juice percentage at peak maturity for this selection was 53.2%.

<u>Tango</u>
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 2 – 1x in Citrusdal. Internally the average juice percentage for Tango was around 55%. At the trial site the Brix: acid ratio was good, with (Brix 12°) and (acid 1.0%).

<u>IRM 1 & IRM 2</u>
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&2 reached peak maturity earlier than normal. IRM 1 reached peak maturity 2 weeks after IRM 2. The fruit size count for both selections ranged between 1 – 1xxx at peak maturity.

IRM 2 had higher juice content between the 2 selections. Internal quality for IRM 1 was better than that of IRM 2. IRM 1, Brix was 14.1° and acid was 1.05% at peak maturity. IRM 2 close to peak maturity was (Brix 12.5° and acid 1.00%). IRM 1&2 seed count peaked at 0.8 seeds per fruit. External colour development was good for both selections IRM 1&2 (T 1 on the colour plate) at peak maturity. IRM 1 & 2 are prone to ribbing.

<u>Gold up</u>
Gold up is an experimental early maturing mandarin hybrid. It was the first crop for Gold up. In this high pollination site Gold up seed count peaked at 1.1 seeds per fruit. The fruit size was small-medium, with count 4 - 1. Brix and acid part of internal quality was fair, Gold up had a Brix of 10.4° and acid of 0.80% towards peak maturity at a 13.0 ratio. The juice percentage was not good. The fruit had a delayed colour development T5 – T6.

<u>Lea</u>
Lea is an experimental early maturing mandarin hybrid selection. It was the fourth selection to reach peak maturity and it was also Lea's first crop. The fruit had a delayed colour development (T4) at peak maturity. The rind colour was orange and the internal colour was deep orange. Lea had a good internal quality, juice % at 50.6%, Brix (12.2°) with acids (1.02%) at peak maturity. The flavour was good and peelability easy. The selection was seedless. Fruit size count ranged 2 – 1x.

<u>RHM</u>
RHM was third to reach peak maturity at the trial site. The fruit size count medium (count 3 – 1). Acid dropped quickly but stayed stable around 0.80% at peak maturity in cold production regions and with the good sugars it contributed to good flavour and eating quality. The external colour development was delayed with T5 - T6 on the colour plate when the fruit was at peak maturity. The selection has good Brix° at 11.9° and acid % of 0.84%. Juice percentage for RHM was low. There were seed for this selection during evaluations with a count of 4.8 seeds per fruit. The fruit is firm with a smooth rind and a deep orange internal colour. Some of the fruit tends to split.

<u>Tanor Late</u>
Tanor Late is a late maturing mandarin hybrid. It was Tanor Late first crop. Fruit size count for the selection was extra-large with a 1xxx count. Tanor Late had a low juice percentage. Tanor Late was virtually seedless. Brix: Acid ratio towards peak maturity was fair. 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. Peelability for Tanor Late is easy. There are small thorns on the bearing branches.

<u>Edit x Nova</u>
Edit x Nova is an experimental early to mid-maturing mandarin hybrid. The internal quality of Edit x Nova was good with juice levels around 50%, no granulation occurs. Brix above 13.5 and good acids (1.04%). The seed count ranged 0.3 - 2.1 seeds per fruit. The fruit size peaked from count 2 - 1X. External colour development was good, being T1 on the colour plate at peak maturity.

Conclusion

IRM 1, IRM 2, Furr, Sirio and Tanor Late had the largest fruit size (1xxx). Samba, Tango, Gold up, Nadorcott ARC, Or, Etna, Gold Nugget and RHM had the smallest fruit size with a count 4 – 3. Furr had the most seeds per fruit on average (4.8 seeds per fruit) followed by Edit x Nova (2.1 seeds per fruit). Lea and Etna were the only selections that were completely seedless. The following selections had a juice percentage over 55% at peak maturity IRM 1 & 2, Mor 26, Furr, Nadorcott, Nadorcott ARC, Or, Tami 2/65, Tango and Gold Nugget. Most of the selections were a T1 on the colour plate range (good colour development) before or at peak maturity.

Table 5.4.16.3. Internal fruit quality data for experimental mandarin hybrid selections from the Citrusdal region of the Western Cape during the 2019 season.

Date	Cultivar	Rootstock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2019-04-29	Edit x Nova	CC	2	51,6	10,1	1,12	9,0	2,1	T 5 - T 6
2019-05-22	Edit x Nova	CC	1 - 1x	48,0	13,9	1,04	13,4	0,3	T 1
2019-03-26	Goldup	CC	4 - 3	44,3	10,2	0,98	10,4	0,9	T 6 - T 7
2019-04-11	Goldup	CC	3 - 1	43,4	10,4	0,80	13,0	1,1	T 5 - T 6
2019-06-18	IRM 1	CC	2 - 1xx	36,5	12,7	1,33	9,6	0,4	T 2
2019-07-09	IRM 1	CC	1 - 1xxx	57,1	14,1	1,05	13,4	0,8	T 1
2019-07-22	IRM 1	CC	1x - 1xxx	62,3	14,5	1,09	13,3	0,5	T 2
2019-05-22	IRM 2	CC	1 - 1xx	38,1	11,6	1,08	10,8	0,3	T 3 - T 5
2019-06-18	IRM 2	CC	1 - 1xxx	43,0	12,5	1,00	12,5	0,3	T 1
2019-07-09	IRM 2	CC	1 - 1xx	63,6	13,9	0,92	15,1	0,8	T 1
2019-04-11	Lea	CC	2	48,6	11,2	1,09	10,3	0,0	T 5
2019-04-30	Lea	CC	2 - 1	50,6	12,2	1,02	12,0	0,0	T 4
2019-05-22	Lea	CC	2 - 1x	40,6	12,6	0,59	21,3	0,0	T 1 & T 5
2019-05-22	Mor 26	CC	2 - 1x	42,4	12,5	1,01	12,4	0,2	T 3 - T 6
2019-06-18	Mor 26	CC	1 - 1x	44,8	14,0	1,08	13,0	0,5	T 2 - T 1
2019-07-09	Mor 26	CC	2 - 1x	61,5	14,7	0,96	15,4	0,2	T 1
2019-05-22	Furr	CC	1x - 1xxx	41,2	13,0	1,04	12,5	1,3	T 1 - T 3
2019-06-18	Furr	CC	1 - 1xx	46,9	15,3	1,15	13,3	0,7	T 1
2019-07-09	Furr	CC	1 - 1xxx	59,5	14,9	1,05	14,2	0,3	T 1
2019-07-09	Nadorcott	CC	2 - 1x	62,3	13,3	1,51	8,8	0,3	T 1
2019-07-22	Nadorcott	CC	2 - 1x	49,8	14,9	1,51	9,9	0,0	T 1
2019-08-28	Nadorcott	CC	2 - 1	56,7	15,2	1,17	12,9	0,3	T 1
2019-07-09	Nadorcott A34 ARC	CC	1 - 1xx	57,8	12,4	1,16	10,7	0,0	T 1
2019-07-22	Nadorcott A34 ARC	CC	2 - 1xx	52,9	13,9	1,19	11,6	0,1	T 1
2019-08-28	Nadorcott A34 ARC	CC	3 - 1	54,5	15,2	1,00	15,2	0,0	T 1
2019-07-09	Or	CC	2 - 1x	61,2	14,9	1,32	11,3	0,2	T 1
2019-07-22	Or	CC	2 - 1	61,0	15,1	1,22	12,3	0,8	T 2
2019-08-28	Or	CC	3 - 1	58,3	16,1	0,98	16,5	0,0	T 1
2019-04-11	RHM	CC	3 - 2	45,9	11,7	1,15	10,2	3,3	T 6 - 7
2019-04-30	RHM	CC	2 - 1	48,4	11,9	0,84	14,2	4,8	T 5 - 6
2019-04-30	Samba	CC	3 - 1	51,5	11,2	0,99	11,3	0,2	T 1, T 2, T 4
2019-06-19	Samba	CC	2 - 1	52,7	12,0	0,93	12,9	1,0	T 1
2019-04-11	Tami 2/65	CC	2 - 1x	55,1	10,2	0,94	10,8	0,0	T 5 - 6
2019-04-29	Tami 2/65	CC	2 - 1xx	55,4	10,6	0,72	14,8	0,2	T 2 - 6
2019-04-29	Etna	CC	2	54,5	9,2	1,00	9,2	0,0	T 6
2019-05-22	Etna	CC	3 - 1	51,0	10,1	0,86	11,7	0,0	T 1 - 4
2019-06-18	Gold Nugget	CC	2 - 1xx	37,4	12,0	1,28	9,4	0,0	T 3 - 5
2019-07-10	Gold Nugget	CC	1 - 1xx	54,2	12,3	0,88	14,0	0,0	T 1

2019-04-29	Sirio	CC	1x - 1xxx	37,7	9,5	0,98	9,7	0,0	T 4 - 7
2019-05-22	Sirio	CC	1 - 1xx	31,9	10,4	0,92	11,3	0,0	T 1 -4
2019-06-18	Sirio	CC	1xx - 1xxx	39,8	10,8	0,82	13,2	0,4	T 1
2019-06-18	Tango	CC	2 - 1x	54,1	12,4	1,19	10,4	0,0	T1 – 3
2019-07-10	Tango	CC	2 - 1	59,4	12,4	1,12	11,1	0,0	T 1
2019-07-22	Tango	CC	2 - 1	52,6	12,7	1,12	11,4	0,0	T 1

Table 5.4.16.4. Internal fruit quality data for experimental mandarin hybrid selections from the Paarl region (Babylonstoren & Lustigaan) of the Western Cape during the 2019 season.

Date	Cultivar	Rootstock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2019-04-30	Samba	CC	4 - 2	49,2	10,9	0,95	11,5	0,0	T 2
2019-05-23	Samba	CC	4 - 2	53,2	11,8	0,93	12,7	0,0	T 1
2019-06-14	Samba	CC	3 - 1	47,2	11,9	0,85	14,0	0,5	T 1
2019-07-03	Tanor Late	CC	Too big	41,2	9,6	1,14	8,4	0,5	T 1
2019-07-26	Tanor Late	CC	1xxx	43,8	9,7	0,72	13,5	0,0	T1
2019-06-14	Gold Nugget	CC	3 - 2	48,5	11,6	1,20	9,7	0,0	T 2 - 4
2019-07-03	Gold Nugget	CC	3 - 1	49,9	12,2	1,14	10,7	0,1	T 2
2019-07-26	Gold Nugget	CC	2 - 1	56,5	12,2	0,81	15,1	0,0	T 1
2019-06-14	Tango	CC	3 - 1	44,8	11,8	1,31	9,0	0,4	T 1
2019-07-03	Tango	CC	3 - 2	55,8	13,4	1,38	9,7	0,3	T 1
2019-07-26	Tango	CC	2 - 1x	59,9	13,7	1,07	12,9	0,6	T 1

5.4.17 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 fairly new trial site in South West Cape. The trial trees had their third crop during the 2019 season. It’s a cultivar 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 follows: Goldup, Tami 2/65, Edit x Nova, RHM, Leanri, Or 4, Taylor Lee LS, IRM 2, Mor 26 and the season ended with IRM 1.

Opsomming

Dit is ŉ nuwe proef perseël in die Suid Wes Kaap. 2019 was die 3de seisoen met vrugte op die bome. Die meeste van die nuwe eksperimentele seleksies van vroeg tot laat rypwordend 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 ŉ perseël bykom wat ook van die nuutste seleksies sal bevat. Die orde van rypwording was as volg gewees: Goldup het die seisoen begin, gevolg deur Tami 2/65, Edit x Nova, RHM, Leanri, Or 4, Taylor Lee LS, IRM 2, Mor 26 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 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, Leanri, Taylor Lee LS, 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 5.4.17.1. List of experimental mandarin hybrid selections evaluated in the Buffeljagsrivier region of the South West Cape during the 2019 season.

Selection	Rootstock	Topwork
Edit x Nova	CC	2014
IRM 1	CC	2014
IRM 2	CC	2014
Mor 26	CC	2014
Tami 2/65	CC	2014
Taylor Lee LS	CC	2014
Goldup	CC	2014
Or 4	CC	2014
RHM	CC	2014
Leanri	CC	2014

Results and discussion

Tami 2/65

Tami 2/65 is an early maturing experimental mandarin. Fruit size for Tami 2/65 was medium – extra-large with fruit size count 2 – 1xx. The internal juice percentage at peak maturity was low. Rind is smooth and the colour is a deep orange. The fruit peels easy. The selection was virtually seedless during the evaluations. Rind colour development was delayed with T2 - T4 on the colour plate at peak maturity. The selection doesn’t have high acid to start with but it had a good acid %, 0.92% at peak maturity with a Brix 11.3

Edit x Nova

Edit x Nova is an early to mid-maturing experimental mandarin hybrid. Edit x Nova had very good Brix: Acid ratio towards peak maturity, Brix° was 10.4° and acid percentage was 1.0% at ratio 10.4. This will give Edit x Nova its good flavour. The seed count peaked at 0.2 seeds per fruit. Edit x Nova colour ranged were delayed at peak maturity. Fruit size for Edit x Nova was favourable with 2 – 1xx count.

IRM 1 & 2

The IRM 1 & 2 are late-maturing experimental mandarin hybrids. IRM 2 reached peak maturity about 2 weeks earlier than IRM 1. IRM 1 & 2 are prone to ribbing and alternate bearing. The fruit size count for IRM 1 was good (count 1 – 1xxx), IRM 2 was smaller (count 5 – 1xx). Internally the juice content for both selections increased towards peak maturity to above 60%. Brix: Acid ratio at peak maturity was very similar for IRM 1 & 2, Brix was high above 16° and acid was good above 1.0%. Seed count peaked at 3.7 seeds per fruit. IRM 1 & 2 was a T1 on the colour plate before peak maturity. The rind was smooth and peelability easy.

Mor 26

Mor 26 fruit had a medium - large size count (count 2 – 1x). Towards peak maturity, Mor 26 juice percentage was 59.2%. Mor 26 internal quality was one of the best of all the selections. Very high sugars and good acids. Towards peak maturity with ratio 11.0 the Brix was already at 16.4° and acid 1.49%. The average seed count for this selection peaked at 1.9 seeds per fruit. Rind colour development was good to reach T1 colour on the colour plate towards peak maturity.

Taylor Lee LS

Taylor Lee LS is mid to late maturing experimental mandarin hybrid. The trees bore medium - extra-large fruit on the trees with count 2 – 1xx, and at peak maturity the juice content was 63.9%. Brix: Acid ratio at 12.8 the selection had Brix 16.0° and acid of 1.25%. The selection seed counts were 4.3 seeds per fruit. Taylor Lee LS reached T1 colour on the colour plate before peak maturity.

Leanri

Leanri is an experimental early – mid maturing mandarin hybrid. The average seed count for Leanri ranged between 0.0 – 0.7 seeds per fruit. Leanri had a good Brix° and acid ratio towards peak maturity (ratio 11.4), the Brix was 12.6° and acid was 1.10%. This gives Leanri its good flavour. The juice percentage for Leanri was low. The fruit was fully coloured up before peak maturity. Fruit size count was (count 2 – 1xx).

RHM

The fruit size count range from large to extra-large (count 1 – 1xx) and the acid dropped quickly towards peak maturity to below 0.80%. The external colour development was delayed with T3 – T4 on the colour plate when the fruit was at peak maturity. Towards peak maturity the selection had low Brix° at 8.7 and acid % of 0.92%. Juice percentage for RHM was low. There were seeds in this selection during evaluations with a count of 0.8 seeds per fruit. The fruit is firm with a smooth rind and a deep orange internal colour.

Or 4

Or is a mid - late maturing mandarin hybrid. The size count for this selection ranged from small to large count 5 – 1. Juice percentage increase towards peak maturity to just above 55%. Internal quality for Or is very good. Brix is high above 13.3° and the acids was still above 1.06% at peak maturity. 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 on the colour plate towards peak maturity. Average seed count was 0.8 seeds per fruit.

Goldup

Goldup is an early maturing experimental mandarin hybrid. It reached peak maturity first in the trial block. Ratio of 12.6 was reached on 2 May. Internal quality was not good. Juice percentage was low, with 9.2 Brix and acid % of 0.73%. The trees are still very young and the internal quality may improve as the trees get older. This selection does tend to have a low acid to start with and it tends to drop quickly. Colour development was delayed at peak maturity of T2 – T4 on the colour plate. Fruit size ranged between 4 – 2 (small – medium). Goldup was virtually seedless during evaluations. Fruit shape is flattish and have a natural shine on them. Peelability is easy and the rind has a prominent aroma when it gets peeled. Rind oil is high.

Conclusion

IRM 1 had the largest fruit size peaking at count (1xxx) and Goldup, Or 4 and IRM 2 had the smallest fruit size count (4 - 5). Taylor Lee LS had the most seeds per fruit on average (3.1). None of the selections were completely seedless. IRM 1&2 and Taylor Lee LS had the highest juice percentage above 60% at peak maturity. Leanri, IRM 1, IRM 2, Mor 26, Or 4 and Taylor Lee LS 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, Taylor Lee LS and Or 4.

Table 5.4.17.2. Internal fruit quality data for experimental mandarin hybrid selections from the Buffeljagsrivier region of the Western Cape during the 2019 season.

Date	Cultivar	Rootstock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2019-05-02	Edit x Nova	CC	2 - 1x	23,5	10,4	1,00	10,4	0,2	T 4 - 6
2019-05-23	Edit x Nova	CC	1x - 1xx	34,0	11,4	0,72	15,8	0,0	T 1
2019-04-08	Goldup	CC	4 - 2	28,3	8,6	0,82	10,5	0,0	T 5 - 6
2019-05-02	Goldup	CC	4 - 2	21,7	9,2	0,73	12,6	0,7	T 2 - 4
2019-07-24	IRM 1	CC	1x-1xxx	60,6	15,2	1,49	10,2	1,7	T1
2019-08-27	IRM 1	CC	1 - 1xx	61,8	16,8	1,16	14,5	3,0	T 1
2019-07-04	IRM 2	CC	5 - 3	57,7	14,0	1,30	10,8	3,7	T 1
2019-07-24	IRM 2	CC	1-1x	62,0	15,5	1,35	11,5	2,7	T1
2019-08-27	IRM 2	CC	2 - 1xx	58,5	16,6	1,08	15,3	2,6	T 1
2019-05-02	Leanri	CC	2 - 1x	17,4	10,9	1,21	9,0	0,3	T 2 - T 4
2019-05-23	Leanri	CC	2 - 1x	34,1	12,6	1,10	11,4	0,7	T 1
2019-06-13	Leanri	CC	1 - 1xx	44,2	13,7	1,04	13,2	0,0	T 1
2019-07-04	Mor 26	CC	5 - 4	46,3	14,9	1,47	10,1	2,5	T 1
2019-07-24	Mor 26	CC	2 - 1x	59,2	16,4	1,49	11,0	1,9	T 1
2019-05-23	Or 4	CC	3 - 2	43,6	11,5	1,01	11,4	0,8	T 3 - 5
2019-06-13	Or 4	CC	3 - 1	43,3	13,3	1,06	12,5	0,2	T 1
2019-07-04	Or 4	CC	5	56,4	14,1	1,02	13,9	0,3	T 1
2019-05-02	RHM	CC	2 - 1	40,9	8,7	0,92	9,5	0,8	T 6
2019-05-23	RHM	CC	1 - 1xx	38,4	10,4	0,70	14,8	0,8	T 3 - 4
2019-04-08	Tami 2/65	CC	2 - 1	37,4	10,7	1,15	9,3	0,7	T 6 - 7
2019-05-02	Tami 2/65	CC	2 - 1x	37,5	11,3	0,92	12,3	0,4	T 2 - 4
2019-05-23	Tami 2/65	CC	1 - 1xx	47,1	11,5	0,56	20,5	0,0	T 1
2019-06-13	Taylor Lee	CC	1 - 1xx	39,8	13,2	1,16	11,4	4,3	T 1
2019-07-24	Taylor-Lee	CC	2 - 1x	63,9	16,0	1,25	12,8	3,4	T1
2019-08-27	Taylor Lee	CC	1 - 1xx	62,9	16,3	0,85	19,1	1,5	T 1

5.4.18 PROGRESS REPORT: Cultivar characteristics and climatic suitability of navel oranges in a cold production region (Sundays River Valley)

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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 second crop on them. For this site the season started with: Habata Early, Lina, LF Early, Fukumoto, Washington, Cara Cara, Painter Early 2, Letaba Early, Trosky Early, Kirkwood Red, KS Early, Navelina, Glen Ora Late 2, Hutton, Clark, HE Late, Suitangi, Autumn Gold, KS Late, Witkrans 3, Carninka and the season ended with Gloudi and Glen Ora Late. The other trial site at Arundel is a late maturing trial and the season kicked off with Hutton, followed with Cambria, Gloudi, Barnfield and the season finished with Lane Late. The third site at Invercloy also had an early to late maturing selection. The season began with De Wet 1, Fischer and the last selection to reach peak maturity was Palmer.

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 tweede drag opgehad in die (2019) seisoen. Die proef bevat die meeste vroee

tot laat rypwordende nawel seleksies. Die seisoen het begin met: Habata Early, Lina, LF Early, Fukumoto, Washington, Cara, Painter Early 2, Letaba Early, Trosky Early, Kirkwood Red, KS Early, Navelina, Glen Ora Late 2, Hutton, Clark, HE Late, Suitangi, Autumn Gold, KS Late, Witkrans 3, Carninka en klaar gemaak met Gloudi en Glen Ora Late. Die ander proef perseel is by Arundel en dit is net laat rypwordende seleksies. Daar het die seisoen begin met Hutton, gevolg deur Cambria, Gloudi, Barnfield en Lane Late het die seisoen afgesluit. Die derde proef perseel is by Invercloy en die perseel bevat ook vroee tot laat rypwordende seleksies. Die seisoen het daar afgeskop met De Wet 1, Fischer en klaargemaak met Palmer.

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, Cambria, Autumn Gold, Barnfield Summer, Fischer, DeWet 1, Fukumoto, Palmer, Carninka Late, Glen Ora Late 2, Habata Early, KS Early.

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 5.4.18.1. List of navel selections evaluated at Sundays River Valley (Endulini) during 2019.

Selection	Rootstock	Planted
Autumn Gold	CC	2015
Cara Cara	CC	2015
Carninka Late	CC	2015
Clark	CC	2015
Fukumoto	CC	2015
Glen Ora Late	CC	2015
Glen Ora Late 2	CC	2015
Gloudi	CC	2015
Habata Early	CC	2015
HE Late	CC	2015
Hutton	CC	2015
Kirkwood Red	CC	2015
KS Early	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 5.4.18.2. List of navel selections evaluated at Sundays River Valley (Arundel) during 2019.

Selection	**Rootstock**	**Planted**
Hutton	CC	1997
Lane Late	CC	1997
Cambria	CC	1997
Gloudi	CC	1997
Barnfield Summer	CC	1997

Table 5.4.18.3. List of navel selections evaluated at Sundays River Valley (Invercloy) during 2019.

Selection	**Rootstock**	**Topworked**
De Wet 1	CC	2012
Fischer	CC	2012
Palmer	CC	2012

Results and discussion

Fukumoto

Fukumoto is one of the early maturing controls. Brix was down this season to 9.5° compared to last season's 11°. Fukumoto still had a very good internal quality at peak maturity. The good Brix: Acid ratio with the good juice % give Fukumoto its good flavour. The colour development was delayed T6 on the colour plate at peak maturity, compared to last season's colour plate T1 at peak maturity. Fukumoto fruit size ranged at count 56 - 64 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.

Lina

Lina was the second to reach peak maturity at this trial site and also one of the early maturing controls. The selection had a delayed colour development with a colour plate T7 when it was over mature. The selection had a good fruit size and peaked at count 64. The fruit shape was more elongated with a large navel-end (fairly open). Lina had a fair internal quality.

Trosky Early

Trosky Early is an experimental early maturing navel. The fruit size count was very good, count 56. Trosky Early had a delayed colour development (colour plate T5) at peak maturity. The selection's juice percentage decreased towards peak maturity to 51.2% at peak maturity. Internal quality was good at Brix:Acid ratio of 10.9, Brix was 10.9 and acid percentage was 0.86%. Crop was fair.

Autumn Gold

The external colour development was delayed (colour plate T6) at peak maturity. It could be that the trees are still young. The selection bore favorably sized fruit and peaked at count 56. Autumn Gold had a high juice percentage of 54.2% at peak maturity. Internal quality was very good at peak maturity especially for a young tree Brix 9.9 and acid percentage (0.93%). The navel ends were small.

Barnfield Summer

Barnfield Summer is a late maturing navel, and it was the second 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 above 50% for this selection. Barnfield Summer has a good external colour development, being a T3 at ratio 9.5. The acid remained good 1.28% until peak maturity and it was supported with high Brix of 12.1. The navel opening was small.

Glen Ora Late & Glen Ora Late 2

The fruit size count of Glen Ora Late was very good with a 56 count preferred export size compared to Glen Ora Late 2 that had fruit size count 48 - 56. The juice percentage for both selections was above 55%, Glen Ora Late juice percentage was slightly higher. Glen Ora Late external colour development was also slightly better T4 on the colour plate compared to Glen Ora late 2, T5. Both selection had very good Brix levels above 10.0° with high acids above (0.85%) towards and at peak maturity, indicating that the fruit can hang slightly longer on the trees. The rind is smooth to slightly coarse with a small to closed navel end. Glen Ora Late 2 reached peak maturity about 3 weeks earlier than Glen Ora Late, and Glen Ora Late was the last selection to reach peak maturity at the trial site.

Lane Late

Lane Late is the control for the late maturing selections. Lane Late was the last selection to reach peak maturity at the Arundel trial site. Lane Late fruit size count was 56, great size for export. Lane Late had good juice percentage above (55%) towards peak maturity. The external colour development was also good on Lane Late, (T1) by peak maturity. Lane Late also kept its acids quite well being 1.19% (good shelf life). The Brix was high 11. The flavour was good. Lane Late had small protruding navel-end on the fruit.

Habata Early

Habata Early is an experimental early maturing navel. Habata Early fruit size peaked at count 64. Habata Early was the first selection in this trial to reach peak maturity with a delayed external colour development (T7). The internal quality at peak maturity was fair, low juice percentage, Brix and acid percentage.

Clarke

Clarke's fruit size count was 56. The young trees at Endulini had a slightly higher juice % at peak maturity 55.6%. Internal quality was good for a young tree having a Brix of 9.6 and acid of 0.88%. The external colour development of Clarke at Endulini was delayed compared to the internal quality. The external colour was T6 on the colour plate.

Gloudi

Gloudi is a promising late maturing navel and it was the second last selection to reach peak maturity. The juice percentage of Gloudi was still good when the fruit was over mature, being 56.3%. Gloudi had a medium fruit size count peaking at 56 count. With a ratio of 12.6 which is considered over mature, internal quality was still good Brix 10.8 and acid percentage 0.86%. Gloudi's acid levels remained high (good shelf life). Colour development was delayed being T4 on the colour plate. Gloudi had a close to small navel end with a good crop.

Witkrans 3

Witkrans is also a very promising late maturing navel. The crop was good. The external colour development was delayed with a T6 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, around 55% and acids were still around 0.90% 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 fruit size ranged at count 64 - 72. The selection had a slight delay in colour development being at colour plate T3 at peak maturity. The juice was low just below 55% at peak maturity and Brix was 12.8° with acids above 1.30%.

De Wet 1

De Wet 1 is an experimental 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 percentage around 1 %.

Caloma

Caloma is an experimental late maturing navel with potential. It bore fruit with large fruit size and peaked at count 56. The crop was good. The external colour development of Caloma was delayed with a T6 on the colour plate at peak maturity. Acids kept quite well the Brix was high even when the fruit was over mature. Juice content was around 55% at peak maturity. Flavour was great.

Washington

Washington is the control for the mid-maturing navel selections. The external colour development was behind the internal quality of the fruit (T6) on the colour plate standards. Washington fruit size count peaked at count 56. The juice content of Washington tested around 52.8%. Brix levels above 9° with acids of 0.80%.

Fischer

Fischer is a very good early to mid-maturing control. The acid levels on Fischer navel were good, averaging around 1.0% for the season. Juice and Brix at peak maturity were 53.4% and 11.2, respectively. Externally the fruit colour development was delayed, and peaked at T5. Fruit size was optimum for navel production, fruit size (count 56). Very good crop on the trees.

Hutton

Hutton had the preferred fruit size for navel production and export (count 56). The juice percentage was 53.2% at peak maturity. Navel ends were open and the rind was coarse. Peak maturity was reached in mid-June – early July. The Brix was around 10 and acid around 0.90%, with T3 colour on the colour plate at peak maturity.

Palmer

The external colour development of the selection was delayed (colour plate T5) at peak maturity. The selection had a good fruit size ranging between counts 48 - 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%. Brix was around 10 with acid of 0.90% at ratio 11.3.

Suitangi

Suitangi was one of the late maturing experimental navel selections evaluated, the external colour development was delayed, T6 on the standard colour plate. The selection normally has deep orange rind colour and it had a fairly small navel end. There was a good crop on the trees this season. Suitangi peaked at count 56. Suitangi internal quality was very good with high juice content; every sample tested was over 54% juice. Brix levels around 10 with acids of 0.90% was achieved at peak maturity, it assured good tasting fruit with good flavour.

Cara Cara

Cara Cara is the control for pigmented navels. Internal quality was good with juice percentage of 54.5%, Brix 9.3 and acid percentage at 0.79% at a Brix: Acid ratio of 11.8 (over maturity). The external appearance was delayed at T7 on the colour plate. Fruit size is uniform to medium large, (count 56). Navel ends are small - medium. 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. 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, juice percentage (54.1%), Brix 9.1 and good acid percentage (0.94%). Fruit is firm and has a smooth peel. Colour development was delayed reaching T6 on the colour plate at peak maturity. 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 May to early June compared to last season 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 considering it was the second crop. Juice percentage was above 55% and the Brix around 10 with an acid percentage of around 1% at peak maturity. Future evaluations will determine the exact maturity period and if the internal quality will stay good. With the acid percentage that stayed stable the fruit can hang on the tree to colour up.

Kirkwood Red

Kirkwood red is a red pigmented navel. The fruit sometimes has external blush and internal colour pigment is dark red. The colour development was delayed being T4 – 6 on colour plate at peak maturity. The cultivar matured slightly later than Cara Cara navel. Kirkwood Red fruit has a relatively high juice content 55.2%. At peak maturity the Brix was 9.4 and acid was 0.88%. The fruit is small with closed end navel and fruit sized peaked at count 56. The tree is more compact compared to Cara Cara. Fruit shape is round to slightly oval.

Letaba Early

It was the second crop on the trees for this selection; future evaluations will give us more info about this experimental variety. Peak maturity was reached in May 2/3 weeks earlier than last season. Fruit size count peaked (count 56). At peak maturity (ratio 10) the internal quality was as follows: juice percentage below (55%), Brix around 9 and acid % around (0.90%). Colour development was delayed reaching T6 – T7 on the colour plate at peak maturity.

LF Early

LF Early is an early maturing experimental navel. LF Early was the third 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 good with count 64. On 08.04.19 the ratio was 9.7 very close to peak maturity. Juice percentage was 51.8%, the Brix was low (8.6) and acid 0.89%. Colour development was delayed at T7 on the colour plate.

Navelina

Navelina is a mid maturing navel like Washington and not an early maturing navel like Lina. In this trial site Navelina reached peak maturity after Washington. The tree is not as vigorous as the Washington tree. Fruit are medium to large (counts 56 - 64) with a small to closed navel. The external fruit colours, with T6 at peak maturity were behind the internal quality. Fruit shape is slightly elongated, and rinds are smooth. The flavour is very good with good sugars, acid levels and a ratio of 10.4:1

Painter Early 2

An early - mid maturing navel. External colour development was delayed at T6 on the colour plate. Fruit size is a uniform medium to large, count 56 - 64. Navel ends are small. Fruit rinds are smooth. Internal flesh colour is orange. The flavour is good with moderate internal quality.

KS Early

KS Early is an experimental mid maturing navel. It shows good potential and the crop this year was good. KS Early have a smooth rind and very small to closed navel end. Fruit is firm. KS Early fruit size count ranged between counts 56 - 64. Colour basically just break T7 on the colour plate at peak maturity. Juice percentage was just below 55%. Brix and acid percentage at peak maturity was moderate. As the trees get older the external colour and internal quality will improve.

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 Glen Ora Late 2, De Wet 1 and Palmer peaking at count 48. All of the selections had delayed colour development. The following selections had juice percentage above 55%: Witkrans 3, Lane Late, Clarke, Glen Ora Late & 2, HE Late, Kirkwood Red, Gloudi, Hutton, Coloma and Suitangi. Fischer, Barnfield, Cambria and Lane Late had the highest Brix above 11.0° for this trial at peak maturity. All the navel selections were seedless.

Table 5.4.18.4. Internal fruit quality data for early to late Navel selections from the Addo (Arundel) region of the Sundays River Valley during the 2019 season.

Date	Selection	Root stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Colour	Avg Seed
2019-06-10	Barnfield	CC	56	52.5	9.2	1.01	9.1	0.0	T 6
2019-07-01	Barnfield	CC	56	51.7	12.1	1.28	9.5	0.1	T 3
2019-06-10	Cal Lane Late	CC	56	54.1	10.1	1.33	7.6	0.0	T 5
2019-07-01	Cal Lane Late	CC	56	57.0	11.0	1.19	9.2	0.0	T 1
2019-06-10	Cambria	CC	64	52.5	11.0	1.44	7.6	0.0	T 5
2019-07-01	Cambria	CC	72	54.4	12.8	1.30	9.8	0.0	T 3
2019-06-10	Gloudi	CC	56	54.0	10.0	1.43	7.0	0.0	T 5
2019-07-01	Gloudi	CC	56	52.3	9.8	0.96	9.7	0.4	T 4
2019-06-10	Hutton	CC	56	46.6	8.9	1.03	8.6	0.0	T 4
2019-07-01	Hutton	CC	56	53.2	9.9	0.91	10.9	0.0	T 3

Table 5.4.18.5. Internal fruit quality data for early to late Navel selections from the Addo/Kirkwood (Endulini) region of the Sundays River Valley during the 2019 season.

Date	Selection	Root stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg Seed	Colour
2019-06-10	Autumn Gold	CC	56	54.2	9,9	0.93	10.6	0.0	T 6
2019-07-01	Autumn Gold	CC	56	54.1	10.6	0.80	13.3	0.0	T 3
2019-04-28	Cara Cara	CC	56	54.5	8.8	1.02	8.6	0.0	T 8
2019-05-20	Cara Cara	CC	56	54.5	9.3	0.79	11.8	0.0	T 7
2019-06-10	Carninka	CC	56	54.1	9.1	0.94	9.7	0.0	T 6
2019-07-01	Carninka	CC	56	53.9	9.8	0.78	12.6	0.0	T 4
2019-05-20	Clark	CC	56	53.0	8.9	0.99	9.0	0.0	T 7
2019-06-10	Clark	CC	56	55.6	9.6	0.88	10.9	0.0	T 6
2019-04-28	Fukumoto	CC	64	54.3	9.5	0.90	10.6	0.0	T 6
2019-05-20	Fukumoto	CC	56	50.5	10.3	0.81	12.7	0.0	T 4
2019-06-10	Glenora Late	CC	56	56.8	9.8	1.04	9.4	0.0	T 6
2019-07-01	Glenora Late	CC	56	60.0	10.7	0.95	11.3	0.0	T 4
2019-06-10	Glenora Late 2	CC	48	55.1	9.5	0.85	11.2	0.0	T 6
2019-07-01	Glenora Late 2	CC	56	57.5	10.3	0.81	12.7	0.0	T 5
2019-06-10	Gloudi	CC	56	54.8	9.7	1.03	9.4	0.0	T 6
2019-07-01	Gloudi	CC	56	56.3	10.8	0.86	12.6	0.0	T 4
2019-04-08	Habata Early	CC	64	48.3	8.4	0.83	10.1	0.0	T 7
2019-04-28	Habata Early	CC	64	51.8	8.6	0.73	11.8	0.0	T 7
2019-06-10	HE Late	CC	56	56.1	9.8	0.90	10.9	0.0	T 6
2019-07-01	HE Late	CC	56	55.9	10.5	0.83	12.7	0.0	T 4
2019-06-10	Hutton	CC	56	54.0	9.6	0.86	11.2	0.0	T 6
2019-07-01	Hutton	CC	56	55.8	10.4	0.76	13.7	0.0	T 3
2019-05-20	Kirkwood Red	CC	56	55.2	9.4	0.88	10.7	0.0	T 6
2019-06-10	Kirkwood Red	CC	56	52.9	10.2	0.90	11.3	0.0	T 4
2019-04-28	KS Early	CC	64	49.8	9.1	1.22	7.5	0.0	T 7
2019-05-20	KS Early	CC	56	54.3	8.8	0.83	10.6	0.0	T 7
2019-06-10	KS Late	CC	56	54.6	10.1	0.96	10.5	0.0	T 6

2019-07-01	KS Late	CC	56	55.7	10.7	0.83	12.9	0.0	T 5
2019-04-28	Letaba Early	CC	56	54.3	8.7	1.03	8.4	0.0	T 7
2019-05-20	Letaba Early	CC	56	51.5	9.5	0.82	11.6	0.0	T 6
2019-04-08	LF Early	CC	64	51.8	8.6	0.89	9.7	0.0	T 7
2019-04-28	LF Early	CC	64	54.3	9.1	0.74	12.3	0.0	T 7
2019-04-08	Lina	CC	64	52.1	8.8	1.02	8.6	0.0	T 8
2019-04-28	Lina	CC	64	54.8	9.3	0.71	13.1	0.0	T 7
2019-04-28	Navelina	CC	64	54.4	8.8	1.16	7.6	0.0	T 7
2019-05-20	Navelina	CC	56	54.8	9.9	0.95	10.4	0.0	T 6
2019-04-28	Painter Early 2	CC	64	54.2	9.2	1.02	9.0	0.0	T 7
2019-05-20	Painter Early 2	CC	56	53.1	9.5	0.81	11.7	0.0	T 6
2019-06-10	Suitangi	CC	56	54.9	9.6	0.90	10.6	0.0	T 6
2019-07-01	Suitangi	CC	56	57.0	10.4	0.79	13.2	0.0	T 5
2019-04-08	Trosky Early	CC	56	53.9	9.0	1.06	8.5	0.0	T 8
2019-05-20	Trosky Early	CC	56	51.2	10.9	0.86	10.9	0.0	T 5
2019-04-28	Washington	CC	56	51.8	9.0	0.96	9.4	0.0	T 7
2019-05-20	Washington	CC	56	52.8	9.2	0.81	11.4	0.0	T 6
2019-06-10	Witkrans 3	CC	56	53.4	9.6	0.92	10.4	0.0	T 6
2019-07-01	Witkrans 3	CC	56	56.7	10.4	0.84	12.4	0.0	T 5

Table 5.4.18.6. Internal fruit quality data for early to late Navel selections from the Kirkwood (Invercloy) region of the Sundays River Valley during the 2019 season.

Date	Selection	Root stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Colour	Avg Seed
2019-04-08	De Wet 1	CC	56	49.2	9.2	1.03	8.9	0.0	T 7
2019-04-28	De Wet 1	CC	56	52.6	10.0	1.01	9.9	0.0	T 6
2019-05-20	De Wet 1	CC	48	50.7	10.6	0.74	14.3	0.0	T 4
2019-04-28	Fischer	CC	56	47.7	10.1	1.17	8.6	0.0	T 6
2019-05-20	Fischer	CC	56	53.4	11.2	0.90	12.4	0.0	T 5
2019-04-28	Palmer	CC	56	45.0	9.4	1.15	8.2	0.0	T 7
2019-05-20	Palmer	CC	48	46.8	10.3	0.91	11.3	0.0	T 5

5.4.19 **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. 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 late maturing selections evaluated in order of ripening consist of Suitangi, Lazyboy and Caloma.

Opsomming

Hierdie proef bestaan uit ŉ paar eksperimentele vroeë-, middel- en laat nawel seleksies. Orde van rypwording was Painter Early 2 gevolg deur Ryan, dit is die 2 vroeë seleksies wat geevalueer was. De Wet 1 is ŉ mid-

rypwordende nawel met ’n ronde vrugvorm. Die vrugte het ’n toe nawel-ent. Die laat nawel seleksie wat ge-evalueer was en orde van rypwording bestaan uit Suitangi, Lazyboy en Caloma.

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: DeWet 1, KS Late, Lazyboy, Painter Early 2, Ryan and Suitangi

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 higher instances of quality and rind issues.

Table 5.4.19.1. List of navel selections evaluated at Loerie site in the Gamtoos River Valley, Eastern Cape during the 2019 season.

Selection	Rootstock	Topworked	Planted
De Wet 1	Carrizo	2012	
Ryan	Carrizo	2013	
KS Late	Carrizo	2012	
Lazyboy	Carrizo	2013	
Painter Early 2	Carrizo	2012	
Suitangi	Carrizo		2016

Results and discussion

<u>De Wet 1</u>
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 ranged at counts 56 - 64; 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 %.

<u>Caloma</u>
Caloma is a promising experimental late maturing navel. The fruit crop was good. Caloma bore fruit with a medium-large fruit size at count 56 - 64. The fruit shape for Caloma is round and the fruit is firm. Caloma’s internal quality was excellent and the best in the trial site. The selection internal quality was as follow at peak maturity, good juice percentage of 55.9%, high Brix 11.5° and a good acid around 1%. The external colour development of Caloma was great, T1 on the colour plate at ratio 11.4. Caloma’s rind is smooth and the navel ends are small to close. Flavour of Caloma is very good.

Ryan

Ryan was the second selection at peak maturity in this trial site. This selection had a good fruit size of count 56 at peak maturity. The external colour development range was delayed T3 (colour plate). Internal quality was moderate.

Painter Early 2

Painter Early 2 was the first selection to mature for this navel trial. Medium large size fruit was on the trees with count 56. Painter Early 2 was at T5 on the colour plate at peak maturity. The juice percentage of Painter Early 2 increased towards peak maturity to around 50%. Painter Early 2 had fair Brix and acid levels at peak maturity. Fruit shape is round with smooth rind and small navel ends.

Suitangi

Suitangi is one of the late maturing experimental navel selections evaluated. It was the first crop on the trees. Crop was fair. The external colour development was delayed; T7 on the standard colour plate at peak maturity. The selection had a small navel end. Suitangi peaked at count 56. Suitangi internal quality was good at peak maturity with juice content around 50%. Brix levels around 10 with acids of 1.0% assured good tasting fruit with good flavour.

Lazyboy

Lazyboy is a late maturing navel with good internal quality. Brix are 10.5 and the fruit hangs well on the tree with acid percentage of 1.03%. Even when the fruit is over mature the acid is still stable before it drops. 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 at peak maturity. The Navel selection with the highest juice percentage was Caloma (55.9%). All of the selections had a delayed external colour development on the colour plate at peak maturity except Caloma with a T1 on the colour plate. Caloma and Lazyboy developed the highest Brix values for this trial.

Table 5.4.19.2. Internal fruit quality data for Experimental Navel selections from the Gamtoos River Valley region of the Eastern Cape during the 2019 season.

Date	Selection	Rootstock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2019-04-15	De Wet 1	CC	56	49.6	8.6	1.03	8.3	0.0	T 8
2019-05-27	De Wet 1	CC	64	51.2	9.2	0.93	9.9	0.0	T 6
2019-06-13	De Wet 1	CC	56	47.0	9.0	0.75	12.0	0.0	T 5
2019-06-13	KS Navel	CC	56	53.5	10.8	1.25	8.6	0.0	T 6
2019-07-10	KS Navel	CC	64	55.9	11.5	1.01	11.4	0.0	T 1
2019-08-21	KS Navel	CC	56	54.2	12.0	0.88	13.6	0.0	T 1
2019-05-27	Lazyboy	CC	56	48.8	9.8	1.12	8.8	0.0	T 7
2019-06-13	Lazyboy	CC	64	44.9	10.5	1.03	10.2	0.0	T 6
2019-07-10	Lazyboy	CC	56	49.4	10.5	0.68	16.2	0.0	T 2
2019-04-15	Painter Early 2	CC	56	50.6	8.8	1.08	8.1	0.0	T 7
2019-05-07	Painter Early 2	CC	56	50.6	9.1	0.80	11.4	0.0	T 5
2019-05-07	Ryan	CC	56	49.7	9.1	0.96	9.5	0.0	T 6
2019-05-27	Ryan	CC	56	51.1	9.9	0.65	15.2	0.0	T 3
2019-05-27	Suitangi	CC	72	50.3	9.8	1.02	9.6	0.0	T 7
2019-06-13	Suitangi	CC	56	48.6	10.5	0.84	12.5	0.0	T 6

5.4.20 **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 recent 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. Fukumoto and Lina are the controls for early maturing navels. Fischer was used as control for the early-mid selections, Washington as control for the mid-maturing navel selections and Cambria for the late navel sections. Cara Cara was used as the control for the pigmented navels. Most of the trees are older and have big tree volumes. Tibshreany Early, Rayno Early, Fukumoto, Lina, 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: Cara Cara, Washington, Navelina, Hutton, Fischer, Summer Gold, Palmer, Glen Ora Late, Chislett Summer, Kirkwood Red, and Clark. The late selections that were evaluated and were last to reach peak maturity were Glen Ora Late 2, Gloudi, Witkrans 3, Barnfield, Cambria, and Carninka.

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 ŉ paar eksperimentele vroee-, middel- en laat nawel seleksies in 2 proef persele. Fukumoto en Lina is as kontrole gebruik vir die vroee seleksies. Fischer is as kontrole gebruik vir die vroee - mid seleksies, Washington word as kontrole gebruik vir die middel seleksies en Cambria dien as kontrole vir die laat nawel seleksies. Cara Cara word as kontrole gebruik vir die gepigmenteerde seleksies. Die meeste van die bome is al ouer en die bome het ŉ groot boom voloume. Die volgorde van rypwording was beginende met die vroee seleksies Tibshreany Early, Rayno Early, Fukumoto, Lina, en Gerhard Early gevolg deur die middel seleksies se volgorde, Cara Cara, Washington, Navelina, Hutton, Fischer, Summer Gold, Palmer, Glen Ora Late, Chislett Summer, Kirkwood Red, en Clark. Die seisoen was afgesluit met die laat seleksies se orde van rypwording Glen Ora Late 2, Gloudi, Witkrans 3, Barnfield, Cambria, en Carninka.

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, Lina, Hutton, Glen Ora Late 2, Clark, Chislett Summer, Barnfield Summer and Summer Gold.

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 various sites in the Citrusdal, Western Cape during the 2019 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	
Barnfield Summer	Carrizo		2016
Chislett Summer	Carrizo	2009	
Clark	Carrizo		2010
Glen Ora Late 2	Carrizo		2014
Hutton	Carrizo	2009	
Lina	Carrizo		2016
Summer Gold	Carrizo		2010
Washington	Carrizo	2009	
Carninka	Carrizo		2010
Navelina	Carrizo		2016
Rayno Early	Carrizo	2009	
Tibs Early	Carrizo		2014
Witkrans 3	Carrizo		2011
Cambria	Carrizo		2011
Palmer	Carrizo		2011

Results and discussion

<u>Barnfield Summer</u>

Barnfield Summer is a late maturing navel, and it was the third last selection to reach peak maturity, it was the selection's first crop. Barnfield Summer fruit size ranged count 40 – 56 (bigger fruit). The juice percentages were below 50% for this selection. Barnfield Summer has a good external colour development, being a T2 – T4 at ratio 11.1. The acid remained good 0.9% until over maturity and it was supported with high Brix of 10. The navel end was small.

<u>Clarke</u>

Clarke's fruit size was medium – large, count 64 - 40. The juice percentage was low. Internal quality was good at peak maturity, having a Brix of 10.2 and acid of 1.02%. The external color development of Clarke was delayed T5 on the color plate compared to the internal quality.

<u>Fukumoto</u>

Fukumoto was the third selection to reach peak maturity. Internal quality at peak maturity (ratio 9.8), the Brix was 10° and very good acid levels (1.02%). The good Brix: Acid ratio give Fukumoto its great flavour. It is also a great eating fruit. The color development was delayed, with color plate T5 at peak maturity. The acids remain stable even when the fruit was over mature (around 0.90% for 3 weeks) fruit will hang well and have a good shelf life. Fukumoto produced a small - medium size fruit which peaked at count 48. The navel-end on the fruit was medium open and protruding, one of the characteristics of the selection.

<u>Chislett Summer</u>

Chislett Summer had a delayed color development T6 at peak maturity. Fruit size count peaked at count 40, larger fruit size count. Juice percentage was slightly on the lower side. The Brix: Acid ratio for Chislett Summer at peak maturity was very good, Brix 10.5 and acid %, 1.01%. The flavour of the selection was very good.

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 trees bore small to large sized fruit that peaked at count 48. Gerhard Early had delayed color development (color plate T5) when the fruit was at peak maturity. Sugars and acids were good at peak maturity, Brix 10.5° and acid 1.10%. Crop was fair.

Fischer

Fischer (control) had a delayed colour development with colour plate T5 at peak maturity. Fischer had a good fruit size which peaked at count 48. Fischer internal quality was very good at peak maturity, Brix around 10° and acid around 0.95%. The flavour was very good. The navel 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 2

Glen Ora Late & Glen Ora Late 2 is late maturing navels with a very good flavour. Glen Ora Late 2 trees are younger than Glen Ora Late. The fruit size count of Glen Ora Late peaked at 48 count (big fruit) while Glen Ora Late 2 was slightly bigger peaking 40 count. The external colour development for Glen Ora Late was delayed (T4 – T6 on the plate) as peak maturity was reached compared to Glen Ora Late 2, T6 on the plate. Both Glen Ora Late selections acids stayed stable after reaching peak maturity. Along with the good and stable acids the Brix was also good around 10°. The rind is smooth to slightly coarse with small to close navel end. Glen Ora Late 2 had a slightly higher juice percentage. Glen Ora Late reached peak maturity before Glen Ora Late 2.

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 T2 – T4 at peak maturity, degreening would have to be done. Gloudi Brix was good 11° with acids around 1.00%. Even when the fruit was well over mature the acid was at 0.95% (good shelf life).

Hutton

Hutton had medium - large fruit size for navel production (count 64 - 40). Navel ends were open and the rind was coarse. Peak maturity was reached in May about 3 weeks earlier than last season. The Brix was around 10 and acid around 0.90%, with T4 – T7 colour on the colour plate at peak maturity.

Washington

Washington was used as control for the mid-maturing navel, and it was the second to reach peak maturity of the mid maturing selections. The external colour development was behind the internal quality of the fruit (T5) on the colour plate at peak maturity. Washington fruit size peaked at count 48. High Brix levels above 10.9° with acids of 0.97%, 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's colour development of T2 – 3 on the colour plate at over maturity, this season the colour development was T5 – T6 at peak maturity. The fruit size ranged between counts 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 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 not as good as last season's T1 on the colour plate. In the 2019 season the colour was delayed T4 – 6, but the good acids allowed us to keep the fruit longer on the trees and it did colour up to T1 on the colour plate. Internal quality for Kirkwood Red was good. The flavour was excellent. Fruit size for Kirkwood Red was medium (count 64) to large (count 40). Flesh colour was deep red; even the fruit stem was red.

Carninka Late

Carninka Late is a late maturing experimental navel. It was the last selection to reach peak maturity at the trial site. Peak maturity was reached in July. Carninka late had fruit size, count ranged between (count 64 – 48). The internal fruit quality at peak maturity, was good, Brix 11 and good acid percentage (0.87%). Fruit is firm and has a smooth peel. Colour development was T1–4 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 a mid-maturing navel. It reached peak maturity about 3 weeks after Lina and 1 week after Washington. It was the selection's first crop. 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 (T6) at peak maturity. Fruit shape is slightly elongated, and rinds are smooth. Due to the young trees and first crop the internal quality was moderate.

Lina

Lina was the fourth to reach peak maturity at this trial site and also one of the early maturing controls. The selection had a delayed colour development with a colour plate of T5 – 6 when it was at peak maturity. The selection had a good fruit size and peaked at count 48. The fruit shape was more elongated with a large navel-end (fairly open). Internal quality at peak maturity for Lina was good; Brix above 10 and acids around 1%.

Rayno Early

Rayno Early is a very early maturing experimental navel. It was the second selection to reach peak maturity at the trial site. Fruit size was small to medium with counts 88 – 56. For an early maturing navel, it managed to get very good Brix above 10 at peak maturity with stable and good acids around 1%. T5 – 7 was the colour on the colour plate when the fruit was over mature. Crop was fair.

Summer Gold

Summer Gold is one of the mid maturing navels in the trial site. Fruit size was large and fruit size count peaked at (count 40). Summer Gold internal quality was good, high Brix and good acid percentage. At ratio 11.8 which is consider over mature the Brix was 11 and acid 0.93%. Colour development was delayed compared to the internal quality, being T5 – T6 on the colour plate.

Tibshreany Early

Tibshreany Early is also an experimental early maturing navel. The crop was fair. It was the first selection to reach peak maturity at the trial site. Peak maturity was reached at the end of March – beginning April, Tibshreany Early fruit size count ranged from 88 (small fruit) - 48 (large fruit). At peak maturity the Brix was around 10 with acid percentage of 1. Tibshreany Early also kept its acid stable. Colour development was delayed with T5 at a ratio of 10.7:1.

Witkrans 3

Witkrans 3 is a very promising late maturing navel. The trees also had a good crop on them. The external colour development was delayed with a T4 – T6 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 around 1% at peak maturity. Brix was good and along with the good acid it will give Witkrans great flavour. It has a closed to small navel end.

Cambria

Cambria is a well-known late navel selection with very good internal quality. Cambria was used as control for the late selections and it reached peak maturity second last. The fruit shape was more elongated compared to the other navel selections. Cambria had small to medium sized fruit and peaked at count 56. The selection had delayed colour development being at colour plate T5 – T6 at peak maturity. The Brix was 9.7° with acids, 0.92% and Brix:acid ratio 10.6:1

Palmer

The external colour development of the selection was delayed (colour plate T5 – T7) towards peak maturity. The selection had a good fruit size and peaked at count 48. The acids were low towards peak maturity but stayed stable until it was over mature. Brix was also low.

Conclusion

The following selections (Rayno Early, Cambria and Witkrans 3) were the only selections that peaked at fruit size count 56. The selections were seedless. The best colour development was from Barnfield Summer and Carninka Late, both late selections. The selections with Brix above 10.5 at peak maturity were Carninka Late, Chislett Summer, Gerhard Early, Glen Ora Late, Gloudi, Hutton, Kirkwood Red, Lina, Summer Gold, Washington and Witkrans. Carninka late had the best juice percentage.

Table 5.4.20.2. Internal fruit quality data for Experimental Navel selections from the Citrusdal region of the Western Cape during the 2019 season.

Date	Selection	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2019-06-18	Barnfield Summer	CC	48 - 40	40,3	10,0	0,90	11,1	0,0	T 2 - 4
2019-07-09	Barnfield Summer	CC	56 - 40	46,9	10,7	0,75	14,3	0,0	T 1 - 4
2019-04-30	Cara Cara	CC	64 - 48	31,0	10,4	0,90	11,6	0,0	T 5 - 6
2019-05-22	Cara Cara	CC	56 - 48	30,2	11,7	0,82	14,3	0,0	T 2 - 4
2019-07-25	Carninka	CC	64-48	52,6	11,0	0,87	12,6	0,0	T4-1
2019-08-29	Carninka	CC	48	50,7	11,1	0,71	15,7	0,0	T 1
2019-05-22	Chislett Summer	CC	64 - 56	17,3	10,5	1,01	10,4	0,0	T 6
2019-06-18	Chislett Summer	CC	64 - 40	42,2	11,9	0,95	12,6	0,0	T 1 - 2
2019-05-22	Clark	CC	64 - 40	24,5	10,2	1,02	10,0	0,0	T 5
2019-06-19	Clark	CC	64 - 48	47,9	11,4	0,93	12,3	0,0	T 2 - 3
2019-03-26	Early Tibshreany	CC	72 - 64	36,6	9,8	1,10	8,9	0,0	T 5 - 7
2019-04-11	Early Tibshreany	CC	88 - 48	29,8	10,4	0,98	10,7	0,0	T 5
2019-04-29	Early Tibshreany	CC	72 - 56	32,2	10,1	0,84	12,0	0,0	T 3 - 5
2019-04-30	Fischer	CC	64 - 48	35,0	8,8	0,98	9,0	0,0	T 5 - 6
2019-05-22	Fischer	CC	64 - 48	30,4	11,4	0,91	12,5	0,0	T 1- 4
2019-03-26	Fukumoto	CC	72 - 56	36,3	9,8	1,20	8,1	0,0	T 5 - 6
2019-04-11	Fukumoto	CC	56 - 48	28,6	10,0	1,02	9,8	0,0	T 5
2019-04-29	Fukumoto	CC	72 - 56	32,0	11,1	0,91	12,2	0,0	T 2 - 3
2019-03-26	Gerhard Early	CC	72 - 48	36,4	10,7	1,22	8,8	0,0	T 5 - 6
2019-04-11	Gerhard Early	CC	64 - 48	26,2	10,5	1,10	9,5	0,0	T 5 - 6
2019-04-29	Gerhard Early	CC	64 - 48	26,3	11,4	1,01	11,3	0,0	T 3 - 4
2019-05-22	Glen Ora Late	CC	56 - 48	26,9	10,6	1,00	10,6	0,0	T 4 - 6
2019-06-18	Glen Ora Late	CC	56 - 48	44,9	11,6	0,95	12,2	0,0	T 1 - 3
2019-05-22	Glen Ora Late 2	CC	56 - 40	29,0	9,7	0,98	9,9	0,0	T 6
2019-06-19	Glen Ora Late 2	CC	56 - 40	48,1	10,4	0,77	13,5	0,0	T 2 - 5
2019-05-22	Gloudi	CC	56 - 48	30,8	10,6	1,16	9,2	0,0	T 5
2019-06-19	Gloudi	CC	56 - 48	51,4	11,5	0,95	12,1	0,0	T 2 - 4
2019-05-22	Hutton	CC	64 - 40	26,0	10,6	0,84	12,7	0,0	T 4 - 7

2019-04-30	Kirkwood Red	CC	64 - 40	34,3	10,1	1,06	9,5	0,0	T 6
2019-05-22	Kirkwood Red	CC	64 - 48	29,6	10,8	1,07	10,1	0,2	T 4 - 6
2019-06-18	Kirkwood Red	CC	64 - 56	44,1	11,6	0,97	12,0	0,0	T 1
2019-03-26	Lina	CC	72 - 56	40,9	10,0	1,21	8,2	0,0	T 5 - 7
2019-04-11	Lina	CC	64 - 48	29,6	10,5	1,09	9,6	0,0	T 5 - 6
2019-04-30	Lina	CC	64 - 48	35,4	11,4	0,88	13,0	0,0	T 2 - 4
2019-04-11	Navelina	CC	72 - 64	43,2	8,4	1,06	7,9	0,0	T 7
2019-04-30	Navelina	CC	56 - 48	39,0	8,9	0,89	10,0	0,0	T 6
2019-05-22	Navelina	CC	64 - 48	35,6	10,0	0,88	11,4	0,0	T 4 - 6
2019-03-26	Rayno Early	CC	72 - 56	40,8	9,7	0,99	9,8	0,0	T 5
2019-04-11	Rayno Early	CC	72 - 56	30,9	10,2	1,00	10,2	0,0	T5 - 7
2019-04-29	Rayno Early	CC	88 - 56	32,3	11,2	0,91	12,4	0,0	T 2 - 5
2019-05-22	Summer Gold	CC	56 - 40	26,5	11,0	0,93	11,8	0,0	T 5 - 6
2019-04-30	Washington	CC	64 - 48	30,3	10,9	0,97	11,2	0,0	T 5
2019-05-22	Witkrans 3	CC	72 - 56	34,3	9,9	1,14	8,7	0,0	T 5 - 7
2019-06-19	Witkrans 3	CC	72 - 56	50,8	10,9	0,87	12,5	0,0	T 4 - 6
2019-06-18	Cambria 3	CC	72 - 64	44,9	9,7	0,92	10,6	0,0	T 5 - 6
2019-07-10	Cambria 3	CC	72 - 56	48,8	10,3	0,83	12,4	0,0	T 2 & T 4
2019-04-29	Palmer	CC	72 - 48	27,6	8,8	0,95	9,2	0,0	T 5 - 7
2019-05-22	Palmer	CC	56 - 48	29,9	9,4	0,85	11,1	0,0	T 5 - 6

5.4.21 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 for a new trial site. The early maturing selection for the trial site is Turkey with Midknight as control. The mid-maturing Valencia selections are Alpha, Gusocora, Benny 2, Henrietta and Midknight 1. The late maturing Valencia selections will be McClean SL, Lavalle and Lavalle 2. At this trial site the season started with Midknight 1, followed by Turkey, Midknight, McClean SL, Henrietta, Benny 2, Gusocora, Lavalle, Lavalle 2, and the season ended off with Alpha. The order of ripening could be influenced by the fact that the area is an intermediate area for Valencias.

Opsomming

Die Valencias wat bespreek word in hierdie proef was in die 2011 seisoen getopwerk. Die bome het hulle eerste drag in die 2015 seisoen gehad. Daar is ŉ moontlikheid om ŉ perseel te begin. Die vroeë seleksie vir die proef perseel bestaan uit Turkey en Midknight wat as kontrole dien. Die mid seleksies is Alpha, Gusocora, Benny 2, Henrietta en Midknight 1. Die laat rypwordende Valencia seleksies was as volg; McClean SL, Lavalle en Lavalle 2. Die proef perseel se seisoen het begin met Midknight 1, gevolg deur Turkey, Midknight, McClean SL, Henrietta, Benny 2, Gusocora, Lavalle, Lavalle 2 en die seisoen het afgeeindig met Alpha. Die volgorde van rypwording kan beinvloed word deur die area wat n intermediere area is vir Valencias.

Objective

To select Valencia cultivars with improved and consistent productivity, fruit size, rind colour, peelability, and internal fruit quality (seedlessness, ratio), and extended harvest period (both earlier and later maturity).

To describe the cultivar characteristics of new Valencia cultivars and to determine the climatic suitability of these cultivars in a cold production region.

Materials and methods

Field evaluations and laboratory analyses were conducted on Alpha, Bennie 2, Gusocora (G5), Henrietta, Lavalle, Lavalle 2, McClean SL, Midknight (control), Midknight 1, and Turkey.

Table 5.4.21.1. Internal fruit quality minimum export requirements for Valencia types.

Cultivar	Juice %	Brix	Min Acid	Max Acids	Ratio	Colour
Valencia EU	48	8.5	0.6	1.8%	7.5:1	Colour plate 3 of set no. 34
Midknight	52	9.5	0.85	1.8%	7.5:1	Colour plate 3 of set no. 34
*Turkey	50	10.0	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34

*Interim internal fruit quality standards.

Table 5.4.21.2. List of Valencia selections evaluated at Panzi (Kirkwood) during 2019 season.

Selection	Rootstock	Topwork
Alpha	CC	2011
Bennie 2	CC	2011
Gusocora G5	CC	2011
Henrietta	CC	2011
Lavalle	CC	2011
Lavalle 2	CC	2011
McClean SL	CC	2011
Midknight	CC	2011
Midknight 1	CC	2011
Turkey	CC	2015

Results and discussion

<u>Alpha</u>

Alpha bore medium size fruit this season on the trees, peaking at count 56. Alpha Valencia were virtually 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 just below 11 supported with good acids by the time of maturity. Juice content peaked at 57.2% at peak maturity. Last selection to reach peak maturity.

<u>Bennie 2</u>

The fruit size peaked from count 56 this season, a good Valencia export fruit size. Bennie 2 has 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 0.0 – 4.3. 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 a moderate internal quality juice percentage just above 55%, Brix:Acid ratio of this selection gave it its good flavour. The crop was very good.

<u>Gusocora</u>

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 on the colour plate. When the selection reached a T1 the Brix was around 11 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 55 - 61% and the fruit size ranged between counts 64 – 56 bigger, the same as 2018 season. The fruit was firm with a round shape and a smooth rind.

Lavalle & Lavalle 2
Lavalle reached peak maturity third last and Lavalle 2 reached peak maturity second last, but with no significant difference. Lavalle & Lavalle 2 had a very good export fruit size at count 56. Lavalle also had fruit with fruit size count 64. Both Lavalle selections had high juice percentage, with Lavalle being slightly higher and it also had 0.5 seeds during one evaluation. All the rest of the evaluations on both selections were completely seedless. Brix: acid ratio for both selections was good around 11 (Lavalle 2 being slightly higher) and the acid percentage were above 1.2% (Lavalle 2 being slightly higher). 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.

Midknight 1
Midknight 1 evaluation seed count was seedless. The fruit size count was count 56, perfect for export. The juice content of Midknight 1 increased towards peak maturity to around 60%. Midknight 1 reached T1 on the colour plate before peak maturity. Brix and acid content compared to Midknight (control) was very much the same, being good with Brix above 10 and acid around 1%.

McClean SL
Fruit shape for McClean SL is a fairly round fruit with a soft fibre strength that peels easily, containing low rind oil levels. All the fruit evaluated remained completely seedless. The trees bore medium sized fruit (count 64 to 56). The internal quality was good with high juice levels for this trial site (60.6%), Brix 11 and acid around 1%. Juice content decreased as the fruit hung but not by much. There was no delay in external colour development being a T1, before peak maturity.

Midknight
Midknight was used as control in this trial site. Midknight trees had medium fruit size with 56 count. The juice content of Midknight peaked at 59.6%. The external colour development of Midknight was very good with a T1 on the colour plate range at peak maturity. Midknight internal quality was good and it gave Midknight its good flavour. The selection was seedless and crop load was good.

Turkey
Fruit size for Turkey was perfect for export with fruit size (count 64). Turkey was the second selection to reach peak maturity. Turkey juice content increased towards peak maturity above 55%; great for export. Brix was above the 10 for export. The external colour of this selection was T1 at peak maturity. Turkey seed count peaked at 3.3 seeds per fruit during the evaluations. Fruit shape was round with coarse rind. The rind colour was deep orange.

Henrietta
Henrietta juice levels peaked above 58% with moderate Brix (up to 10.4) and acids 1.01% at peak maturity. The external colour development was good; T 1 for the season. The average seeds per fruit were 0.3 seeds per fruit.

Conclusions

None of the Valencia selections had a problem with external colour development, all of them reached T1 on the colour plate at peak maturity. All of the selections' internal and external qualities complied with the minimum export requirement for Valencia types. Benny 2 had the highest count of 4.3 seeds per fruit followed by Turkey 3.3 seeds per fruit. All the other selections were virtually seedless. All of the selections had a fruit size count of 64 - 56. The following selections developed a juice content above 60% at peak maturity; McClean SL, Lavalle, Gusocora and Midknight 1.

Table 5.4.21.3. Internal fruit quality data for Valencia selections at Panzi (Sundays River Valley) during the 2019 season.

Date	Cultivar	Root stock	Count	Juice %	Brix°	Acid %	Ratio	Colour	Avg. Seed
2019-07-03	Alpha	CC	64	58.7	10.0	1.53	6.5	T 2	0.1
2019-08-13	Alpha	CC	56	57.2	10.2	1.21	8.4	T 1	0.1
2019-09-10	Alpha	CC	56	53.8	10.9	1.20	9.1	T 1	0.0
2019-09-17	Alpha	CC	56	56.7	10.9	1.07	10.2	T 1	0.0
2019-06-10	Benny 2	CC	64	55.2	9.7	1,48	6.6	T 5	0.0
2019-07-03	Benny 2	CC	64	56.8	10.3	1.46	7.1	T 1	4.3
2019-09-10	Benny 2	CC	56	56.7	10.5	1.02	10.3	T 1	2.4
2019-07-03	Gusocora	CC	64	56.4	10.0	1.31	7.6	T 3	0.0
2019-07-29	Gusocora	CC	56	61.9	10.3	1.16	8.9	T 1	0.0
2019-08-13	Gusocora	CC	56	55.9	10.8	1.15	9.4	T 1	0.0
2019-09-10	Gusocora	CC	64	56.0	11.1	1.13	9.8	T 1	0.0
2019-09-17	Gusocora	CC	56	55.2	10.0	0.92	10.9	T 1	0.1
2019-07-03	Henrietta	CC	64	58.1	9.4	1.48	6.4	T 5	0.0
2019-07-29	Henrietta	CC	64	58.3	9.8	1.28	7.7	T 1	0.3
2019-08-13	Henrietta	CC	64	58.5	10.3	1.26	8.2	T 1	1.0
2019-09-10	Henrietta	CC	56	58.9	10.4	1.01	10.3	T 1	0.0
2019-07-29	Lavalle	CC	56	59.6	10.1	1.42	7.1	T 4	0.0
2019-08-13	Lavalle	CC	56	59.3	10.1	1.28	7.9	T 2	0.0
2019-09-10	Lavalle	CC	64	60.8	10.9	1.26	8.7	T 1	0.5
2019-09-17	Lavalle	CC	56	58.7	10.8	1.08	10.0	T 1	0.0
2019-07-29	Lavalle 2	CC	56	58.4	10.6	1.57	6.8	T 5	0.0
2019-08-13	Lavalle 2	CC	56	58.0	10.8	1.58	6.8	T 1	0.0
2019-09-10	Lavalle 2	CC	56	57.2	11.3	1.37	8.2	T 1	0.0
2019-07-03	Mc Clean SL	CC	56	57.6	10.2	1.36	7.5	T 2	0.0
2019-07-29	Mc Clean SL	CC	64	60.6	10.4	1.28	8.1	T 1	0.0
2019-08-13	Mc Clean SL	CC	64	59.7	10.7	1.21	8.8	T 1	0.0
2019-09-10	Mc Clean SL	CC	64	58.9	11.1	1.05	10.6	T 1	0.0
2019-09-17	Mc Clean SL	CC	64	59.2	11.2	1.01	11.1	T 1	0.0
2019-06-10	Midknight	CC	64	54.3	9.1	1.37	6.6	T 5	0.0
2019-07-03	Midknight	CC	56	55.9	10.2	1.28	8.0	T 1	0.0
2019-08-13	Midknight	CC	56	58.0	10.3	1.11	9.3	T 1	0.4
2019-09-10	Midknight	CC	56	57.2	10.5	0.90	11.7	T 1	0.1
2019-09-17	Midknight	CC	56	59.6	10.9	0.87	12.5	T 1	0.2
2019-06-10	Midknight 1	CC	64	55.1	9.1	1.20	7.6	T 5	0.0
2019-07-03	Midknight 1	CC	56	59.6	9.9	1.13	8.8	T 2	0.0
2019-07-29	Midknight 1	CC	56	60.9	10.1	0.94	10.7	T 1	0.0
2019-08-13	Midknight 1	CC	56	60.7	10.8	0.94	11.5	T 1	0.0
2019-09-10	Midknight 1	CC	56	60.1	10.7	0.85	12.6	T 1	0.1
2019-09-17	Midknight 1	CC	56	59.0	10.6	0.78	13.6	T 1	0.0
2019-07-03	Turkey	CC	64	55.0	11.9	1.38	8.6	T 1	3.3
2019-07-29	Turkey	CC	64	56.0	12.0	1.28	9.4	T 1	2.1

5.4.22 **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 an intermediate region to farm Valencia oranges. The Valencia fruit tends to get high sugars, but acids also stay high in this region. It gives fruit with good flavour and shelf life. 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 ripening order was as follows starting with Turkey, Midknight H14, Midknight, Midknight F17, Delta, McClean SL, Val Late, Gusocora, Henrietta, Alpha, Benny, Alpha 2, Ruby Val, Louisa and the season finished off with Lavalle 2 and Lavalle.

Opsomming

Die klimaat en die grond maak die verbouing van Valencias ʼn intermediere area. Hoë suikers word verkry, maar die suur bly hoog. Dit maak vrugte met goeie geure en hou vermoe. 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, Turkey, Midknight H14, Midknight, Midknight F17, Delta, McClean SL, Val Late, Gusocora, Henrietta, Alpha, Benny, Alpha 2, Ruby Val, Louisa en die seisoen is afgesluit met Lavalle 2 en Lavalle.

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 Midknight H14 and Midknight F17, Turkey, Gusocora, McClean SL, Val Late, Henrietta, Midknight, Benny, Alpha, Louisa, Delta, Ruby Valencia, Lavalle, Lavalle 2, and the last one was Alpha 2.

Table 5.4.22.1. Internal fruit quality minimum export requirements for Valencia types.

Cultivar	Juice %	Brix	Min Acid	Max Acids	Ratio	Colour
Valencia EU	48	8.5	0.6	1.8%	7.5:1	Colour plate 3 of set no. 34
Midknight	52	9.5	0.85	1.8%	7.5:1	Colour plate 3 of set no. 34
*Turkey	50	10.0	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34

*Interim internal fruit quality standards.

Table 5.4.22.2. List of Valencia selections evaluated at Kweekkraal (Citrusdal) during 2019 season.

Selection	Rootstock	Topwork
Alpha	CC	2009
Alpha 2	CC	2016
Benny	CC	2009
Henrietta	CC	2010
Delta	CC	2009

Gusocora	CC	2009
Turkey	CC	2009
Valencia Late	CC	2009
McClean SL	CC	2009
Midknight	CC	2009
Ruby Valencia	CC	2009
Lavalle	CC	2009
Lavalle 2	CC	2011
Midknight H14	CC	2011
Midknight F17	CC	2016
Louisa	CC	2011

Results and discussion

Alpha & Alpha 2

It was Alpha 2's first crop. The internal quality for both selections was good, Brix for Alpha 2 was between 10.1 – 11.6, and Brix for Alpha was slightly higher between 11.1 – 12.3. Acids on both selections were below the max acids of 1.8% to export. Fruit size for Alpha varied from count 88 to 56, slightly on the smaller size but still good for Valencia production and export. Alpha 2 fruit size was slightly bigger and varied from count 72 – 48, it could be due to the first crop. External colour peaked on T1 for both selections. As the fruit hung on the trees the internal quality improved for export fruit of higher standards. During the three evaluations Alpha had only one seed count of 1.5 seeds per fruit. Juice percentage for both selections peaked at 57%.

Benny

The fruit size count peaked at count 72 to 56, medium fruit size and a good Valencia export size. Benny had a soft fibre strength compared to the other Valencia selections. But due to the high acid the fruit was left to hang so that the acids can come down to 1.2% and Brix up to above 11. This made that the selection matured much later but also gave it a good flavour. The selection had a much better internal quality. Seed count ranged between 0.3 – 1.3 seeds per fruit. There was no delay in external colour development (T1) before peak maturity. Bennie developed a high juice content of 60.3% towards peak maturity.

Gusocora

The fruit was left on the trees to the end of August and the fruit coloured up completely to T1 on the colour plate. The Brix and juice percentage also went up and the acids also dropped to get a fruit with very good export internal quality. The Brix was 12 and acids were still good at 1.15% 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 1.1 seeds per fruit. Fruit size count ranged from count 88 – 56. Henrietta had no problem with external colour development (T1) at peak maturity. Henrietta produced a moderate juice content below 55%, but good Brix:Acid ratio.

Lavalle and Lavalle 2

There was no major difference between Lavalle and Lavalle 2. Lavalle selections' fruit size this season was slightly larger peaking at count 48 for Lavalle, and 40 for Lavalle 2 compared to last season's very good export fruit size count peaking at 56. Lavalle selections were virtually seedless and the juice content of this selection increased towards peak maturity to a high of (57%) with a Brix: acid ratio around 8. Brix was also good along with the high acids. Lavalle 2 had a slightly higher Brix. There was a delay with the external colour development when Lavalle selections developed a T2 -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. Lavalle 2 reached peak maturity slightly earlier than Lavalle. Both selections completed the season at the trial site.

Louisa

Fruit size count for Louisa was very good ranging from count 88 – 64. The fruit has smooth rinds, but rind oil is high. Fruit shape is round to slightly elongated. Louisa's internal quality complies with all the export standards. Low juice content of 50.6%, Brix at 11 and acid 1.29%. Colour development was delayed being a T2 on the colour plate. Fruit rind colour is more of a yellow colour. Louisa were completely seedless.

Valencia Late

Valencia Late was the control for the late maturing selections. The Valencia Late produced small – medium size fruit at count 72 - 56. The internal and external quality was moderate, the juice content was low just above 50%, and Brix started off good 13 with acid content 1.43%, but colour development was delayed T4 – T2. When the fruit was left to hang to reach T1 on the colour plate, the Brix and acid percentage went down. Seed counts were between 0.6 – 0.8 seeds per fruit.

Delta

Delta, as the control cultivar, produced completely seedless fruit and a good yield on the trees. Fruit size peaked between count 88 – 56. Good internal quality (end of August), Brix of 10.9 and acid content of 1.09% and juice content 57.5%. The external colour of the fruit was T2. The fruit was round with a smooth rind and peeled fairly easily.

McClean SL

McClean SL tree bore fruit with fruit size count ranging from count 72 - 56. The selection was seedless. External colour development reached T1 on the colour plate before peak maturity. Brix was good (above 11) peaking at 10.5:1 and acids remained stable towards the end of the season around 1%, resulting in 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 did vary for Turkey with count ranging between 72 – 40 (small – large). Brix was around 11 and acids around 1.3%. This will meet the export standards as well as the external colour that was at T1 on the colour plate. Turkey were seedless. Fruit characteristics for Turkey were round fruit shape, with a very good flavour, soft rag, fairly thin rind and easy 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.

Midknight & Midknight F17 and Midknight H14

Midknight was used as control for this trial site but also as control for the other two Midknight selections. The fruit size development for Midknight peaked at count 48 followed by Midknight H14 and Midknight F17 peaking at count 56. The smallest fruit size count for Midknight and Midknight H14 was count 72, and Midknight F17 was count 88. All the Midknight selections bore round fruit on the trees with a medium to coarse rind, fibre strength was fairly soft and the fruit peeled easy. The Midknight selections were seedless. The colour development towards peak maturity was slightly delayed for all the selections, but at peak maturity all the selections were T1 on the colour plate. The trees produced well. All the Midknight selections had a good Brix of more or less the same above 10 and increasing towards peak maturity to above 11. Midknight H14 was first to reach peak maturity, followed by Midknight and Midknight F17 was the last selection to reach peak maturity.

Ruby Valencia

Ruby Valencia bore small - medium fruit that peaked at count 88 - 56. The juice content of Ruby Valencia at peak maturity was below 55%. At peak maturity Ruby's external colour development was T2 – T1 on the colour plate range. Fruit seed count ranged between 0.7 – 1.3 seeds per fruit. At peak maturity the internal quality was good. Brix was just above 11 and acid content around 1.3%. The colour of the flesh was red, and the selection has a unique taste.

Conclusions

None of the selections had problems with their external colour development because with the high acids, 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. Alpha, Ruby Valencia, Valencia Late, Benny, 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 and peaked around count 56. All the selections produced a Brix above 10, except Alpha, Benny, Gusocora, Lavalle 2, Midknight H14 and Valencia Late with Brix around 12. Acid content for the selections varied around 1.5% but when the fruit was left to hang, it reached 1.1%. Benny had the highest juice content at 60.3%.

Table 5.4.22.3. Internal fruit quality data for Valencia selections at Kweekkraal (Citrusdal) during the 2019 season.

Date	Cultivar	Root stock	Count	Juice %	Brix°	Acid %	Ratio	Avg. Seed	Colour
2019-07-09	Alpha	CC	72 - 56	54,4	11,1	1,60	6,9	0,0	T 1
2019-07-25	Alpha	CC	64	55,2	11,7	1,53	7,7	0,0	T1
2019-08-29	Alpha	CC	88 - 56	57,0	12,3	1,45	8,5	1,5	T 1
2019-07-09	Alpha 2	CC	64 - 48	52,9	10,1	1,55	6,5	0,0	T 1
2019-07-25	Alpha 2	CC	72-56	57,0	10,7	1,52	7,0	0,0	T1
2019-08-29	Alpha 2	CC	72 - 64	55,4	11,6	1,20	9,6	0,0	T 1
2019-07-09	Benny	CC	72 - 56	52,9	10,0	1,40	7,1	1,3	T 1
2019-07-25	Benny	CC	64-56	57,5	10,9	1,45	7,5	0,3	T1
2019-08-29	Benny	CC	72 - 56	60,3	11,8	1,29	9,1	0,3	T 1
2019-07-09	Delta	CC	88 - 72	54,7	10,3	1,35	7,6	0,0	T 1 & T5 - T6
2019-07-25	Delta	CC	72-56	53,5	10,1	1,22	8,3	0,0	T5-3
2019-08-29	Delta	CC	88 - 64	57,5	10,9	1,09	10,0	0,0	T 2
2019-07-25	Gusocora	CC	72-56	52,7	11,3	1,31	8,6	0,0	T3-1
2019-08-29	Gusocora	CC	88 - 56	55,4	12,0	1,15	10,4	0,0	T 1
2019-07-25	Henrietta	CC	72-56	52,5	10,7	1,33	8,1	1,1	T3-1
2019-08-29	Henrietta	CC	72 - 64	54,4	11,4	1,24	9,2	0,0	T 1
2019-07-25	Lavalle	CC	64-48	56,5	10,1	1,54	6,6	0,0	T4
2019-08-29	Lavalle	CC	72 - 56	57,8	10,7	1,41	7,6	0,0	T 3
2019-07-25	Lavalle 2	CC	72-48	52,7	11,2	1,67	6,7	0,2	T5-4
2019-08-29	Lavalle 2	CC	64 - 40	57,3	12,3	1,54	8,0	0,0	T 2
2019-07-25	Louisa	CC	88-64	51,3	10,2	1,38	7,4	0,0	T4-T2
2019-08-29	Louisa	CC	88 - 72	50,6	11,0	1,29	8,5	0,0	T 2
2019-07-25	Mc Clean SL	CC	72-56	55,2	11,2	1,17	9,6	0,0	T1
2019-08-29	Mc Clean SL	CC	72 -56	55,6	11,2	1,07	10,5	0,0	T 1
2019-07-09	Midknight	CC	72 - 56	53,8	10,6	1,25	8,5	0,0	T 1 & T 3
2019-07-25	Midknight	CC	64-48	52,5	10,7	1,17	9,1	0,0	T4-T1
2019-08-29	Midknight	CC	72 - 56	54,7	11,7	1,09	10,7	0,0	T 1
2019-07-09	Midknight F17	CC	72 - 56	54,1	10,0	1,31	7,6	0,0	T 1 - 2
2019-07-25	Midknight F17	CC	72-64	54,3	10,6	1,26	8,4	0,0	T1
2019-08-29	Midknight F17	CC	88 - 64	55,4	11,5	1,08	10,7	0,0	T 1
2019-07-09	Midknight H14	CC	72 - 56	54,0	10,2	1,07	9,5	0,0	T 1 - 3
2019-07-25	Midknight H14	CC	64-56	53,8	11,3	1,06	10,7	0,0	T1
2019-08-29	Midknight H14	CC	72 - 56	54,9	11,9	0,88	13,5	0,0	T 1
2019-07-25	Ruby val	CC	72-56	53,3	9,6	1,35	7,1	0,7	T4

2019-08-29	Ruby val	CC	88 - 56	53,8	11,2	1,26	8,9	1,3	T2 -1
2019-06-19	Turkey	CC	56	52,8	10,7	1,39	7,7	0,0	T 1
2019-07-09	Turkey	CC	64 - 40	50,4	10,9	1,33	8,2	0,0	T 1
2019-07-25	Turkey	CC	64-48	54,0	11,7	1,29	9,1	0,0	T1
2019-07-25	Val Late	CC	72-56	50,3	13,3	1,43	9,3	0,6	T4-2
2019-08-29	Val Late	CC	72 - 64	52,4	11,8	1,16	10,2	0,8	T 1

5.4.23 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

Only some of the selections were evaluated. 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. There is a new exciting site that will come into production in a few years with all the latest selections. Some of the selections are on interstock. The season started with Basol, Clemenpons, followed by Nules, Esbal, Early Esbal and ended with Large Esbal.

Opsomming

Die seleksies wat geevalueer was die seisoen is net ŉ paar van die seleksies. 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. Daar kom ŉ nuwe opwindende perseel by met al die nuwe seleksies. Van die seleksies is op ŉ tussen stam. Die seisoen het begin met Basol, Clemenpons, gevolg deur Nules, Esbal, Early Esbal en ge-eindig met Large 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 cultivars were evaluated: Basol, Clemenpons, Early Esbal, Large Esbal, 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 5.4.23.1. List of Clementine selections evaluated at Invercloy (Kirkwood) during 2019.

Selection	Rootstock	Planted
Esbal	Carrizo	2012
Nules	Carrizo	2012

Table 5.4.23.2. List of Clementine selections evaluated at Invercloy (Kirkwood) during 2019.

Selection	Rootstock	Topworked
Clemenpons	Carrizo with Midknight as interstock	2015
Basol	Carrizo with Midknight as interstock	2015
Early Esbal	Carrizo with Midknight as interstock	2015
Large Esbal	Carrizo with Midknight as interstock	2015

Results and discussion

Basol

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. This one is with an interstock. Fruit size for Basol was small with count 3. Internal quality was excellent for Basol at ratio 14.4:1 the juice content was 65.6%, Brix 12.8 and acid 0.89%. This selection was seedless. External colour break was delayed T2 on the colour plate. The fruit peels easily. Basol tends to have a very short harvest period before the fruit is over mature and starts to granulate.

Clemenpons

Clemenpons was the second 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 an interstock to see if it will help with the galls. So far there are no galls present. Fruit shape is round and the flesh is pale. Crop was good. Seed count peaked at 0.3 seeds per fruit during evaluations. Fruit size peaked at count 3. Clemenpons had a very good internal quality with juice around 60% and Brix above 10 and acids around 0.90% at peak maturity. Colour development was delayed, peaking on T5 – T6 on the colour plate.

Esbal & Early Esbal & Large Esbal

Esbal was used as control for the two experimental Esbal selections. Early Esbal is selected to mature before Esbal and Large Esbal is selected to crop bigger fruit than Esbal. The order of ripening for the 3 selections were Esbal first, followed by Early Esbal and Large Esbal finished the season. Factors that can contribute to the order of ripening is: Esbal trees are older and were topworked directly onto CC rootstock. Early – and Large Esbal trees were topworked onto Valencia interstock with CC as rootstock, and it was also the second crop for these trees. Early Esbal had slightly smaller fruit peaking count 2 while Esbal and Esbal Large fruit size peaked at count 1. Esbal had the best internal quality, highest juice content, and the best Brix:acid ratio towards peak maturity. Early Esbal were completely seedless, Large Esbal seed count peaked at 1.3 seeds per fruit and Esbal seed count peaked at 1.2 seeds per fruit. Early Esbal were the only selection to reach T1 on the colour plate towards peak maturity, Esbal and Large Esbal were T6 on the colour plate. Fruit was round to oblate with a smooth to pebbly rind

Nules

Nules were the third selection to reach peak maturity. The selection had a high juice percentage (56%) at peak maturity. Nules had a favourable fruit size count 2 - 1. Internal quality for Nules at peak maturity was good; Brix (10.8°) and acid (0.9%). Nules also kept its acids well. This contributes to Nules good flavour. Nules average seed count was 1.16 seeds per fruit. Rind colour development was not good with T7 on the colour plate at peak maturity, it did improve to T1 when the fruit were left to hang. Peelability is easy and the internal colour is orange.

Conclusion

Nules had the highest seed count of all the selections that were evaluated, seed count of 1.9 seeds per fruit. Most of the selections had delayed colour development at peak maturity except Early Esbal with T1. Degreening practices will be essential after harvesting to ensure optimal colour development. Basol had the highest Brix (12.8°) above Esbal (11.3). Basol and Clemenpons had the smallest fruit size count 3. All the selections had a good juice percentage: all over 50%, with Basol, Clemenpons and Nules the highest above (55%).

Table 5.4.23.3. Internal fruit quality data for Clementine selections in the Sundays River Valley region of the Eastern Cape during the 2019 season.

Date	Cultivar	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2019-04-08	Basol	CC	3	65.6	12.8	0.89	14.4	0.0	T 2
2019-04-08	Clemenpons	CC	3	60.4	10.2	0.91	11.2	0.3	T 6
2019-04-28	Clemenpons	CC	3	58.1	10.6	0.82	12.9	0.0	T 5
2019-04-28	Early Esbal	CC	3	57.3	9.2	0.92	10.0	0.0	T 2
2019-05-20	Early Esbal	CC	2	50.2	9.2	0.88	10.5	0.0	T 1
2019-04-28	Large Esbal	CC	2	56.0	10.0	1.10	9.1	1.3	T 7
2019-05-20	Large Esbal	CC	1	50.2	9.7	0.99	9.8	0.3	T 6
2019-04-28	Esbal	CC	2	59.8	10.9	1.03	10.6	1.2	T 6
2019-05-20	Esbal	CC	1	54.6	11.3	0.81	14.0	0.8	T 5
2019-04-08	Nules	CC	3	53.9	10.0	1.18	8.5	1.9	T 8
2019-04-28	Nules	CC	2	56.0	10.8	0.90	12.0	1.6	T 7
2019-05-20	Nules	CC	1	52.0	10.8	0.81	13.3	0.0	T 1

5.4.24 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 were two 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. Cultifort started the season in Citrusdal followed by Basol, Basol with interstock, Early Esbal, Clemenpons, Octubrina, Clemenpons new, Nules, Esbal, Saratoga, Clemenluz and Large Esbal 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 vroee-, mid – en laat seleksies. Die vroee seleksies gaan nog ŉ belangrike rol speel in die toekoms soos wat dit begin oorvleul met Satsumas. Cultifort het die seisoen in Citrusdal begin, gevolg deur Basol, Basol met tussenstam, Early Esbal, Clemenpons, Octubrina, Clemenpons new, Nules, Esbal, Saratoga, Clemenluz en Large Esbal 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, Basol, Basol with interstock, Cultifort, Early Esbal, Large Esbal, Octubrina, Clemenluz 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 5.4.24.1. List of Clementine selections evaluated at Kweekkraal and Stargrow (Citrusdal) during 2019.

Selection	Rootstock	Planted	Topworked
Clemenpons	Carrizo	2010	
Saratoga	Carrizo		2012
Nules	Carrizo	2009	
Basol	Carrizo		2010
Basol interstock	Carrizo		2011
Esbal	Carrizo	2009	
Clemenpons new	Carrizo		2010
Cultifort			2016
Early Esbal			2011
Large Esbal			2011
Octubrina			2016
Clemenluz			2009

Results and discussion

Clemenpons and Clemenpons new
Clemenpons is an 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 were higher compared to Clemenpons. Clemenpons had higher Brix and acid percentage compared to Clemenpons new selection at peak maturity. Internal quality was good for both selections. The colour development was delayed for both at peak maturity but the Clemenpons selection had better colour development. Clemenpons had a higher seed count peaking at 3.3 seeds per fruit compared to Clemenpons new selections peaking at 0.6 seeds per fruit.

Cultifort
Cultifort is a new early maturing experimental Clementine selection. It was the first selection to reach peak maturity at the trial site. Peak maturity was reached by the end of March. It was the first crop for the trees. Internal quality and juice content were good, Brix was high 11.6 with an acid percentage of 0.98% at peak maturity (ratio 12). Fruit were left to hang to reach over maturity (ratio 13) and the acids were 0.88%, still good and stable. Colour development was delayed, being T6 at peak maturity and T5 on the colour plate when the fruit was over mature. Fruit size ranged count 3 – 1xx. Average seed count during all the evaluations was 0.4 seeds per fruit. Rind was smooth and easy to peel.

Basol and Basol interstock
Basol is one of the earliest maturing Clementine selections. Basol trees tend to develop galls on the trunk but the Basol trees with the navel interstock do not develop galls. Fruit size on Basol was bigger compared to Basol on the interstock, Basol fruit size was large – extra-large (count 1 – 1xx) compared to Basol interstock fruit size small to medium with count (4 – 2). Basol with interstock had better internal quality compared to Basol on CC at peak maturity: Juice percentage was better, Brix was higher 13.2 compared to 11.9. Acid percentage in ratio with the good Brix were 1% for Basol with the interstock and 0.85% for Basol on CC. Acid content stayed stable after peak maturity. The selection on CC had a lower seed count 0.1 seeds per fruit and Basol with interstock had 0.9 seeds per fruit. External colour break for Basol on CC at peak maturity were T1 – T4 compared to Basol with interstock T2 – T5 on the colour plate. Basol has a very short harvest period before the fruit is over mature and starts to granulate.

Esbal & Early Esbal & Large Esbal

Esbal was used as control for the 2 experimental Esbal selections. Early Esbal is selected to mature before Esbal and Large Esbal is selected to crop bigger fruit than Esbal. The order of ripening for the 3 selections was Early Esbal first, followed by Esbal and Large Esbal finished the season at this trial site. Early Esbal had slightly smaller fruit peaking count 3 while Esbal Large fruit size peaked at count 2 and Esbal fruit size peaked at count 1. Juice content for all 3 selections were more or less the same between 50% - 55% at peak maturity. Large Esbal had the best internal quality, the best Brix:acid ratio at peak maturity Brix 12.8 and acid 1.10% (ratio 11.6:1). Early Esbal were virtually seedless, Large Esbal seed count peaked at 0.8 seeds per fruit and Esbal seed count peaked at 4.4 seeds per fruit. All 3 selections had delayed colour development at peak maturity, Large Esbal had the best colour development T1 – T4, Early Esbal T3 – T6 and Esbal T6 on the colour plate. The fruit was round to oblate with a smooth to pebbly rind

Nules

Nules was used as control. At peak maturity fruit size count was 3 – 1 (medium – large). The internal quality was good with Brix (11.1°) and acid (1.01%) towards peak maturity ratio 11:1. Acids stayed stable 0.8% even when the fruit was well over mature. Internal colour development was delayed, being T6 on colour plate at peak maturity. Those acids will give the fruit good shelf life and the high sugars with acids give Nules its good flavour. The rind is smooth and thin and it peels easily. The seed count was 3.4 – 5.9 seeds per fruit.

Octubrina

Octubrina is a new experimental early maturing Clementine. It must have sweet orange as an interstock. It reaches peak maturity about 2 – 3 weeks before Nules. It was the first crop for the trees and it was good for a first crop. Fruit size range was very good for Clementine production, with fruit size count 3 – 1. At peak maturity (ratio 12:1), the Brix was 11 and acid percentage was 0.91%. Acids stayed stable after peak maturity, which is very good. Seed count ranged between 0.8 – 1.1 seeds per fruit, it will be seedless in an isolated block. External colour development was delayed at peak maturity being T4 – T6 on the colour plate. The selection does degreen very well. The fruit is round to flattish and peelability is easy.

Saratoga

Fruit size for Saratoga was large to extra-large with a fruit size count of 1 – 1xxx. Fruit size was bigger than last season due to the lighter crop. The juice content was low; it could be due to granulation. External colour development was delayed for Saratoga, T5 – T6 on the colour plate at peak maturity. The internal quality for Saratoga was moderate at peak maturity, Brix 9.3, with acid percentage being 0.77%. The selection did have seeds during the evaluations, 0.5 – 0.6 seeds per fruit.

Clemenluz

Clemenluz is an early maturing Clementine selection. Nules was used as a control for this section. The Clemenluz reached peak maturity after Nules according to the ratio. Compared to Nules, Nules had better internal quality, higher juice percentage, higher Brix and better acids at peak maturity. Clemenluz seed count peaked at 1.6 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 completely seedless, because they were planted in mixed trial blocks. Early Esbal was the selection with the lowest seed count 0.0 – 0.1 seeds per fruit. Clemenluz had the highest seed count 3.4 – 5.9 seeds per fruit. Most of selections had delayed colour development. Degreening practices will be essential after harvesting to ensure optimal colour development. Early Esbal was the only selection to reach T1 – T2 on the colour plate at peak maturity. Basol with interstock had the highest Brix (13.2°) and acid (1.0%) at peak maturity. Clemenpons had the smallest fruit size and peaked at count 5 - 3. Saratoga had the largest fruit size count 1 - 1xxx in Citrusdal. The selection that had the best juice percentage was Early Esbal.

Table 5.4.24.2. Internal fruit quality data for Clementine selections in the Citrusdal region (Kweekkraal and Stargrow) of the Western Cape during the 2019 season.

Date	Cultivar	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2019-04-10	Basol	CC	1 - 1xx	28,8	11,9	0,85	14,0	0,1	T 1 & T 4
2019-04-29	Basol	CC	1 - 1xx	29,2	12,2	0,80	15,2	0,0	T 1
2019-04-10	Basol M7	CC	4 - 2	32,2	13,2	1,00	13,3	0,9	T 2 - T 5
2019-04-29	Basol M7	CC	4 - 3	48,1	13,7	0,96	14,3	0,0	T 1
2019-03-26	Clemenpons	CC	5 - 4	46,1	12,0	1,18	10,2	3,1	T 6 & T 7
2019-04-10	Clemenpons	CC	5 - 3	33,1	13,0	1,06	12,3	1,2	T 4 - 6
2019-04-29	Clemenpons	CC	5 - 2	45,9	13,3	0,90	14,8	3,3	T 4 - 5
2019-03-26	Clemenpons nuwe	CC	5 - 3	41,9	10,6	1,08	9,9	0,1	T 7
2019-04-11	Clemenpons nuwe	CC	4 - 2	46,8	11,4	0,98	11,7	0,6	T 6 - 7
2019-04-29	Clemenpons nuwe	CC	4 - 1	48,2	12,2	0,88	13,8	0,2	T 5
2019-03-26	Cultifort	CC	3 - 1	45,9	11,6	0,98	11,8	0,8	T 6
2019-04-10	Cultifort	CC	1 - 1xx	25,7	11,6	0,88	13,2	0,0	T 5
2019-03-26	Early Esbal	CC	5 - 4	50,1	10,5	0,96	10,9	0,1	T 5
2019-04-10	Early Esbal	CC	4 - 3	39,1	10,8	0,87	12,4	0,0	T 3 - 6
2019-04-29	Early Esbal	CC	4 - 3	53,4	11,1	0,87	12,7	0,0	T 1 - 2
2019-05-22	Early Esbal	CC	3	52,8	12,3	0,94	13,1	0,0	T 1
2019-04-10	Esbal	CC	5 - 3	33,1	10,7	1,31	8,2	3,3	T 6 -7
2019-04-29	Esbal	CC	4 - 2	50,5	11,6	0,94	12,3	4,4	T 6
2019-05-22	Esbal	CC	3 - 1	54,3	12,4	0,95	13,0	3,3	T 1
2019-04-10	Large Esbal	CC	5 - 3	29,1	10,9	1,46	7,5	0,8	T 6
2019-04-29	Large Esbal	CC	5 - 3	47,1	11,7	1,13	10,4	0,3	T 6
2019-05-22	Large Esbal	CC	3 - 2	52,8	12,8	1,10	11,6	0,1	T 1 - 4
2019-04-10	Nules	CC	3 - 2	29,7	11,1	1,01	11,0	3,4	T 6
2019-04-29	Nules	CC	3 - 1	44,1	12,5	0,83	15,0	5,9	T 6
2019-04-10	Octubrina	CC	3 - 1	34,4	11,0	0,91	12,1	1,1	T 5 - 6
2019-04-29	Octubrina	CC	2 - 1	47,1	11,7	0,93	12,6	0,8	T 4 - 6
2019-04-11	Saratoga	CC	1 - 1xx	41,4	8,8	0,90	9,8	0,6	T 6 - 7
2019-04-30	Saratoga	CC	1x - 1xxx	41,8	9,3	0,77	12,1	0,5	T 5 - 6
2019-04-09	Clemenluz	CC	3 - 2	41,1	10,1	0,95	10,7	1,6	T 6 - 7
2019-04-29	Clemenluz	CC	4 - 1	42,1	9,7	0,85	11,4	0,8	T 5- 6
2019-05-22	Clemenluz	CC	3 - 1x	40,6	11,2	0,71	15,8	0,6	T 1

5.4.25 **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 range of selections at the moment. Buffeljagsrivier region will be one of the biggest Clementine trial sites in the future with another site on the way. The season started with Basol, followed by Nules, Esbal and ended with Early Esbal.

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. Daar is nog 'n perseel oppad. Die seisoen het begin met Basol, gevolg deur Nules, Esbal en ge-eindig met Early 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 Buffeljagsrivier region of the South West Cape; the planting date was 2014. The following cultivars were evaluated: Basol, Esbal, Early 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 5.4.25.1. List of Clementine selections evaluated at Olivedale (Buffeljagsrivier) during 2019 season.

Selection	Rootstock	Planted
Basol	Carrizo	2014
Esbal	Carrizo	2014
Nules	Carrizo	2014
Early Esbal	Carrizo	2014

Results and discussion

Basol
Basol is an early maturing Clementine selection. Fruit size count for Basol was 2 - 3. Basol juice percentage increased towards peak maturity. There were barely seeds in this selection. This selection reached T1 – T2 at peak maturity. Basol had the highest Brix (11.3°) and acid (0.91%) at peak maturity. The fruit peels easily. Basol's rind colour is deep orange. Basol has a very short harvest period before the fruit is over mature and starts to granulate.

Esbal & Early Esbal

Esbal was used as control for the experimental Early Esbal Clementine that was selected to reach peak maturity earlier than Esbal. In this trial site Esbal reached peak maturity before Early Esbal. Esbal mature later than Nules. Fruit size count for Esbal was 4 – 2, slightly bigger than Early Esbal fruit size count 5 - 3. Internal quality for Early Esbal was slightly better that Esbal's internal quality. Rind colour development was also slightly better for Early Esbal T1 – T3 compared to Esbal T2 – T4 on (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 2.2 seeds per fruit compared to Early Esbal seed count that peaked at 0.6 seeds per fruit.

Nules

Nules was the second selection to reach peak maturity. The selection's juice percentage increased towards peak maturity. Internal quality for Nules towards peak maturity was good. Brix (10.4°) and acid (1.04%). This contributed to Nules good flavour. Nules seed count ranged from 3.2 to 4.3 seeds per fruit. Rind colour development was not good for Nules with a T3 – T6 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

Early Esbal had the lowest seed count and Nules had the highest seed count. Basol and Early 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. Early Esbal had the highest Brix (11.3°). Early Esbal had the smallest fruit size and peaked at count 5 - 3. Nules had the biggest fruit size count 3 - 1. Basol and Nules had the best juice percentage.

Table 5.4.25.2. Internal fruit quality data for Clementine selections in the Buffeljagsrivier region (Olivedale) of the South West Cape during the 2019 season.

Date	Cultivar	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg. Seed	Colour
2019-04-08	Basol	CC	3 - 2	36,8	11,3	0,91	12,4	0,7	T 1 - 2
2019-04-08	Early Esbal	CC	5 - 3	26,9	10,6	1,31	8,1	0,5	T 5
2019-05-02	Early Esbal	CC	5 - 4	26,5	11,3	1,05	10,8	0,6	T 1 - 3
2019-04-08	Esbal	CC	4	28,5	9,6	1,15	8,3	1,1	T 5 - 6
2019-05-02	Esbal	CC	4 - 2	32,6	10,4	0,87	12,0	2,2	T 2 - 4
2019-04-08	Nules	CC	3 - 2	30,7	10,4	1,04	10,0	4,3	T 6 - 7
2019-05-02	Nules	CC	3 - 1	36,7	10,6	0,76	13,9	3,2	T 3 - 6

5.4.26 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 medium to large tree canopies. The order of ripening was as follows; Miho Wase started the season, followed by Miyagawa Wase, Sugiyama, Aoshima, Ueno, Imamura, and Bela was 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: Miho Wase het die seisoen begin gevolg deur Miyagawa Wase, Sugiyama, Aoshima, Ueno, Imamura, 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 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 Satsuma selections from the Citrusdal region of the Western Cape. The following selections were evaluated: Aoshima, Imamura, Miho Wase, Miyagawa Wase, Sugiyama, Ueno, and 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 5.4.26.1. List of Satsuma selections evaluated at Kweekkraal and Stargrow (Citrusdal) during 2019.

Selection	Rootstock	Topworked
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

<u>Aoshima</u>
Aoshima is a mid – late maturing Satsuma. Fruit size count for Aoshima ranged between counts 1x - 1xxx. Aoshima was one of the selections with the biggest fruit size count. Ratio 11.9:1 the Brix was good (10.3°) as well as the acid percentage (0.86%). There were some seeds in the fruit 0.0 - 1.2 seeds per fruit. The external colour development of the Aoshima was not good at all, with a T4 – T6 on the colour plate. Juice percentage increased towards peak maturity. The fruit is not pleasant to look at and was very pebbly with some damage due to sunburn.

<u>Miho Wase</u>
Miho Wase was the first 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 T6 – T7 at peak maturity. Fruit matured internally, rind colour development was delayed. Sugar at peak maturity was around 8.6° with an acid percentage of around 0.88%. Juice content decreased towards peak maturity.

Imamura

Imamura is a late maturing Satsuma. In this cold production region, it reached peak maturity from mid-May. It was the second last selection to reach peak maturity. For a Satsuma, Imamura had a good Brix: Acid ratio, 9.8° and 1.12 % respectably (ratio 8.7 towards peak maturity). One of the selections with a better juice percentage. Seed count was 0 seeds per fruit. External colour development was T7 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 beginning of June. The fruit is large with a fruit size count of 1 – 1xx. 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 around 9° and acid above 0.80%. External colour development was delayed compared to the internal maturity with T4 - T6 on the colour plate. Bela was seedless.

Miyagawa Wase

Miyagawa Wase was the second selection to reach peak maturity. The fruit size of Miyagawa Wase at peak maturity was count 1 – 1xx. Brix: acid ratio 8.8:1; Brix was 8.5° and acid was 0.97%. Colour development was not good and delayed compared to internal maturity. The colour on the colour plate at peak maturity was T5 – T6. This selection was seedless and the juice percentage increased towards peak maturity. The 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 had the second highest juice content. Ueno had a medium to extra-large fruit size count with a 2 – 1xx count. The Brix° and acid percentage for Ueno at peak maturity were very good, 10° and 0.94% respectively. There were 0.0 – 0.1 seeds per fruit and Ueno colour on the colour plate at peak maturity was T6 – T7. Fruit peeled easily.

Sugiyama

Sugiyama is a mid to late maturing Satsuma. At this trial site it reached peak maturity in mid-April. Sugiyama had a very good fruit size count for export at 2 - 1x. The Brix° and acid percentage of Sugiyama were around 9° and 0.85% respectively at peak maturity. Sugiyama had the highest juice percentage. Seed count for this selection ranged 0.0 – 0.3 seeds per fruit. There was also a delay in colour development with a T5 – T6 on the colour plate at peak maturity.

Conclusion

Most of the selections peaked with an extra-large fruit size (count 1xx - 1xxx). Sugiyama had the smallest fruit size count peaking (count 1x). Aoshima, Ueno and Imamura had the highest Brix° of all the Satsuma selections above (10°). Aoshima had the highest seed count with 1.2 seeds per fruit. Rind colour development was not good for any of the selections at peak maturity. Bela had the best internal colour. Sugiyama had the highest juice percentage.

Table 5.4.26.2. Internal fruit quality data for Satsuma selections in the Citrusdal region of the Western Cape during the 2019 season.

Date	Cultivar	Root-stock	Count	Juice (%)	Brix°	Acid (%)	Ratio	Avg, Seed	Colour
2019-04-10	Aoshima	CC	1x - 1xx	28,5	10,0	1,09	9,1	0,0	T 5 - 6
2019-04-29	Aoshima	CC	1x - 1xxx	40,9	10,3	0,86	11,9	1,2	T 4 - 6
2019-05-22	Bela	CC	1 - 1xx	46,4	8,7	1,08	8,1	0,0	T 7 - 8
2019-06-18	Bela	CC	1 - 1xx	40,8	9,7	0,75	12,9	0,0	T4 - 6
2019-04-29	Imamura	CC	1x - 1xx	42,9	9,8	1,12	8,7	0,0	T 7

2019-03-26	Miho Wase	CC	1 - 1xxx	45,7	8,3	1,01	8,2	0,0	T 6 - 8
2019-04-09	Miho Wase	CC	1 - 1xxx	38,0	8,6	0,88	9,8	0,0	T 6 - 7
2019-04-29	Miho Wase	CC	1xx - 1xxx	38,0	8,4	0,60	13,9	0,0	T 5 - 6
2019-04-09	Miyagawa Wase	CC	1 - 1xx	36,7	8,5	0,97	8,8	0,0	T 6 - 7
2019-04-29	Miyagawa Wase	CC	1 - 1xx	42,4	9,0	0,66	13,6	0,0	T 5 - 6
2019-04-09	Sugiyama	CC	2 - 1	35,6	8,9	1,15	7,7	0,3	T 6 – 7
2019-04-29	Sugiyama	CC	1x	46,0	9,3	0,79	11,7	0,0	T 5 - 6
2019-04-09	Ueno	CC	2 - 1xx	32,5	9,5	1,30	7,3	0,0	T 6 - 7
2019-04-29	Ueno	CC	1x - 1xx	44,6	10,0	0,94	10,6	0,1	T 6 - 7

5.4.27 PROGRESS REPORT: Evaluation of new University of Florida (UF) rootstocks

Project 1246 (2019/20 - 2021) by PJR Cronje, J Joubert, J van Niekerk (CRI)

Summary

The UF/CREC rootstock programme has been breeding rootstocks for 30 years to address various problems in this production area. In the recent past and due to the massive natural screening as the HLB epidemic spread in Florida, some potential tolerant/resistant rootstocks with commercial potential, were identified. During 2019, negotiations with the University of Florida were successfully concluded in a signed MOU with CRI. The full list of relevant rootstock selections will, therefore, be made available to CRI for evaluation purposes when seed becomes available for the first rootstocks in early 2020 (harvest of Florida fruit). In collaboration with the Florida researchers selections of the most relevant rootstocks will be made and seed supplied for propagation. There have also been successful negotiations with three producers in Nelspruit, SRV and Citrusdal to host the planting. However due to a limit in seed availability the Nelspruit experimental site will be the first to be developed. The aim of this long term project is to have production information available of rootstocks with reputed HLB tolerance on two standard cultivars and in three main production areas to enable plantings in integrated systems once the disease reaches SA production areas.

Opsomming

Die UF/CREC teel en ontwikkel al vir die laaste 30 jaar onderstamme om verskillende probleme in hierdie area aan te spreek. As gevolg van die massiewe natuurlike sifting wat plaasgevind het soos die HLB-epidemie in Florida versprei het, is enkele potensiële verdraagsame/weerstandige onderstokke geïdentifiseer. Gedurende 2019 is onderhandelinge met die Universiteit van Florida suksesvol afgehandel en 'n MOU met CRI geteken. Hiervolgens is die volledige lys van onderstam kultivars beskikbaar vir evalueringsdoeleindes deur CRI soos saad beskikbaar raak. Die eerste groep onderstamme wat saad lewer sal in die vroeë 2020 ge-oes word in Florida. In samewerking met die Florida navorsers sal die mees relevante onderstamme gekies word waarna saad gelewer sal word aan CRI vir voortplanting. Drie produsente in Nelspruit, SRV en Citrusdal areas, was ook suksesvol genader om die aanplanting te huisves. Vanweë die beperkte beskikbaarheid van saad, sal die Nelspruit-proefperseel egter eerste ontwikkel word. Die doel van hierdie langtermynprojek is om produksie-inligting te ontwikkel oor hierdie onderstamme met ʼn bekende HLB-verdraagsaamheid. Die projek sal fokus op twee standaardkultivars en in drie hoofproduksiegebiede, om sodoende aanplantings in geïntegreerde beheer stelsels te kan ontwikkel na die siekte in SA produksiegebiede vestig.

5.4.28 PROGRESS REPORT: Studies into the high incidence of chimeras of Valencia orange cultivars, specifically Valencia Late.

Project 1185 (2019/20 - 2021) by P.J.R. Cronje, J. Joubert, W. Swiegers, J. Niemann and V. White (CRI)

Summary

This progress report aims to give background on the development of chimeras in citrus fruit. In addition, it summarises the research and available information from various efforts by CRI. It is clear from the literature and the available information in the South African context that this phenomenon in citrus is not well understood on either a scientific basis or a practical citriculture level. This cellular mutation process of the shoot meristem has been used in the citrus industry to generate a significant portion of new cultivars and thereby adding value. However, once the incidence of chimeras on trees and orchards reaches problematic commercial levels, a clear lack of any management options for even the long term is evident. A preliminary observation that can be made of the current data shows that in some Valencia selections, high incidence could occur in production areas with different climatic conditions. The high prevalence at various producers, who use different crop and pest management strategies, indicate that orchard level actions cannot be responsible. Therefore, a wider, long term research strategy needs to be adopted to try to ascertain the reason behind the high occurrence in some orchards of certain cultivars.

Opsomming

Hierdie vorderingsverslag het ten doel om agtergrond inligting te verskaf oor die ontwikkeling van chimeras in sitrus vrugte. Daarmee saam word die beskikbare inligting en informasie van verskeie CRI ondersoeke opgesom. Wat duidelik uitstaan uit beskikbare literatuur asook die beskikbare informasie in die Suid Afrikaanse konteks is dat die fenomeen glad nie goed verstaan word nie. Nie op ŉ wetenskaplike of ŉ praktiese sitrusproduksie vlak nie. Hierdie sellulêre mutasie proses wat geskied in die knop-meristeem is suksesvolle g ebruik in die sitrus bedryf om ŉ betekenisvolle hoeveelheid kultivars na vore te bring en so waarde toe te voeg. As die voorkoms van chimera egter toeneem in ŉ boord tot kommersiële problematiese vlakke is dit duidelike dat daar nie eens vir die lang termyn bestuursposisies beskikbaar is nie. Voorlopige waarnemings wat gemaak kan word uit die data wys dat seker seleksies van Valencia lemoene ŉ baie hoe voorkoms van chimera kan ontwikkel in uiteenlopende klimaat en produksie areas. Die hoë voorkoms by verskeie produsente, wat almal eie boom en pesbestuur strategie volg, dui daarop dat boordvlak aksies nie kon verantwoordelik gewees het nie. ŉ Langtermyn navorsing strategie wat weier kyk na ander faktore word benodig om die voorkoms van die hoë vlakke in seker kultivars uit te pluis.

6. CITRUS IMPROVEMENT SCHEME (CIS)

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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 three record budwood supply years in 2016/17, 2017/18 and 2018/19, which were mostly dominated by lemon and mandarin supply, certified budwood supply declined in 2019/20. A total of 6.1 million buds were supplied by the CFB or authorized for cutting in certified nurseries, 1.1 million less than in 2018/19. Lemon demand increased from 6.1% to 12.0%, and Valencia from 19.1% to 20.9%, whilst mandarin supply decreased from 47.9% to 42.0%. High demand for Midknight Valencia in 2017/18 (700 thousand buds) and 2018/19 (705 thousand buds) has declined to 470 thousand in 2019/20. An increase in 'Star Ruby' demand was also experienced, from 299 thousand in 2017/18, to almost 349 thousand buds in 2018/19, stabilising at 332 thousand in 2019/20. In 2017/18, 72 thousand RHM (Royal Honey Mandarin) buds were supplied, which increased to 414 thousand in 2018/19 and to 418 thousand in 2019/20. Amidst the rise in budwood and rootstock seed demands, CFB's ability for primary supply in 2018/19 decreased from 67.9% in 2017/18 to 62.7% in 2018/19, but has increased to 75.7% in 2019/20; this can be attributed to a decrease in demand, new multiplication trees of high demand cultivars that came into production, and increased demand for lemons. Budwood stock of the 439 cultivars at CFB must be constantly managed to meet demand of sought-after cultivars. In 2019/20, 20-thousand new multiplication trees were produced, 11-thousand redundant trees were

removed, and the rootstock seed orchards expanded with another 1 030 rootstock trees of high demand rootstock cultivars.

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. Na drie agtereenvolgende rekord jare in 2016/17, 2017/18 en 2018/19, wat hoofsaaklik oorheers is deur aanvraag na suurlemoene en mandaryne, was daar ŉ afname in die okuleerhout aanvraag in die huidige 2019/20 seisoen, maar was die aanvraag steeds baie hoog. 'n Totaal van 6.1 miljoen ogies is deur die Grondvesblok en in samewerking met gesertifiseerde kwekerye verskaf. Die aanvraag na suurlemoene het van 6.1% na 12.0% gestyg, en Valencias van 19.1% tot 20.9%, terwyl mandaryne van 47.9% tot 42.0% gedaal het. Die vraag na Midknight Valencia het van 381-duisend in 2016/17 tot 700-duisend in 2017/18 en 705-duisend ogies in 2018/19 gestyg, maar het vanjaar afgeneem tot 470-duisend. 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, met ‘n bestendige aanvraag van 332-duisend in 2019/20. In 2017/18 is 72-duisend ogies van RHM (Royal Honey) mandaryn verskaf word, wat na 414-duisend in 2018/19 en weer tot 418-duisend in 2019/20 gestyg het. Te midde van die verhoogde aanvraag na okuleerhout en saad het die primêre verskaffing vanaf die Grondvesblok van 67.9% in 2017/18 tot 62.7% in 2018/19 gedaal, maar het verhoog na 75.7% in 2019/20. Hierdie styging kan toegeskryf word aan die daling in aanvraag, nuwe vermeerderingsbome wat in produksie gekom het, asook ŉ hernude aanvraag na suurlemoene. Okuleerhout voorraad van 439 kultivars moet konstant bestuur word om soveel as moontlik van die hoë aanvraag kultivars te kan verskaf. In 2019/20 is 20-duisend nuwe vermeerderingsbome gemaak, 11-duisend onnodige bome verwyder. Die saadbron is vergroot deur nog 1 030 hoë aanvraag onderstam bome te vestig.

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 - Tropical and Subtropical Crops (ARC-TSC), DALRRD’s Directorate of Plant Health (DPH) and citrus nurseries represented by the South African Citrus Nurserymen's Association (SACNA). 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. Additionally, Cultivar and Pathology sub-committees co-ordinate the respective CIS activities.

The phytosanitary status of the CIS is ensured by virus-elimination and diagnostic services of cultivars prior to CIS introduction and routinely confirmed through re-indexing of mother trees as well as multiplication blocks.

6.2 **Budwood**

This report summarises the seasonal supply of budwood from 1 July 2019 to 30 June 2020. Following the record number of 7.23 million buds in 2018/19, 6.14 million were supplied by the Citrus Foundation Block (CFB) and authorised for cutting in certified nurseries (BCIN). The decline in budwood supply of almost 1.1 million 2019/20 is 17.9% less buds than in the same period of 2018/19 and 16.6% less buds than in the same period of 2017/18. During this period 40 190 buds were exported to neighbouring countries.

Budwood demand generally decreased in volume and was mostly from Western Cape (33.8%), Limpopo (31.0%), followed by the Eastern Cape (16.9%), Mpumalanga (10.0%) and the other provinces ranging from 2.8% to 0.9% (Table 6.2.1).

Mandarin (42.0%) was the most popular citrus type, followed by Valencia (20.9%), lemon (12.0%), navel (8.3%), Clementine (8.1%), and grapefruit (5.9%); in 2018/19 this proportion was 47.9%, 19.1%, 6.1%, 12.3%, 7.5% and 4.9%, respectively (Table 6.2.2). Valencia demand was stable from 2014/15 – 2016/17 with a 3-year average of 560 thousand buds; however, in the past three seasons, we experienced an unexpected increase and 1.3, 1.4 and 1.3 million buds were supplied in 2017/18, 2018/19 and 2019/20, consequently surpassing the lemon demand. Whilst supply of lemon budwood is still lower than 2017/18 and 2016/17 levels, it increased from 6.1% to 12%, with Eureka ranking as the second most popular cultivar in 2019/20. Navel demand decreased with 8.3% to 508 thousand buds, compared to the 887 thousand buds in 2018/19. The high demand for Clementine buds remained at around 500 thousand buds per season, which is 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-, 358- and 361 thousand buds supplied in 2017/18, 2018/19 and 2019/20, respectively. This high demand is significant, but recent supply figures are still low when compared to the 10 year average of 535 thousand buds during the 1990's. A break-down of buds per variety type to nurseries in different provinces is given in Table 6.2.3.

The top 30 varieties comprised 91.3% of total number of buds supplied. ARC Nadorcott LS (ARCCIT9) was the most popular cultivar, followed by Eureka, Tango, Midknight, RHM, Star Ruby, Nules, Jassie, Nova and Leanri (Table 6.2.4). ARC Nadorcott LS supply levels have increased year on year from 29 160 to 267 422 to 513 582 to 1 080 328 to 1 133 797 in 2018/19, slightly decreasing to 920 281 in 2019/20. The protected mandarin varieties in the top 10 (ARC Nadorcott LS (ARCCIT9), Tango, RHM and Leanri) contributed to 32% to the total budwood supply, with a large proportion (45.1%) of these buds BCIN supplied in 2019/20: RHM (70.1%), ARC Nadorcott LS (49.0%) and Tango (29.7%). BCIN supply for Or 4 decreased from 14.2% to 0%, and for Leanri from 37.3% to 5.0% in 2019/20. The top 10 cultivars (4.08 million) comprised 66.4% of all of the budwood supplied, of which 69.2% were supplied from the CFB (Table 6.2.4).

Primary CFB supply significantly improved from 67.8% and 61.8% in 2017/18 and 2018/19, respectively, to 75.7% in 2019/20. BCIN proportion per variety type: mandarins (64.6% of which ARC Nadorcott LS (ARCCIT9), RHM and Tango comprised of 59.3%, and Sigal, Nova, Queen, PE1, Lea and Leanri a further 5.2%), Valencia's (20.8% of which Midknight and Jassie comprised 13.9%), Clementine (4.9% of which Nules comprised 3.9%), grapefruit (4.3% comprising only of Star Ruby), navel (2.4%) and other (3.0%) (Figures 6.2.1-7).

Table 6.2.1. Buds supplied during the period July to June 2017/18 – 2019/20.

Area	**2017/18**	**Dist %**	**2018/19**	**Dist %**	**2019/20**	**Dist %**
Local	**7 134 440**	**99.7%**	**7 207 920**	**99.6%**	**6 099 824**	**99.3%**
Eastern Cape	1 450 017	20.3%	1 105 534	15.3%	1 037 927	16.9%
Gauteng	120 267	1.7%	245 575	3.4%	174 473	2.8%
KwaZulu Natal	47 600	0.7%	17 840	0.2%	58 100	0.9%
Limpopo	2 491 852	34.8%	2 040 987	28.2%	1 904 192	31.0%
Mpumalanga	460 315	6.4%	612 206	8.5%	614 506	10.0%
North West	164 000	2.3%	139 610	1.9%	142 410	2.3%
Northern Cape	275 950	3.9%	299 629	4.1%	94 200	1.5%
Western Cape	2 124 439	29.7%	2 746 539	37.9%	2 074 016	33.8%
International	**24 315**	**0.3%**	**31 589**	**0.4%**	**40 190**	**0.7%**
Botswana	11 000	0.2%	500	0.0%		0.0%
India		0.0%	1 250	0.0%	1 250	0.0%
Mozambique	3 965	0.1%		0.0%		0.0%
Nigeria	4 000	0.1%	1 000	0.0%		0.0%
USA		0.0%	139	0.0%	200	0.0%
Zambia		0.0%		0.0%	1 100	0.0%
Zimbabwe	5 350	0.1%	28 700	0.4%	37 640	0.6%
Total	**7 158 755**	**100.0%**	**7 239 509**	**100.0%**	**6 140 014**	**100.0%**

Table 6.2.2. Buds supplied during the period July to June 2017/18 – 2019/20.

Variety Type	2017/18	Dist %	2018/19	Dist %	2019/20	Dist %
Local	**7 134 440**	**99.7%**	**7 207 920**	**99.6%**	**6 099 824**	**99.3%**
Mandarin Hybrid	3 114 568	43.5%	3 470 954	47.9%	2 581 461	42.0%
Valencia	1 306 702	18.3%	1 385 710	19.1%	1 284 925	20.9%
Lemon	1 056 903	14.8%	440 877	6.1%	737 758	12.0%
Navel	784 523	11.0%	887 277	12.3%	508 286	8.3%
Clementine	459 217	6.4%	542 195	7.5%	495 190	8.1%
Grapefruit	335 450	4.7%	358 214	4.9%	361 610	5.9%
Satsuma	29 840	0.4%	35 130	0.5%	44 248	0.7%
Lime	29 830	0.4%	25 385	0.4%	36 560	0.6%
Midseason	1 480	0.0%	34 860	0.5%	18 419	0.3%
Kumquat	8 300	0.1%	12 355	0.2%	14 590	0.2%
Diverse	2 107	0.0%	5 528	0.1%	8 970	0.1%
Pummelo	5 320	0.1%	4 925	0.1%	7 685	0.1%
Ellendale		0.0%		0.0%	100	0.0%
Rootstock	200	0.0%	4 510	0.1%	22	0.0%
International	**24 315**	**0.3%**	**31 589**	**0.4%**	**40 190**	**0.7%**
Total	**7 158 755**	**100.0%**	**7 239 509**	**100.0%**	**6 140 014**	**100.0%**

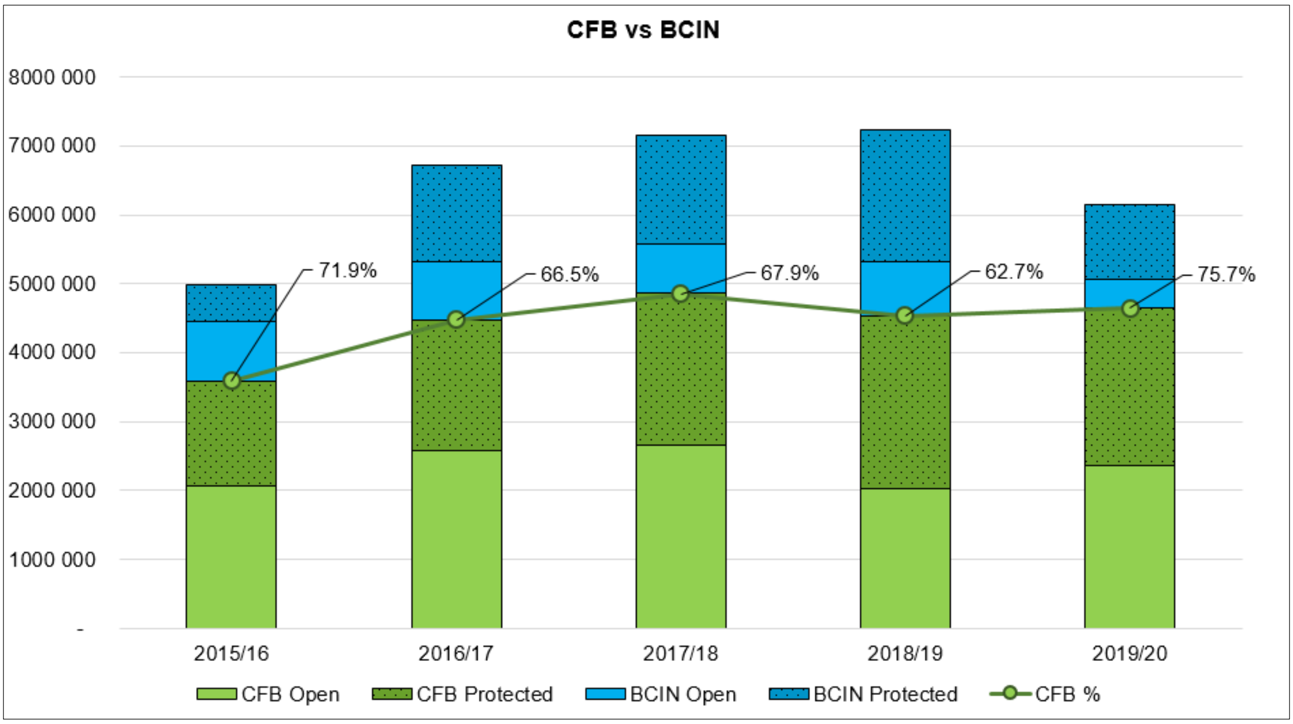

Figure 6.2.1. Budwood of open and protected cultivars (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2015/16 – 2019/20.

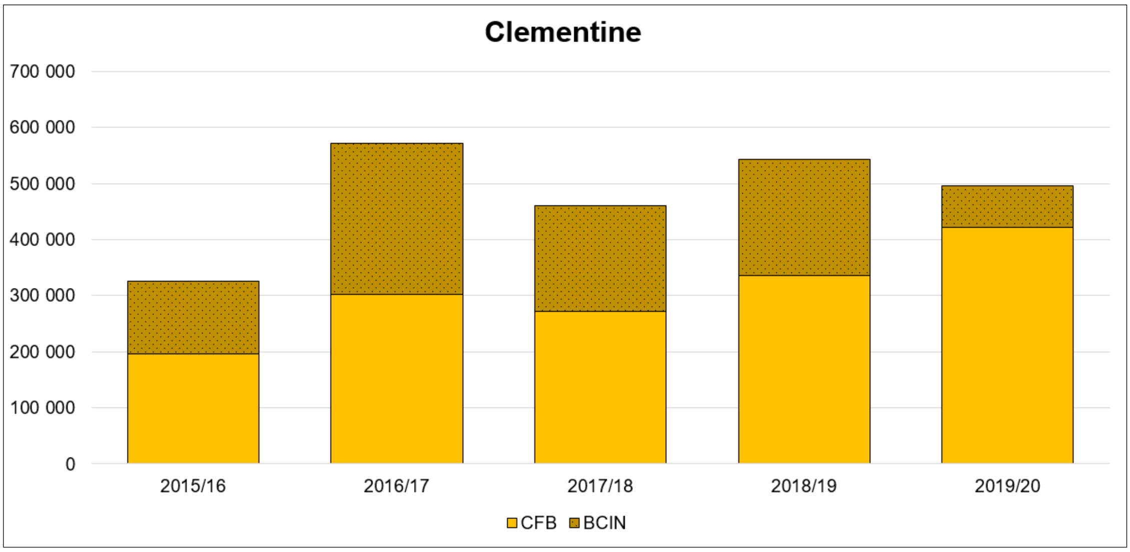

Figure 6.2.2. Clementine budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2015/16 – 2019/20.

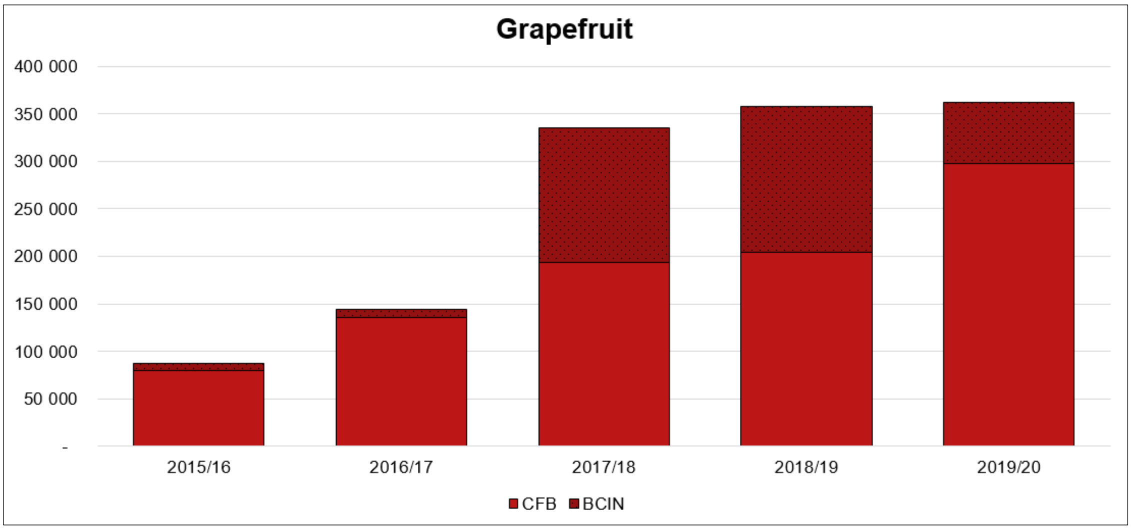

Figure 6.2.3. Grapefruit budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2015/16 – 2019/20.

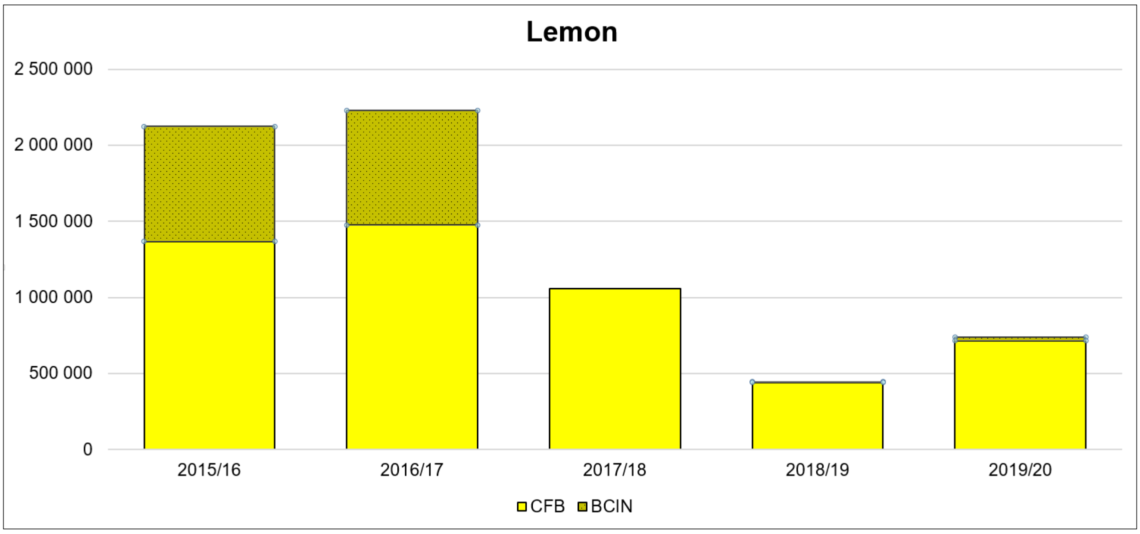

Figure 6.2.4. Lemon budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2015/16 – 2019/20.

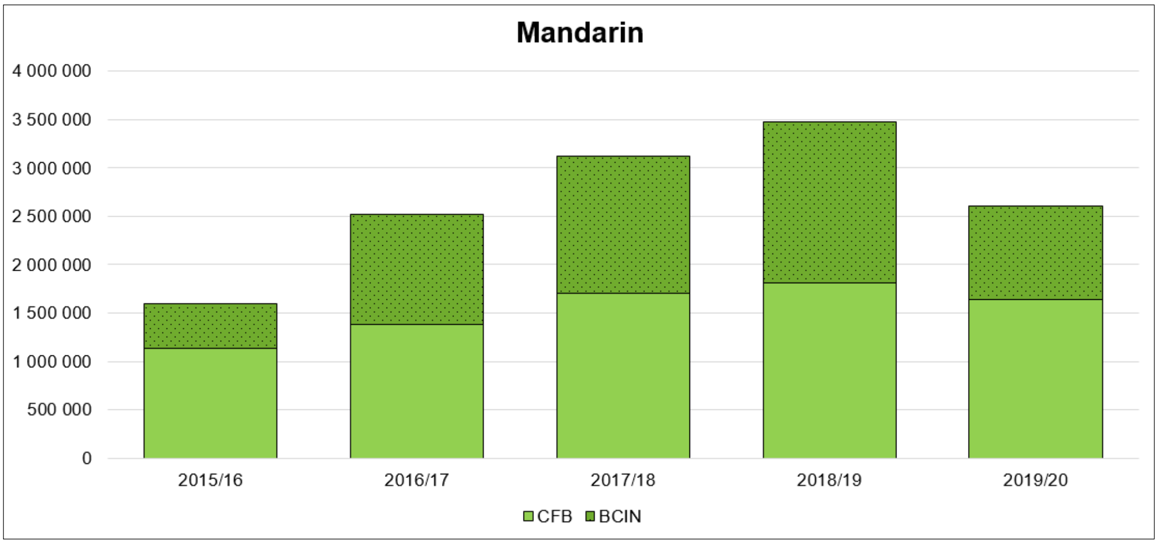

Figure 6.2.5. Mandarin hybrid budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2015/16 – 2019/20.

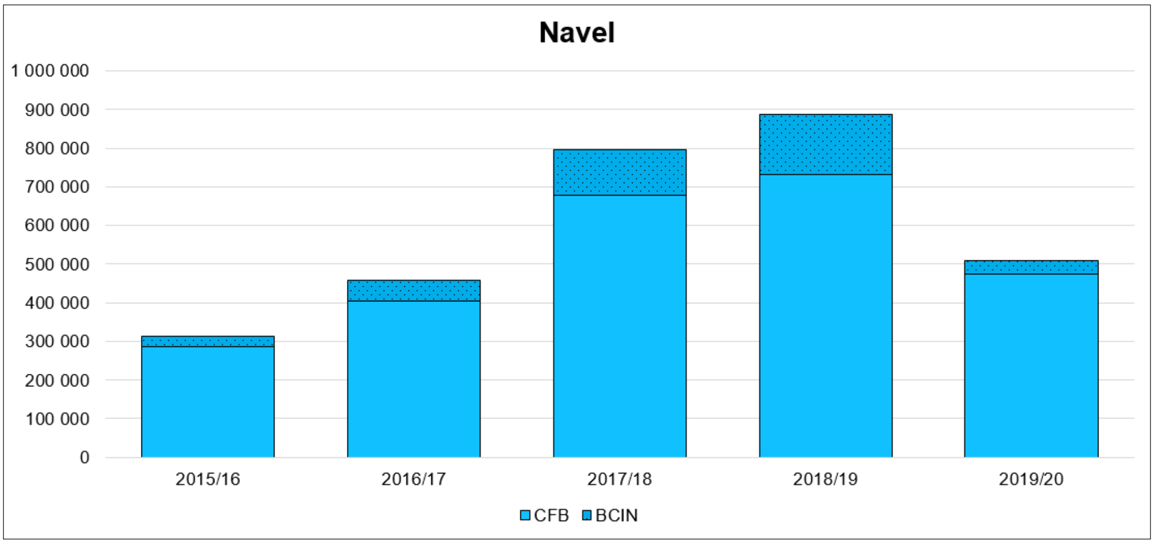

Figure 6.2.6. Navel budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2015/16 – 2019/20.

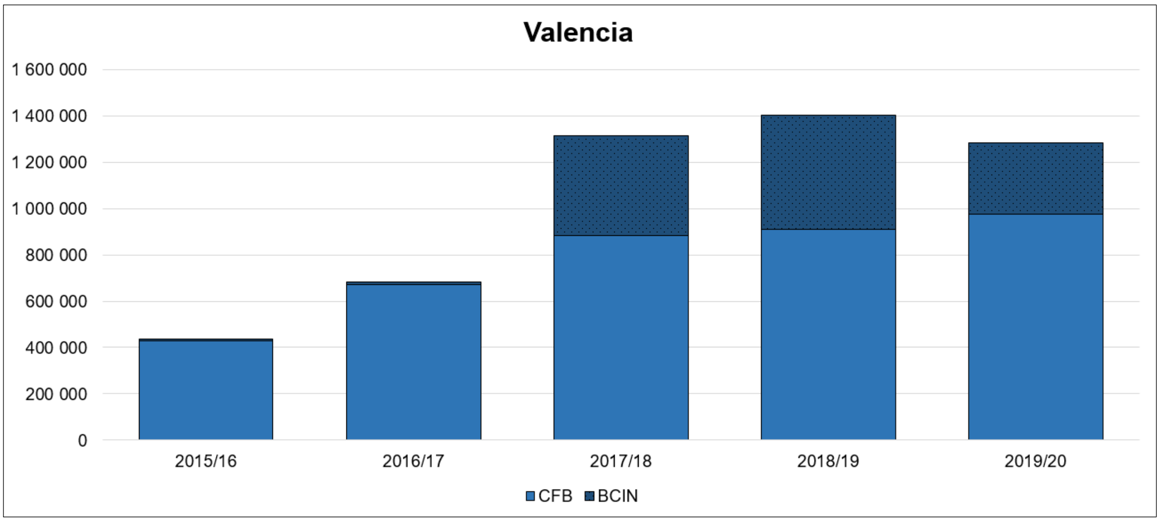

Figure 6.2.7. Valencia budwood (total number of buds per season) supplied by the CFB and authorised for cutting in nurseries (BCIN) during the periods July to June from 2015/16 – 2019/20.

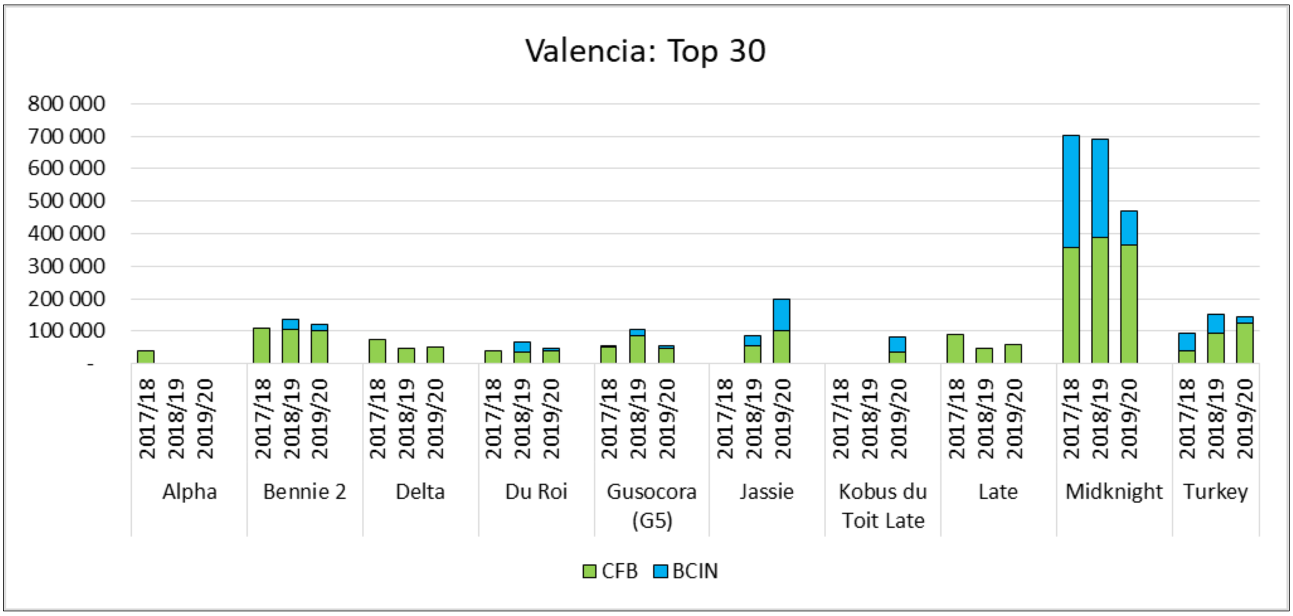

Figure 6.2.8. Budwood supply (BCIN/CFB) of the Valencia cultivars in the top 30 in 2017/18 – 2019/20.

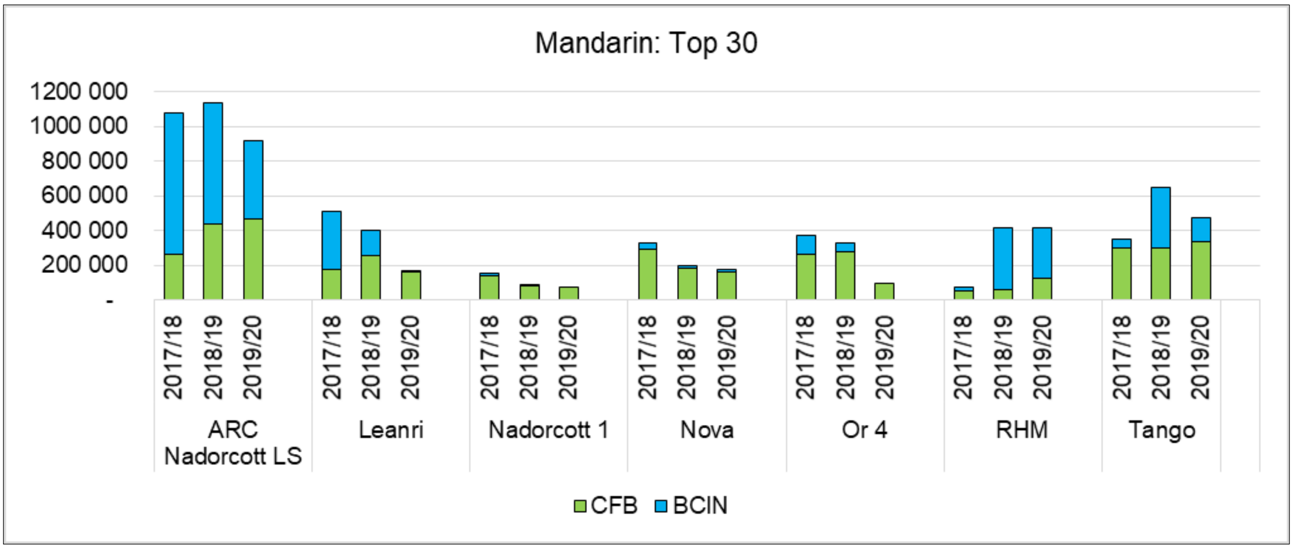

Figure 6.2.9 Budwood supply (BCIN/CFB) of the mandarin hybrid cultivars in the top 30 in 2017/18 – 2019/20.

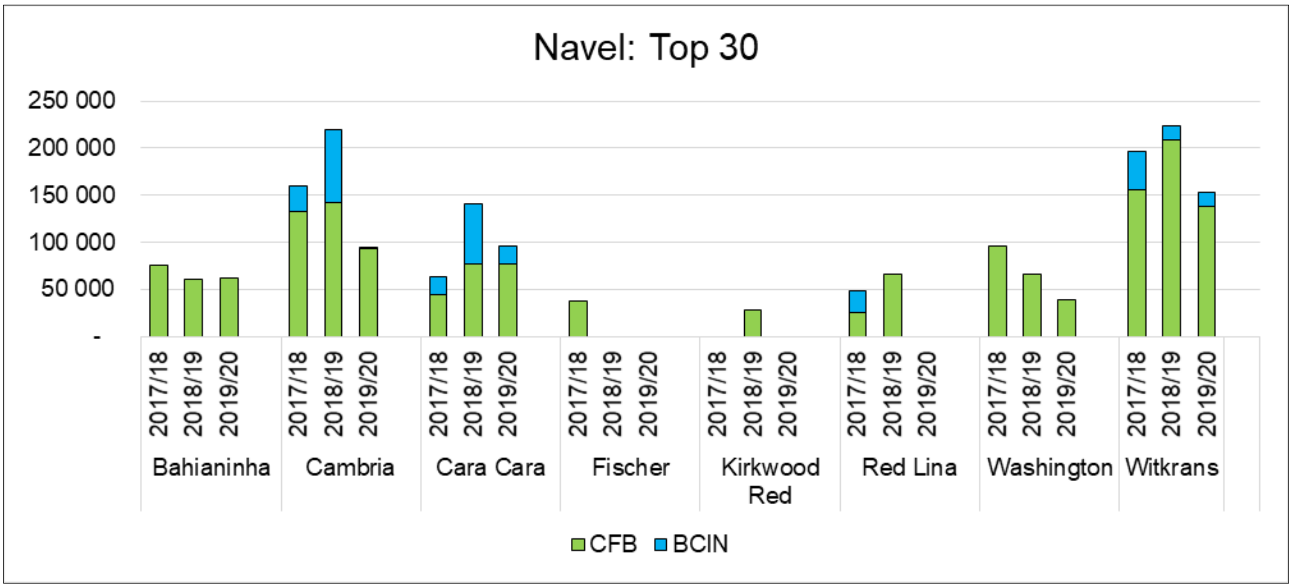

Figure 6.2.10 Budwood supply (BCIN/CFB) of the navel cultivars in the top 30 in 2017/18 – 2019/20.

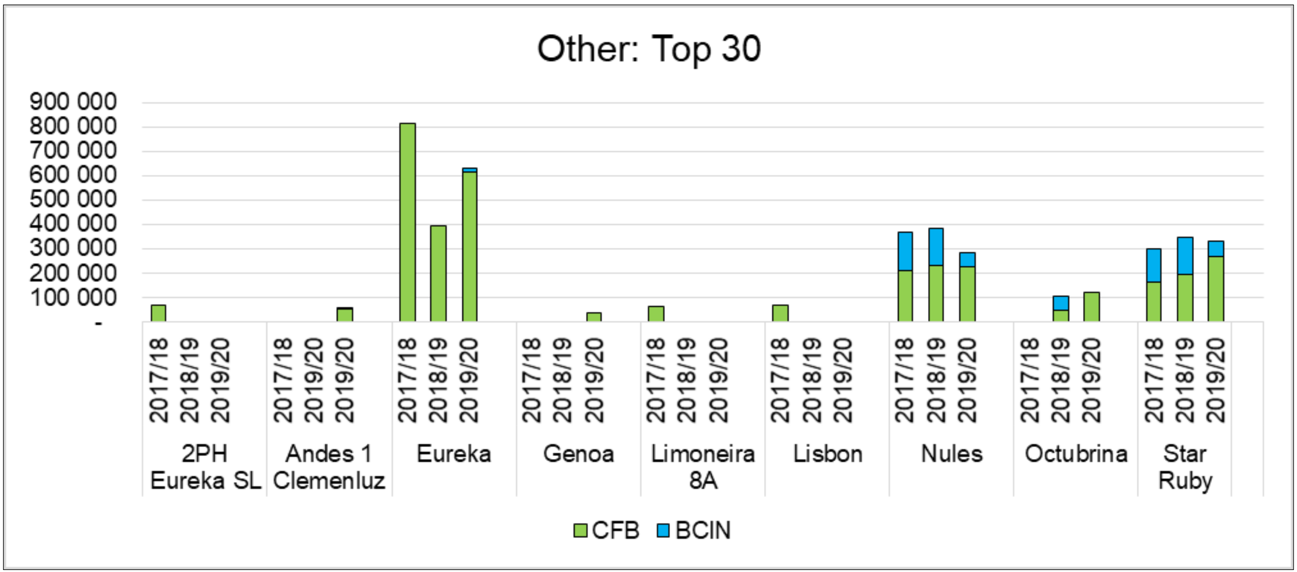

Figure 6.2.11 Budwood supply (BCIN/CFB) of the other variety types in the top 30 in 2017/18 – 2019/20.

6.3 **Seed**

CFB is the primary supplier of rootstock seed in SA, supplied around 90% of certified seed. A significant decrease in fruit yield, as well as number of seed per fruit yield were experience during 2018/19, leading to a reduction in supply of most rootstock varieties in that season. In 2019/20, yields were in line with the estimated yield potential: Carrizo citrange yield increased with 92.5% from 2018/19, but slightly less than 2017/18 (a record Carrizo year); C35 citrange (32.1% less); Swingle citrumelo (33.8% more); Rough Lemon similar to 2017/18 and 2018/19.

The shift in demand from lemons to mandarins, Valencia's and navels has resulted in higher orders of trifoliate hybrid cultivars and the demand exceeded the supply in most cases in 2019/20 (Figure 6.3.1). The supply data for 2018/19 were influenced by the reduced yield.

During May to April 2020, 6358 litres of seed were supplied locally (Table 6.3.1) and 42 litres of seed were exported to SADC countries (Table 6.3.1). Carrizo citrange remains the most popular rootstock (42.9%), followed by Swingle citrumelo (18.3%), Rough lemon (5.2%), C35 citrange (9.8%), X639 (4%) and Troyer citrange (5.2%) (Table 6.3.2).

Because of the higher demand when compared with previous seasons, supply levels did not meet the high demand (Table 6.3.1) and in 2019/20 CRI imported Carrizo citrange (CC), Rough lemon (RL) and C35 (C35) on behalf of nurseries from the USA. However, the seed quality was very poor, resulting in big losses.

Table 6.2.3. Buds supplied per variety type per area (total number of buds per season) during the periods July to June from 2017/18 – 2019/20.

Variety Type	Season	EC	GP	KZN	LP	MPU	NW	NC	WC	International	Total
Clementine	2017/18	89 782	22 000		45 475	11 080	2 000	4 200	284 680	500	459 717
	2018/19	43 490	24 500		20 880		4 000	18 600	430 725	1 400	543 595
	2019/20	43 644	25 500		18 600	6 150	2 000	14 000	385 296		495 190
Diverse	2017/18	100	1 087		200	100	200		620		2 307
	2018/19	6 108	630	500	750			450	1 600		10 038
	2019/20	400	5 428		2 384	40			840		9 092
Grapefruit	2017/18	22 150	9 000	6 200	141 060	115 800	5 800	14 200	21 240	300	335 750
	2018/19	18 520	29 254		135 042	76 798	9 570	17 410	71 620		358 214
	2019/20	2 800	3 000	2 000	196 760	78 550	9 900	2 900	65 700		361 610
Kumquat	2017/18		880			1 200	2 000		4 220		8 300
	2018/19	1 000	1 285	2 000	1 700	600	5 000		770		12 355
	2019/20	900	5 050		1 730		3 000	500	3 410		14 590
Lemon	2017/18	189 239	22 500	18 400	515 050	68 265	40 700	35 800	166 949	3 000	1 059 903
	2018/19	213 050	7 960	7 320	44 850	24 487	15 000	39 000	89 210	2 350	443 227
	2019/20	243 982	35 820	29 500	264 500	13 817	29 700	8 000	112 439	1 350	739 108
Lime	2017/18	3 830	1 870		3 950	7 540	1 000	1 000	10 640		29 830
	2018/19	2 350	1 795		3 900	8 880	600		7 860	100	25 485
	2019/20	2 210	6 515		4 550	14 500	2 075	500	6 210		36 560
Mandarin Hybrid	2017/18	471 630	2 105	3 000	1 285 141	152 825	33 190	125 550	1 041 127	3 650	3 118 218
	2018/19	389 052	6 668	20	1 299 065	243 639	28 940	106 271	1 397 299	10 000	3 480 954
	2019/20	425 227	21 300		684 435	343 058	43 450	14 000	1 049 991	37 740	2 619 201
Midseason	2017/18					60		600	820		1 480
	2018/19	250				240			34 370		34 860
	2019/20	550					400		17 469		18 419
Navel	2017/18	289 732	9 800	4 500	123 180	39 575	32 260	33 700	251 776	10 600	795 123
	2018/19	286 733	7 840	3 500	90 700	55 897	34 710	30 900	376 997	639	887 916
	2019/20	87 776	31 260	2 500	144 040	13 597	24 900	39 800	164 413	1 100	509 386
Pummelo	2017/18	970			4 350						5 320
	2018/19				3 750	1 100			75		4 925
	2019/20				7 600				85		7 685
Satsuma	2017/18	19 475		3 000	2 000	35			5 330		29 840
	2018/19	19 770		2 500	7 250	400			5 210		35 130

	2019/20	17 203		1 000	6 500		2 000	3 500	14 045		44 248
Valencia	2017/18	363 109	51 025	12 500	371 446	63 835	46 850	60 900	337 037	6 265	1 312 967
	2018/19	125 211	165 643	2 000	433 100	200 165	41 790	86 998	330 803	17 100	1 402 810
	2019/20	213 235	40 600	23 100	573 093	144 794	24 985	11 000	254 118		1 284 925

Table 6.2.4. Top 30 cultivars based on total number of buds supplied for seasons July to June from 2017/18 – 2019/20.

2017/18			**2018/19**			**2019/20**		
Cultivar	**BCIN**	**CFB**	**Cultivar**	**BCIN**	**CFB**	**Cultivar**	**BCIN**	**CFB**
ARC Nadorcott LS MAN *	813 829	266 499	ARC Nadorcott LS MAN *	694 075	439 722	ARC Nadorcott LS MAN *	451 392	468 889
Eureka LEM		814 031	Midknight VAL	299 985	389 045	Eureka LEM	18 605	613 739
Midknight VAL	343 749	356 781	Tango MAN	346 000	301 198	Tango MAN	141 100	333 742
Leanri MAN	334 150	177 509	RHM (Royal Honey) MAN	351 738	62 535	Midknight VAL	106 348	363 737
Or 4 MAN	108 360	266 852	Leanri MAN	150 344	253 507	RHM (Royal Honey) MAN	292 893	125 211
Nules CLE	157 661	212 663	Eureka LEM		395 272	Star Ruby GFT	64 050	268 160
Tango MAN	50 000	303 580	Nules CLE	150 000	233 133	Nules CLE	58 065	223 889
Nova MAN	37 300	294 375	Star Ruby GFT	153 685	195 049	Jassie VAL	97 274	102 755
Star Ruby GFT	136 550	162 610	Or 4 MAN	46 600	281 334	Nova MAN	16 200	160 161
Witkrans NAVEL	40 950	156 240	Witkrans NAV	14 950	209 222	Leanri MAN	8 545	162 059
Cambria NAVEL	26 875	133 127	Cambria NAV	76 697	142 707	Witkrans NAV	14 419	137 983
Nadorcott 1 MAN	17 000	140 700	Nova MAN	16 900	184 799	Turkey VAL	19 000	124 545
Bennie 2 VAL		110 148	Turkey VAL	58 143	94 670	Bennie 2 VAL	20 360	101 718
Washington NAV		96 270	Cara Cara NAV	63 596	76 621	Octubrina CLE		120 602
Turkey VAL	53 600	38 400	Bennie 2 VAL	31 433	105 100	Cara Cara NAV	19 750	76 285
Late VAL		90 681	Gusocora (G5) VAL	19 176	85 215	Or 4 MAN		95 014
Bahianinha NAV		75 980	Octubrina CLE	53 570	49 853	Cambria NAV	1 100	93 647
Delta VAL		75 203	Nadorcott 1 MAN	8 500	80 484	Kobus du Toit Late VAL	47 203	35 000
RHM (Royal Honey) MAN	20 000	52 359	Jassie VAL	32 103	55 842	Nadorcott 1 MAN		73 185
Lisbon LEM		69 065	Queen MAN		67 629	Sigal MAN	17 846	46 994
2PH Eureka SL LEM		67 700	Du Roi VAL	33 050	34 176	Bahianinha NAV		62 350
Limoneira 8A LEM		63 470	Washington NAV		65 870	Late VAL		59 970
Cara Cara NAV	19 030	44 151	Red Lina NAV		65 396	Andes 1 Clemenluz CLE	4 700	50 600
IR M2 MAN	18 300	33 899	Bahianinha NAV		60 120	Gusocora (G5) VAL	6 000	47 400
Gusocora (G5) VAL	2 120	49 635	Lea MAN	49 320	5 995	Delta VAL		49 490
Red Lina NAV	23 100	25 070	Delta VAL		48 379	Du Roi VAL	9 362	37 938

Alpha VAL		39 520	Late VAL		46 925	Furr (Clem x Murcott) MAN		43 175
Queen MAN		39 010	Gold Nugget MAN		29 800	Washington NAV		39 510
Fischer NAV		37 801	Kirkwood Red NAV		28 213	PE 1 MAN	10 880	25 710
Du Roi NAV		37 780	IR M2 MAN		24 585	Genoa LEM		36 354
Top 30	**2 202 574**	**4 331 109**	**Top 30**	**2 649 865**	**4112396**	**Top 30**	**1 425 092**	**4 179 812**
> Top	**98 508**	**526 564**	**> Top**	**48 590**	**428658**	**> Top**	**67 880**	**467 230**
Total	**2 301 082**	**4 857 673**	**Total**	**2 698 455**	**4541054**	**Total**	**1 492 972**	**4 647 042**
	32.1%	**67.9%**		**37.3%**	**62.7%**		**24.3%**	**75.7%**

* ARCCIT9 (ARC Nadorcott LS) MAN

Table 6.3.1. Seed (litres) supplied by the CFB during the periods May to April 2017/18 – 2019/20.

Area	2017/18	Dist %	2018/19	Dist %	2019/20	Dist %
Local	**6508**	**99.0%**	**5130.3**	**98.6%**	**6358**	**99.3%**
Eastern Cape	653	9.9%	788.0	15.1%	547	8.5%
Gauteng	133	2.0%	103.0	2.0%	158	2.5%
KwaZulu Natal	70	1.1%	75.0	1.4%	96	1.5%
Limpopo	2523	38.4%	2066.3	39.7%	2437	38.1%
Mpumalanga	417	6.3%	325.0	6.2%	455	7.1%
North West	179	2.7%	83.0	1.6%	136	2.1%
Northern Cape	694	10.6%	360.0	6.9%	522	8.2%
Western Cape	1841	28.0%	1330.0	25.6%	2006	31.3%
International	**64**	**1.0%**	**73.7**	**1.4%**	**42**	**0.7%**
Botswana	4	0.1%		0.0%		0.0%
Chile	12	0.2%		0.0%		0.0%
Congo	3	0.0%		0.0%	3	0.0%
India		0.0%	0.2	0.0%	0.2	0.0%
Mauritius	1	0.0%		0.0%		0.0%
Mozambique	6	0.1%		0.0%		0.0%
Swaziland	32	0.5%	14	0.3%		0.0%
Zambia	6	0.1%	9	0.2%		0.0%
Zimbabwe		0.0%	51	1.0%	39	0.6%
Total	**6572**	**100.0%**	**5204**	**100.0%**	**6400**	**100.0%**

Table 6.3.2. Seed (litres) supplied by the CFB during the periods May to April 2017/18 – 2019/20.

Rootstock cultivar	2017/18	Dist %	2018/19	Dist %	2019/20	Dist %
CFB	**6145**	**93.5%**	**4467**	**85.8%**	**5717**	**89.3%**
Benton citrange (BC)	20	0.3%	15	0.3%	13	0.2%
C35 citrange (C35)	878	13.4%	923	17.7%	627	9.8%
Carrizo citrange (CC)	3105	47.2%	1426	27.4%	2744	42.9%
Cleopatra mandarin (CM)		0.0%	33	0.6%	22	0.3%
Mineola X Trifoliate (MXT)	179	2.7%	151	2.9%	60	0.9%
Rough lemon (RL)	284	4.3%	303	5.8%	297	4.6%
Swingle citrumelo (SC)	898	13.7%	874	16.8%	1169	18.3%
US812 Sunki X Benecke (SXB)	26	0.4%	67	1.3%	45	0.7%
Troyer citrange (TC)	347	5.3%	294	5.6%	330	5.2%
Volckameriana (VA)	108	1.6%	114	2.2%	143	2.2%
X639	283	4.3%	253	4.9%	257	4.0%
Yuma citrange (YC)	19	0.3%	15	0.3%	6	0.1%
Other	0	0.0%	0	0.0%	4	0.1%
Imported		**0.0%**	**239**	**4.6%**	**452**	**7.1%**
SPIN**	**427**	**6.5%**	**499**	**9.6%**	**231**	**3.6%**
Total	**6572**	**100.0%**	**5204**	**100.0%**	**6400**	**100.0%**

**Seed produced in nurseries

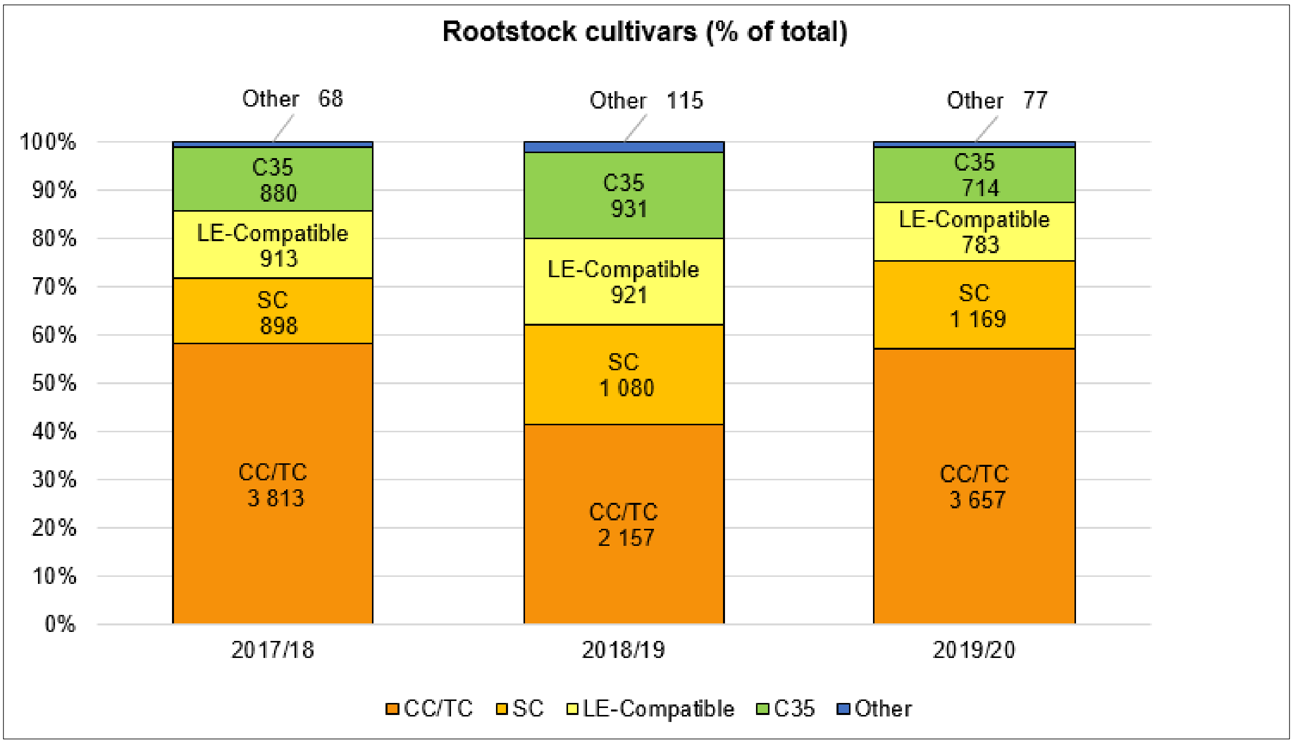

Figure 6.3.1. Rootstock cultivars grouped by type (percentage of total litres of seed per season) supplied by the CFB and seed produced by nurseries (SPIN) during the periods May to April from 2017/18 – 2019/20.

6.4 **Production**

Budwood: CFB presently maintains more than 149 thousand multiplication trees of 439 cultivar lines with a potential annual budwood stock of >10 million buds. In the past season, the STG facilities at CRI-Nelspruit released 22 new cultivars to the CFB and re-introduced 2 existing cultivar lines, and ARC-TSC introduced 10 new cultivars and re-introduced 6 existing cultivars to the CFB (Table 6.4.1). Introduced cultivars are budded to rootstock seedlings in CFB's rapid multiplication tunnels, whereafter new multiplication trees are planted out into the insect-protected greenhouses.

In order to timeously address budwood demand, CIS obtains budwood demand estimates from the CIS Cultivar Committee, private cultivar owners/agents and nurseries. This feedback is considered with historical supply and BCIN records, and multiplication tree stocks are managed accordingly. In 2019/20, 20 475 new multiplication trees, representing 51 cultivar lines, were made, and these trees should contribute to an estimated 1.4 million buds in the next 1-2 years. To address space constraints, >11 thousand redundant multiplication trees were removed. Importantly, CIS needs a lead time of 2 years to meet demand levels, and considering that stocks of many different cultivars must be managed, and that budwood supply of newly introduced cultivars are limited, supply levels realistically requires a number of years to meet high demand levels. Cases of short supply, and reliance on the BCIN system in such cases, is therefore a reality.

Budwood shortages were experienced for 21 cultivars in the top 30; however, reliance on the BCIN system declined from 37.3% in 2018/19 to 24.3% in 2019/20.

A three-bay, polycarbonate nucleus block facility, was completed. Two bays will be used to house a duplicate of each nucleus block accession while one bay will be dedicated to pre-immunised sources. The nucleus and pre-immunised block will be populated in Spring 2020.

Table 6.4.1. Cultivar introductions from 2015/16 – 2019/20.

Area	**2015/16**	**2016/17**	**2017/18**	**2018/19**	**2019/20**
ARC: New introductions	14	4	14	9	10

ARC: Re-introduction (from Nucleus Block/new CTV strain)		2	14	5	6
CRI: New introductions		6	7	7	22
CRI: Re-introductions (from Nucleus Block/new CTV strain)			21		2
CFB: Re-multiplication of existing cultivar lines	58	35	84	57	51

Seed: During 2019/20 the CFB seed harvest was in line with forecasted yields, significantly better than the unexpectedly low yields experienced on some of the varieties in 2018/19. Carrizo, the main rootstock variety ordered, yielded as expected and estimated yield for the upcoming season looks promising. Gibb sprays was prioritised during the spring of 2019 and bees were introduced during the flowering season. Due to early flowering, CFB seed harvest is expected to commence early in April 2020. Planned orchard expansion with high demand seed cultivars were completed in November; 817 trees was established on low ridges and another 213 trees planted in available open space. This 1 030 trees increased the number of seed trees from 3731 to 4761; the number of seed source trees prior to the recent expansions which started in 2015 was 2624 trees. When these are in full production some older trees will be removed to make space for greenhouse expansion.

Despite these recent expansions, it was proposed to move the CFB seed source to another farm >5km away from CFB. Removal of all trees outside insect-secure structures from CFB is a necessary measure to ensure the sustained biosecurity of the CFB budwood sources, considering the threat of exotic pests and diseases and the potential quarantine implications. A CAPEX proposal to acquire and develop a seed farm was approved, but is subject to approval of the next CGA levy cycle.

6.5 **Tree Certification**

There were 4 520 609 trees certified during April to March 2019/20 (Table 6.5.1). This is 181 365 more than in 2018/19. Of the applications received, the trees not meeting the certification requirement were 52 165 (1.1% of the applications in 2019/20), 35 339 (0.9%) and 30 893 (0.7%) in 2017/18 and 2018/19, respectively (Table 6.5.2). 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 indicates the nursery tree certification criterion of trees certified as a percentage of budwood supplied during the 2-year period prior to the maximum age certifiable age of trees in the nursery (30 months). Of the 35 certified nurseries, 6 nurseries certified tree above the 80% benchmark, 8 nurseries above the 67% benchmark and 17 nurseries below the benchmark (note that new nurseries might not have certified trees in this period yet). This criterion was included in the updated nursery certification guideline and nurseries will be penalised if they do not meet the 80% benchmark.

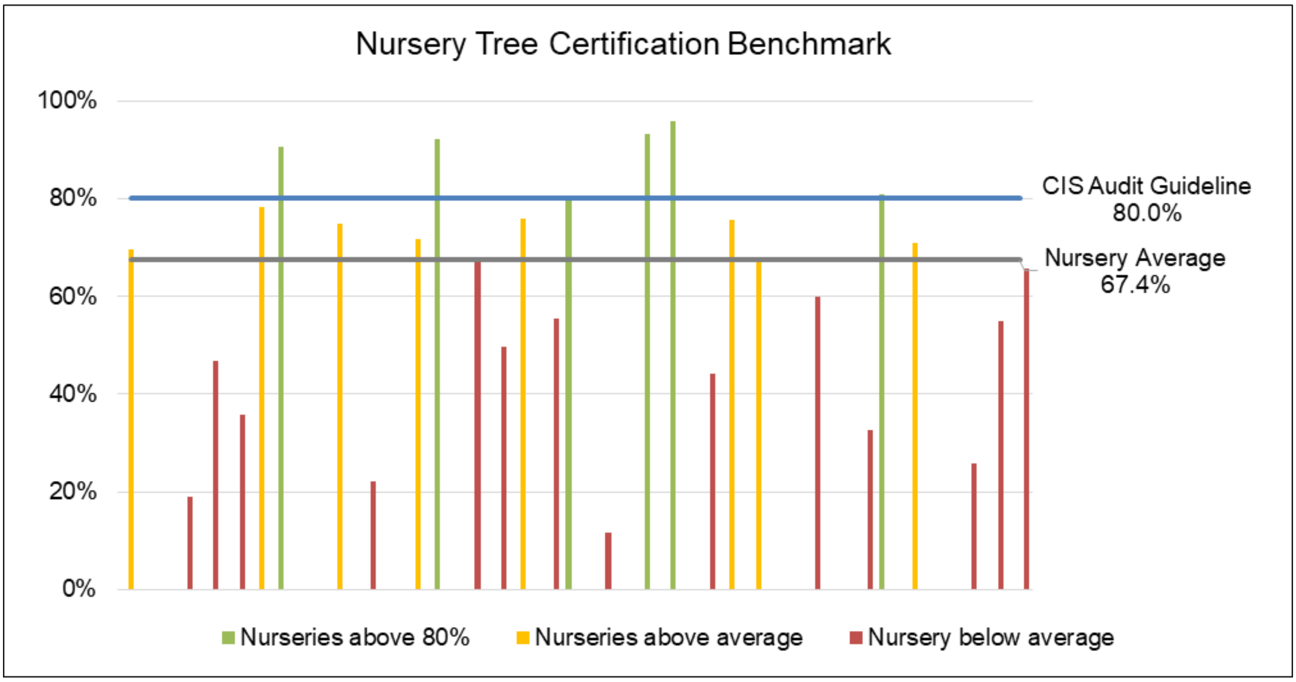

Figure 6.5.1. Nursery tree certification as a percentage of budwood supplied during the 2-year period prior to the maximum age certifiable age of trees in the nursery (30 months). The updated CIS audit guidelines required a tree certification percentage of >80.

Table 6.5.1. Trees certified during the period April to March from 2017/18 - 2019/20.

Variety Type	Season	EC	FS	GP	KZN	LIM	MPU	NC	NW	WC	EXPORTED	TOTAL
Clementine	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
	2019/20	60 938				19 913	10 130	2 681	2 500	259 021	2 765	357 948
Diverse	2017/18									1 700		1 700
	2018/19	75				275	550					900
	2019/20					1 035						1 035
Grapefruit	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
	2019/20	6 756		19 805	67 700	104 716	60 551	11 515		4 600	11 550	287 193
Lemon	2017/18	501 724		3 051	21 250	354 266	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
	2019/20	106 689		10 728	5 500	104 037	19 447	7 000	3 317	81 489	7 419	345 626
Lime	2017/18					500				4 688	200	5 388
	2018/19	155			769	2 814	150			1 612	2 695	8 195
	2019/20	3 077		100		1 462				900		5 539
Mandarin Hybrid	2017/18	385 070	351	28 205	2 000	384 525	317 089		33 003	530 299	700	1 681 242
	2018/19	433 949	220	11 189	1 300	745 768	471 464	13 738	26 994	424 846	49 908	2 179 376
	2019/20	303 837		3 530		755 087	415 647	17 250	14 577	519 413	1 800	2 031 141
Midseason	2017/18									164		164
	2018/19									5 868		5 868
	2019/20									570		570
Navel	2017/18	60 945		2 700		20 285	55 312	3 174	6 680	33 538	4 800	187 434
	2018/19	124 265	1 660	21 970	6 660	51 098	31 495	3 050	21 585	47 512	19 010	328 305
	2019/20	260 849	50	3 220		60 750	64 981	7 090	30 425	79 507	31 334	538 206
Satsuma	2017/18	1 661				300				12 162	3 650	17 773
	2018/19	12 265				1 813	110			16 394		30 582
	2019/20	6 135								2 661		8 796
Valencia	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
	2019/20	185 416		5 620	26 850	434 519	91 090	44 333	4 186	115 605	33 896	941 515

Table 6.5.2. Trees not meeting the certification criteria during the period April to March from 2017/18 - 2019/20.

Certification Status	Season	EC	FS	GP	KZN	LIM	MPU	NC	NW	WC	EXPORTED	Total
Certified	2017/18	1 058 709	351	56 524	33 500	993 853	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
	2019/20	933 697	50	43 003	100 050	1 484 029	662 376	89 869	55 005	1 063 766	88 764	4 520 609
Not Certified	2017/18	18 601					177	2 745	120	13 696		35 339
	2018/19	15 035			107	204	1 321		3 030	11 196		30 893
	2019/20	30 870				359	1 622			17 016	2 298	52 165

6.6 **Nursery Certification**

Of the 36 nurseries visited in May 2019, 31 were fully certified and 5 were provisionally certified. Provisionally certified nurseries were either new nurseries that have not fully implemented the quality management systems required for CIS certification, or were old nurseries that encountered certain problems that needed to be addressed in order to retain full certification status. The standard varied 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 were reviewed, as some nurseries supply trees topped on triangular wood at 70-80 cm tree height and not on round wood, and other nurseries supplied mini-trees instead of standard whip trees. In general a decline in tree orders was experienced; however, the large nurseries were still fully booked. Most nurseries have not started the required improvements needed for an HLB Safe System. The impression was that they will react when the vector or disease was found in South Africa.

Of the 36 nurseries visited in November 2019, 31 were fully certified and 5 were provisionally certified and 1 nursery required a re-audit. This round of audits was done using the updated audit guidelines and ratings, as in the draft version circulated prior to the visits. Several improvements to the current draft and rating criteria were identified and will be communicated to the nurseries prior to the next audits. Audit criteria that made the biggest impact on the final score (higher weights given their importance), were tree status and control (standard and uniformity of seedlings and trees in the nursery), *Phytophthora* status of trees and irrigation water, quality management systems and tree certification and sales register. Nursery standard is generally high and the average nursery score during the November 2019 audit was 68%. Five nurseries scored >80% (proposed "5-star" rating), 9 nurseries scored 70-79% (proposed "4-star" rating), 18 nurseries scored 60-69% (proposed "3-star" rating), 2 nurseries 50-59% (proposed "2-star" rating), and 1 nursery <50% (proposed "1-star" rating). The low-scoring nurseries clearly had problems and were either provisionally certified, or will be re-audited.

It is clear from the discussions during the audits that all of the nurseries are considering the implications of the HLB/ACP Action Plan, but few have implemented the required changes.

Table 6.6.1: CIS Certified Nurseries in November 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@augsburgnursery.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	eugene@cgrow.co.za
Casmar Kwekery	Mooinooi	NW	Neville Wenhold Jnr		082 881 4189	casmarnursery@absamail.co.za
Cederberg Tree Nursery	Citrusdal	WC	Patricia Willemse	022 921 3526	076 622 7007	trish@cederbergtreenursery.co.za
Dodhill Nursery	Chegutu	ZIM	Pete Breitenstein		+263 77 222 1046	dodhill@iwayafrica.co.zw
Du Roi Kwekery	Letsitele	LP	Zylon McGaffin	015 345 1650	076 227 6704	zylon@duroi.co.za
Du Roi Halls Nursery	Nelspruit	MPU	Arve Grindstad	013 004 0462	071 411 0131	arve@duroihalls.co.za

Esselen Kwekery	Malelane	MPU	Louis Esselen	013 790 0160	078 803 7010	esselenkwekery@gmail.com
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@capespanfarms.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	tremper@hdsnursery.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	rian@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
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	jeanroeleveldrs@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
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	rudyvdmeulen@gmail.com
Witkrans Kwekery	Boshoek	NW	Linda Grobler	014 573 3036	082 414 4739	Witkrans1@mweb.co.za

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 supports a compulsory statutory CIS.

The new Plant Improvement Act (PIA) 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 DALRRD and the designated authority, CGA, has been drafted and will be tabled for discussion by stakeholders at CISAC-2019.

In the face of imminent biosecurity challenges, the urgency in proceeding with promulgation was stressed at CISAC-2019. CISAC agreed to the request for the PCMCs and SACNA to be granted opportunity to make final inputs before 18 September 2019. However, this deadline was subsequently waived in light of constructive follow-up meetings during which parties agreed on the way forward. This culminated in the amendment of the CISAC Terms of Reference (ToR). Notably, the inclusion of a “voting block” of CISAC members was addressed (voting members identified), and the dispute resolution procedures available to the various CIS stakeholders were summarised as an appendix to the CISAC ToR. It is anticipated that CISAC-2020 will approve the submission of the proposal to convert the CIS to a compulsory statutory scheme under the PIA.

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 and CRI-CIS have made several follow-up visits to one owner refusing to remove 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, orders were issued and most home owners have removed their trees. Currently, two owners are refusing to remove their trees.

6.9 PROGRESS REPORT: Shoot tip grafting and CIS diagnostic services at CRI-Nelspruit

Project 1144 by J. H. J. Breytenbach, C. Steyn, R. de Bruyn 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. Eleven new selections were received for STG and 22 were released to the CFB and added to the gene source. The gene source maintained at CRI currently comprises 400 accessions. The screening of the CFB multiplication blocks for citrus viroids in greenhouse structures 1 and 3 was completed and no infections were detected. General diagnostics and investigations of *ad hoc* problems or outbreaks, relating to graft transmissible diseases, are additionally conducted and these ongoing activities are reported.

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 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 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 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-TSC, were duplicated in part at CRI Nelspruit as back-up sources.

Indexing, or establishing whether GTD disease agents are present, is 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 aphid 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 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 suit 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. Indexing for CTV and Citrus viroids (CVds) are done biennially. Screening for other GTD such as citrus psorosis virus (CPsV), citrus tatter leaf virus (CTLV) and Citrus Impietratura disease (CID) are done every 10 years.

Objectives

A. Cultivar introduction (administration, establishment, STG, diagnostics, pre-immunisation, submission to the CFB and Nucleus Block)
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-TSC 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 a 20% solution of household bleach, which contains 3.5% 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 28ºC in continuous darkness. When the seedlings have reached a height of 30 to 40 mm, they are stored at 4ºC in darkness.

Scion preparation: Method 1: Buds of the source plant are budded on a standard rootstock in the glasshouse. After bud growth and maturation (approximately 3 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 a 7.5% solution of household bleach and then rinsed three times in sterile distilled water.

Method 2: If sufficient budwood of the source plant is supplied, numerous buds are budded to a few rootstocks each with 2 buds. As shoots develop from the buds they are used directly for STG and only one bud is left to grow as the reference source plant. The time from budding to STG is reduced by at least 3 months. Shoot harvesting and preparation for STG is done as above.

STG: The rootstock seedling is aseptically decapitated about 50 mm above the cotyledons. The cotyledons and their auxiliary buds are removed and a balcony incision is made, 1 mm horizontally and 1 – 2 mm diagonally 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 surface of the incision on the rootstock. The grafted plant is transferred to sterile MS liquid medium and cultured at constant 28ºC exposed to 16 hours of light per day.

STG plant propagation: The shoot tip normally starts growing 3 to 4 weeks after STG. The shoot tip is micro-grafted with the seedling rootstock onto a vigorously-growing virus-free rootstock in the glasshouse. After micro-grafting, the graft is covered with a plastic bag for 8 days to prevent desiccation. Once the graft has sufficiently grown, the plant is screened for GTD pathogens.

Virus indexing: The micro-graft is pre-screened for CTV and CVds by RT-PCR. If the plant is negative, buds are taken for biological indexing, but if the plant is positive, it is the new source plant from which further STGs are done.

Elimination of GTD pathogens is confirmed by biological indexing on sensitive indicators as described by van Vuuren and Collins (1990). Biological indexing results are thereafter verified using molecular diagnostics to detect CVd, CTV, CPsV, CTLV and Liberibacter spp that cause Citrus Greening and Huanglongbing.

On average, it takes 24 to 30 months to obtain a virus-free accession, which includes the biological 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 established on virus-free rootstocks and maintained in an insect-free tunnel. Material derived from the gene source is 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 5 years as part of the routine maintenance. Photographic records of fruit from each cultivar/selection are kept on the database to confirm cultivar identifications. The purpose is to ensure that the correct citrus fruit type is produced from each accession and as an additional confirmation that no mix-ups have occurred. Cultivar identification of the gene source accessions at the ARC are also done with the assistance of a CRI cultivar evaluator.

C. Biological and molecular re-indexing of mother trees and multiplication blocks at the CFB

All CFB mother trees are re-indexed every second year to establish if the CTV pre-immunized source is maintained. Re-indexing of CFB mother trees for CVds is done simultaneously every second year. All CFB mother trees and seed source trees are inspected annually for symptoms of citrus greening disease by ARC-TSC and CRI virologists. PCR and/or biological indexing are conducted on plants showing suspicious symptoms. Most other citrus viruses are transmitted by infected budwood 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 CFB multiplication trees for CVds is done yearly on a third of the multiplication trees. Therefore all the CFB multiplication trees are screened every third year. The screening 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 pooled 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 viroid-specific tests for citrus bent leaf viroid (CBLVd), hop stunt viroid (HSVd), citrus dwarfing viroid (CDVd), citrus bark cracking viroid (CBCVd), citrus viroid V (CVd V) and citrus exocortis viroid (CEVd).

D. Collaboration and duplicate indexing with ARC-TSC laboratory

Shoot tip grafting for the CIS is done at both 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 by both laboratories.

E. *Ad hoc* diagnostics for GTDs for growers and external institutions

Field material received for diagnostics is generally budded to 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 suspected disease being indexed. The indicators are monitored for symptoms for a minimum of 3 months post inoculation. Direct molecular tests are also done, depending on the diagnostic requirement.

F. *Ad hoc* investigations as required by CIS

Diseases 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

Routine maintenance and improvements at the CIS Nelspruit facilities are done to ensure the safekeeping of accessions.

Results and discussion

Objective / Milestone	Achievement
A. Cultivar introduction and STG pipeline	• 21 accessions in STG pipeline • 11 new accessions received • 22 accessions released to the CFB • A conviron was acquired and heat therapy is used to assist in pathogen elimination
B. Maintenance of the virus-free gene source	• 400 cultivars maintained • Renewal of gene source in progress • CRI personnel assisted in citrus type verification of fruiting trees of the ARC gene source
C. Biological and molecular re-indexing of mother trees and multiplication blocks at the CFB	• Screening multiplication blocks for viroids: greenhouses 1 and 3 completed (882 samples processed, representing 40 6400 trees) • Severe stem-pitting in 3 grapefruit cultivars at CFB investigated
D. Collaboration and duplicate indexing with ARC-TSC laboratory	• All accessions sent to the CFB were duplicate tested prior to release
E. *Ad hoc* diagnostics for GTDs for growers and external institutions	• Approx. 75 *ad hoc* samples analysed

F. *Ad hoc* investigations as required by CIS	• CTLV seed transmission study published • Viroid related stunting investigated on two farms linked to supply of uncertified trees • C35 incompatibility investigated • SSR markers used to differentiate new rootstock lines • SSR markers used to assist in cultivar identifications to resolve mix-ups
G. Facility management	• Drip irrigation installed in gene source tunnel • Tunnel entrances were sealed and covered with psylla screen • Trapping and monitoring as per the HLB/ACP Action Plan implemented • Breaches in tunnels were sealed • The UV water sterilisation system was serviced • Fans were installed in all tunnel double-door, vestibule entrances • Electrical supply problems were rectified

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 2015 to date are summarised in Table 6.9.1. Eleven new selections of four variety types were submitted for STG in this report period. Twenty-one accessions are at various stages in the STG pipeline. A total of 291 STGs were done within this period, including failed grafts. Seventy-four STGs were successfully micro-grafted, which is a 25% STG success rate.

Table 6.9.1. STG submissions in the pipeline for graft transmissible disease elimination and indexing.

Variety type[2]	**STG introductions and releases 2015 to 2019[1]**															
	2015			2016			2017/18			2018/19			2019/20			
	Bf	New Introductions	Releases to CFB	Bf	New Introductions	Releases to CFB	Bf	New Introductions	Releases to CFB	Bf	New Introductions	Releases to CFB	Bf	New Introductions	Releases to CFB	Balance
C	4	0	1	3	0	1	2	1	1	2	2	1	3	0	2	1
G	0	0	0	0	1	0	1	0	0	1	0	0	1	0	1	0
L	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1
Li	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Mi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ma	5	1	1	5	2	5	2	3	3	2	1	1	2	3	1	4
N	13	2	2	13	1	4	10	3	1	12	2	3	11	1	9	3
V	7	1	0	8	2	1	9	2	1	10	1	2	9	5	7	7
Or	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rs	0	0	0	0	0	0	0	2	0	2	0	0	2	1	2	1
Total	**30**	**4**	**4**	**30**	**6**	**11**	**25**	**11**	**6**	30	6	7	29	11	22	18

[1] Bf = Brought forward from previous year; Balance = Balance for the current reporting year.

[2] Variety type: C = Clementine; G = Grapefruit; L = Lemon; Li = Lime; Mi = Midseason; Ma = Mandarin; N = Navel;

R = Reticulata; V = Valencia; Or = Ornamental; Rs = Rootstock.

In the 2017 annual cycle, nine cultivars in the nucleus block (NB) tested positive for GTD pathogens, which were re-introduced to the STG pipeline for pathogen removal. Six of these cultivars went through STG of which three tested clean by direct RT-PCR, the remainder will be processed in 2020.

To facilitate a faster turn-around with the STG process, new introductions are tested directly via 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 seven successful STGs for CTV, CTLV and CVds were finalised and confirmed negative by RT-PCR. Biological indexing of 30 STGs was completed for CPsV and CiVA and confirmed negative by RT-PCR. Twenty-two cultivars were interim-released to the CFB.

To gauge the efficiency of the STG progress over time, some parameters are graphically presented in Figures 6.9.1 to 4, which provide additional information to the numbers provided in Table 6.9.1.

The supply of new cultivars allows the industry to remain competitive in a global market. The STG process was recognised as a hindrance as some cultivars remained in the STG pipeline for lengthy periods. Improvements to the STG process are assessed in how quickly accessions are released to the CFB. Figure 1 shows the average time from introduction of cultivars to interim release to the CFB since 2015. A significant reduction in the average STG pipeline time was achieved since 2017. These averages are for 3, 5, 10 and 3 accessions in 2015, 2016, 2017 and 2018, respectively. However, problem accessions are occasionally encountered for which pathogen elimination was not successful and which remain in the STG pipeline for lengthy periods. The reasons for these failures are not always clear. However, a conviron (growth chamber) was acquired in this report period. The conviron allows for controlled, fluctuating light and temperature regimes, which was shown to assist in pathogen elimination by affecting plant growth and simultaneously reducing the pathogen titres close to the meristem.

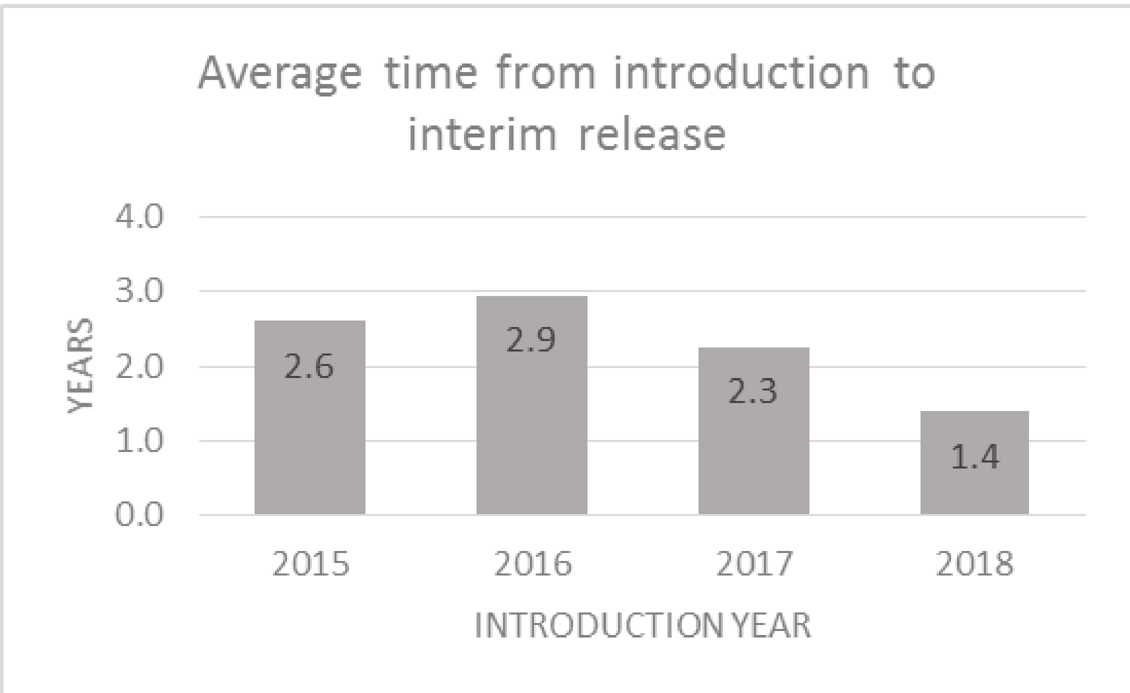

Figure 6.9.1. The average time (years) that accessions were in the STG pipeline from introduction to interim release is shown per introduction year for the period 2015-2018.

The graph of Figure 6.9.2 shows 4 accessions that have been in the STG pipeline for up to 8 years. Accession 5107 originally contained both CTV and CVds. To date 31 STGs were done on this accession and 3 micro-grafts are still positive for viroids, although the CTV component was eliminated. Despite 48 STGs, no successful micro-grafts were obtained for accession 5121. These two accessions will be placed in the conviron to hopefully assist in pathogen elimination.

Accession 5128 contains citrus tatter leaf virus (CTLV), which is notoriously difficult to eliminate. Seventy-four STGs were performed on this accession. However, an ex-plant from one STG, still containing CTLV, was placed in the conviron. STGs done from this plant generated 3 ex-plants which are negative for CTLV in initial tests. These results must still be confirmed by biological indexing.

Thirty-four STGs were performed from accession 5131 and 6 ex-plants were obtained. A recent micro-graft was negative for pathogens in initial tests. Biological indexing will commence to confirm these results.

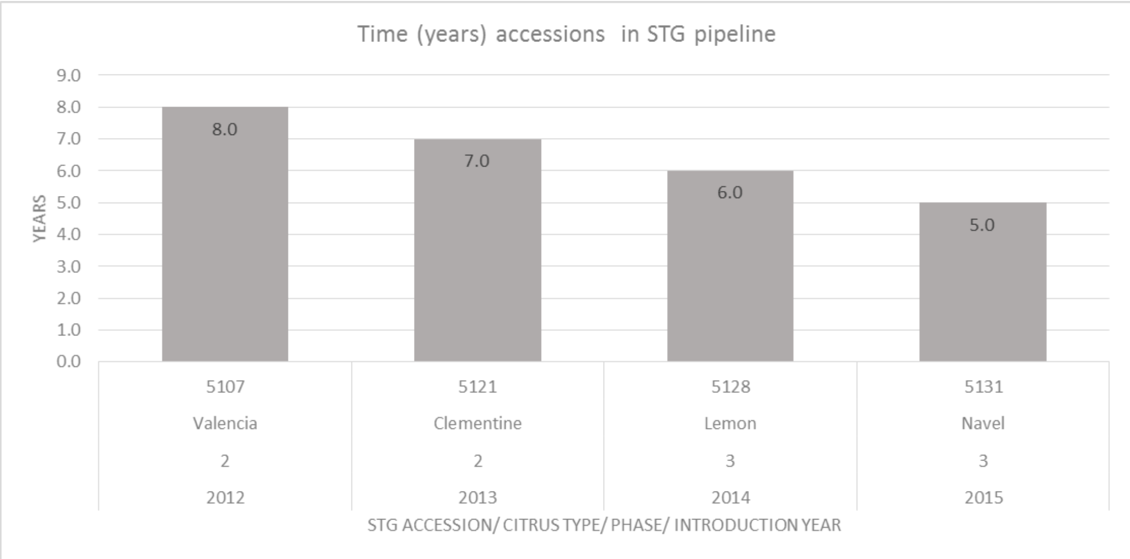

Figure 6.9.2. Problematic accessions that remain in the STG pipeline

As a measure of productivity, the number of STGs performed on finalised accessions, per citrus type and per year finalised, is shown in Figure 6.9.3. As mentioned, a total of 291 STGs were done for the period April 2019 - March 2020. The graph indicates the year, not restricted to this report period, but shows a high output of STGs.

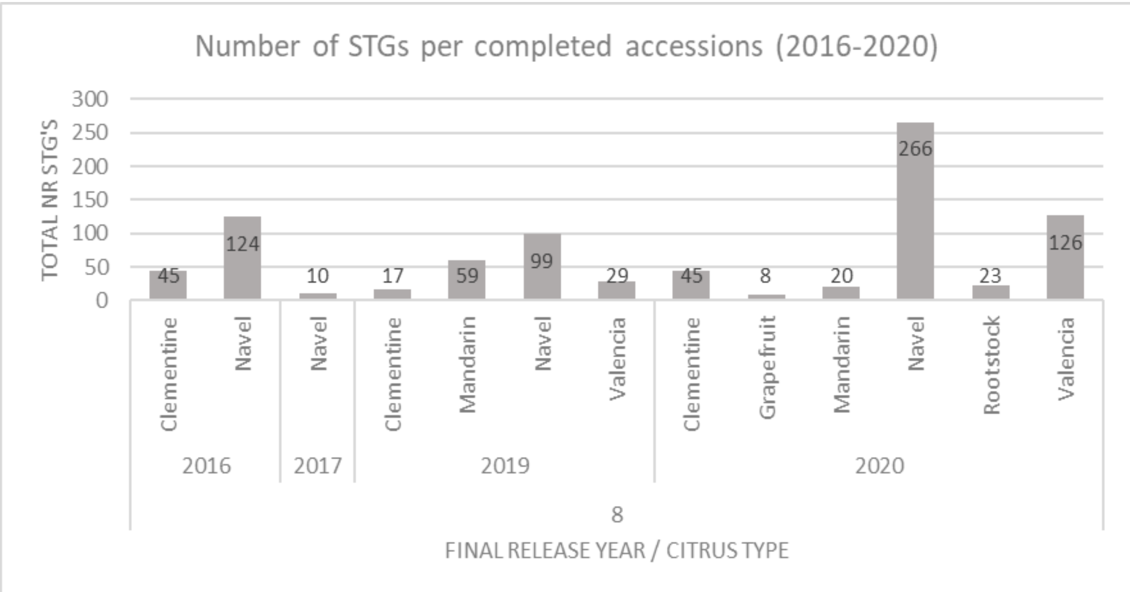

Figure 6.9.3. The number of STGs done for finalized accessions per citrus type and per year in which the accessions were final released to the CFB.

The efficiency and unpredictability of the STG process can be assessed by the number of STG's that was done to obtain a pathogen-free accession. Figure 6.9.4 shows the average number of STGs performed per citrus type for accessions released per year. The values indicate that the STG efficiency has remained constant and the number of STGs required to obtain a successful plant is within the expected range.

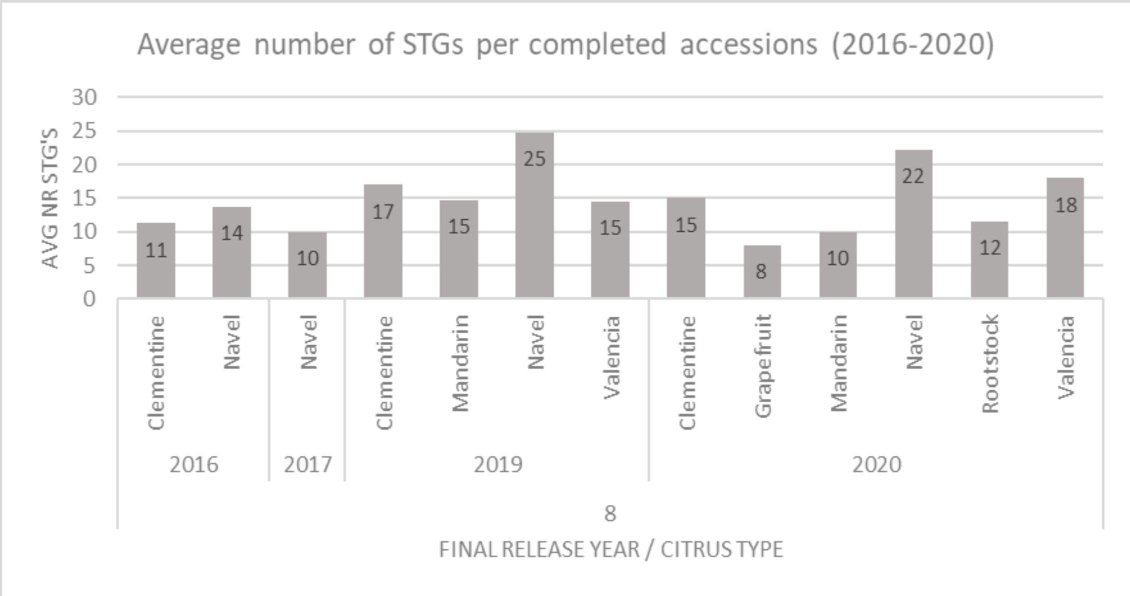

Figure 6.9.4. The average number of STGs performed per citrus type for accessions released per year.

A further improvement to the STG system was the use of all additional buds. Extra buds are budded to available rootstocks and the bud-shoots are used directly for STG. This eliminates the time required for a nurse plant to grow sufficiently before defoliation can occur. Figure 6.9.5 shows that since implementing this approach the average time before the first STG can be performed was reduced to 1 month.

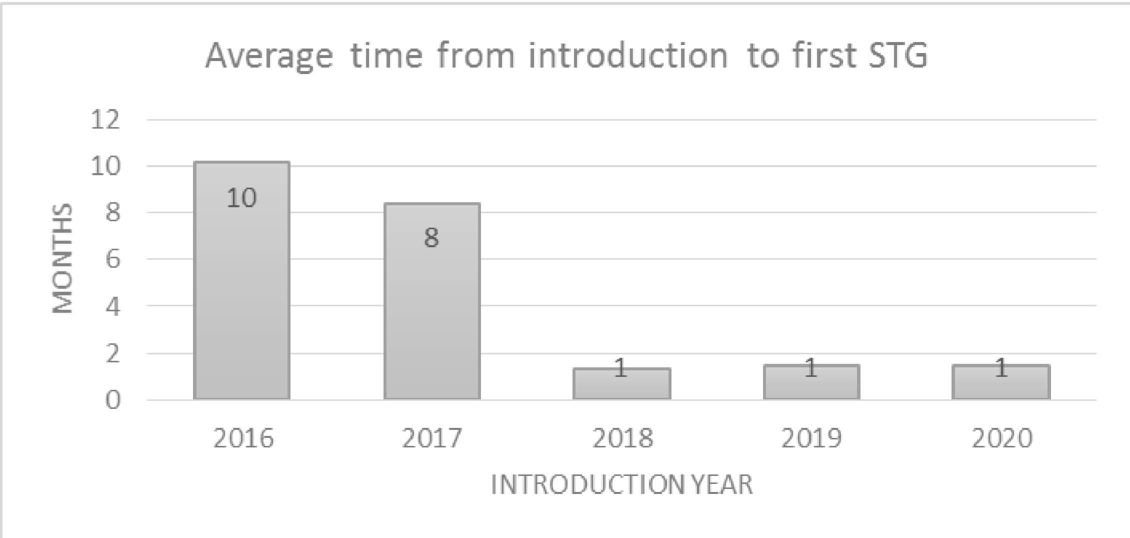

Figure 6.9.5. The average period (months) from receipt of accession to the first STG.

B. Maintenance of the virus-free gene source

The nine accessions which tested positive for CVds and/or CTV during the routine indexing in 2017 are currently in the STG pipeline for pathogen elimination.

The CRI gene source currently comprises 400 accessions and the number of selections per variety type is shown in Table 6.9.2. A Phytophthora outbreak in the CRI Nucleus Block is being addressed by a total renewal of all accessions. As a result of the Phytophthora root rot and losses during re-budding, some accessions were lost and will be replaced from the ARC nucleus block (20 cultivars) or re-STGd from CFB lines (12 cultivars). Larger pots with better drainage were acquired and budding to the new rootstocks has started. A drip irrigation system was installed for controlled water management.

CRI's nucleus block was established in 2004 with material from the ARC nucleus block. It became evident that 3 of these cultivars were not TtT after re-introduction to CFB from the CRI nucleus block. Based on tree morphology comparisons between the ARC and CRI nucleus blocks, we suspect that these mistakes originated from the ARC nucleus block. One other cultivar appears not to be TtT, and differs from the ARC nucleus block tree; this case is being investigated.

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. No additional accessions were verified during the 2019 season. The number of accessions confirmed to be the correct citrus type is presented in Table 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 date as the correct citrus type.

Variety Type	*No. of cultivars at CRI*	*Citrus type confirmed by fruit*
Clementine	34	9
Diverse (Citron, Sour orange, etc.)	2	1
Ellendale	4	-
Grapefruit	24	15
Kumquat	2	2
Lemon	23	21
Lime	4	2
Mandarin hybrid	67	32
Midseason	34	17
Navel	97	16
Ornamental	4	4
Pumelo	8	5
Rootstock	25	19
Satsuma	8	6
Valencia	65	22
Total	**400**	**171**

Insect monitoring in all CRI growth rooms and tunnels commenced as stipulated in the action plan for HLB and ACP.

Cultivar identification of the NB accessions at the ARC was conducted with the assistance of the CRI cultivar evaluator to ensure that the citrus fruit type observed corroborates the database. This evaluation is reported by the ARC.

C. Biological and molecular re-indexing of mother trees and multiplication blocks at the CFB

The screening of the CFB multiplication blocks for citrus viroids in greenhouse structures 1 and 3 was completed. This entailed a representative sub-sampling of 40 640 trees and processing of 882 samples. No viroids were detected in this screening.

Trees of 3 grapefruit accessions in the CFB evaluation block showed severe stem pitting, twig dieback and small fruit, typical of CTV stem pitting decline. These trees were sampled in July 2019 and CTV strain analysis indicated that the trees were not challenged from an external source, but are sensitive to the current CTV pre-immunisation source. Trees from certain of these cultivars in trial sites did not exhibit these symptoms, and we suspect that the cooler climate in the CFB evaluation block was more conducive to CTV. The investigation is ongoing.

We are in the process of pre-immunizing the commercial grapefruit with an approved single-strain CTV source, B390-5. The previous source and the current source will be maintained in parallel until it is confirmed that the new source shows no adverse effects.

SSR marker analyses were done to confirm various cultivars at CFB.

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 by both laboratories. The number of accessions tested for specific pathogens are presented in Table 6.9.3.

Table 6.9.3. Sample numbers of duplicate tests of ARC introductions, for various pathogens.

Pathogen	ARC-TSC accessions
CTV [3]	29
CVd	14
CTLV	14
CPsV	14
'*Ca*' Liberibacter species	18

[3] Includes testing to confirm CTV pre-immunization

E. *Ad hoc* diagnostics for GTDs for growers and external institutions

- Approximately 75 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. Positive samples are further tested to determine the Liberibacter species. Thirty-eight suspect samples were received of which 16 tested positive for 'Ca. L. africanus'. All samples were negative for 'Ca. L. asiaticus'.
- Twenty-seven samples were submitted for CVd testing.

F. *Ad hoc* investigations as required by CIS

- Viroids were identified as the causative agents of stunted trees of a mandarin hybrid on C35 rootstock at two farms. Supply was traced to a nursery that used uncertified budwood. Approximately 140 samples were processed during this investigation.
- An incompatibility problem with C35 and a mandarin was investigated. The rootstock was confirmed as C35 with Simple Sequence Repeat (SSR) markers. No pathogens other than CTV were detected. This incompatibility complaint is further investigated by the Citriculture department.
- SSR markers were used to differentiate 10 rootstocks acquired from Argentina.
- A study to test seed transmission of citrus tatter leaf virus (CTLV) was concluded. Findings of this study were not supportive of CTLV seed transmission in citrus. A manuscript reporting this work was published.

G. Facility management

- Routine maintenance and internal audits were done on a weekly basis and two external audits were conducted by the CIS manager.
- All tunnel entrances were sealed with silicon and covered with psylla screen. All tunnel roofs and walls were inspected and sealed with silicon where necessary.
- Trapping and monitoring as per the HLB/ACP Action Plan was implemented.
- The UV water sterilisation system was serviced.
- Fans were installed in all tunnel double-door, vestibule entrances to minimise insect entry.

- A drip irrigation system was installed for controlled water management in one gene source tunnel.
- Power failure to the facility, caused by uneven electrical phases, was rectified.
- Delay switches for fans were installed in all tunnels to prevent power surges that tripped supply.

Conclusion

Efficient pathogen detection and elimination enables supply of healthy budwood to the industry and is the primary objective of this project. Successful elimination of GTDs from new selections were achieved and these were added to the gene source and supplied to the CFB. The CRI gene source maintenance is an ongoing function. Diagnostic services were provided and analysis of industry problems relating to graft transmissible diseases were addressed.

Technology transfer

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6.10 **PROGRESS REPORT: Diagnostic and technical services for the Citrus Improvement Scheme by the ARC-TSC**
Project P03000127 by E. Jooste (ARC)

1. PEQ for citrus propagation material, including pathogen-therapy using shoot-tip-grafting (STG), conventional and molecular diagnostics

Data were captured for 19 new international budwood introductions and 6 seed introductions, ARC 2079-2103 were captured from April 2019 to March 2020. The plants were established in the quarantine glasshouse. The following outputs for are recorded for April 2019-March 2020:

- A total of 935 STGs performed on 56 selections with 72 successful STGs, which is a success rate of 7.7%.
- Direct PCR for CTV and viroids performed on 26 nurse plants before biological indexing
- Ongoing biological indexing on 17 selections
- Selections in the pipeline are listed in Table 6.10.1 indicating the different phases.

Table 6.10.1. Number of STG's done and successes. Outstanding selections in the STG pipeline are marked in green and selections that are currently in the biological indexing phase are indicated in yellow. STG successes are cumulative and are carried over from quarter to quarter.

ARC#	STG's done Q1	Success April to June	STG's done Q2	Success July to October	STG's done Q3	Success October to Dec	STG's done Q4	Success Jan to March	Comment
1973			11		6			Yes (1)	Micro-grafted
1978	3	-	30		17	Yes(1)	5		Growing to start index
1983			27	Yes(1)			2		Growing to start index
1988	35	Yes (3)							Indexing
1991			15		11		8		
1992	6	Yes (2)*							Meyer lemon
1996			7		7		6		
2006					3		5		
2007	@13	Yes (1) +CTV	17		4	Yes(1)			Growing to start index
2012	10	Yes (5)*		Yes(2)					Indexing
2013	12	Yes (4)							Indexing
2014			10		18		8		
2018	8	Yes (5)		Yes(2)					Indexing
2024			16	Yes(1)	1	Yes(1)			Growing to start index
2026	11	Yes (1)							Indexing
2029	5	-	34	Yes(2)					Growing to start index
2034	22	-	6	Yes(2)					Indexing
2036	4	Yes (2)		Yes(1)					Indexing
2042	30	Yes (1)	17	Yes(1)		Yes(2)			Indexing
2043	4	Yes (2)							Indexing

2044			15	Yes(1)		Yes(1)			Indexing
2046	20	Yes (2)							Indexing
2047	6	Yes (2)							Indexing
2048	2	-	14		6		5		
2049	15	-	14		7		7	Yes (1)	Died
2050	15	Yes (1)	4	Yes(1)	2	Yes(1)			Indexing
2051			6		8	Yes(3)			Growing to start index
2052	3	Yes (2)							Indexing
2053			25	Yes(2)					Indexing
2054			12		13		9		
2063			6		17				
2064			22			Yes(1)			Growing to start index
2065			15			Yes(1)			Growing to start index
2066			6		10				
2067			19		9				
2070			6		15		3		
2074			8	Yes(1)		Yes(2)			Growing to start index
2075					7		5		
2076			15		2				
2077			5		8		8		
2079			1		5		5		
2080			4		6	Yes(1)			Growing to start index
2081			2	Yes(1)	2				Ready for index
2086			1		4		4		
2087					4	Yes(1)	4		Growing to start index
2089			3						
2090			6		5		6	Yes(1)	Growing to start index
2091			2		6		1		
2092									
2093							6	Yes (1)	Micro-grafted
2095							5		
2097							5		
2098									
2099							5	Yes (1)	Micro-grafted
2100									
2101							2		

2102							2		
2103									
Total	**227**	**34**	**401**	**18**	**194**	**15**	**113**	**5**	
Success%	**15%**		**4.5%**		**7.7%**		**4.4%**		

*1992-1: CTLV; *1992-2:CTV, CTLV; *2012-2:CTV; *2012-5: Negative

STG success rate vs number of STGs done:

Seventy of the established nurse plants were pathogen-free after STG. A nurse plant from accession 2012 was infected with CTV after STG, but another nurse plant tested clean and is now in the biological indexing phase.

Four imports from a so-called virus-free repository tested positive for CTV, when budwood was screened and tests for other pathogens are currently underway. This emphasizes the importance of STG to ensure virus-free material is released to industry. Fast tracking of such samples will take longer in the end.

Two successful STGs from 1992 (Meyer lemon) tested positive after STG, one with CTV and both with CTLV. ARC-TSC laboratory are not proceeding with this accession for now, as this is a local accession that was successfully STGd at CRI.

Efforts were wasted on accession 1983 after the selection was withdrawn from the PEQ pipeline due to duplication with another accession.

Analysis of introductions, processes and timeframes relating to citrus PEQ

Yearly introductions

The number of accessions introduced to the PEQ process since 2013 to 2019 is shown in Figure 6.10.1. Figure 6.10.2 and 3 show the number of accessions released for interim (phase 6) and final (phase 8) release. The PEQ workshop agreed that final quarantine release after 6 months bio-index can be implemented when material has tested free from quarantine-pests.

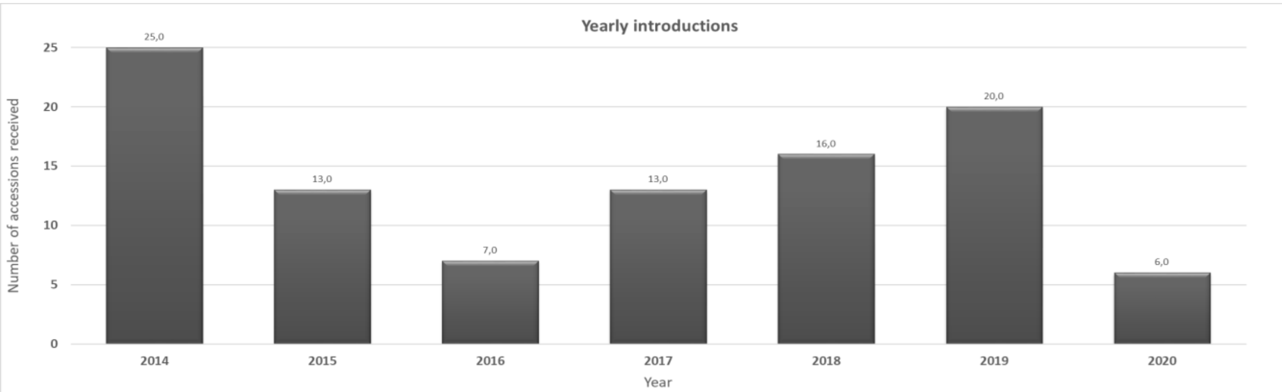

Figure 6.10.1. Yearly introductions from 2014 to March 2020

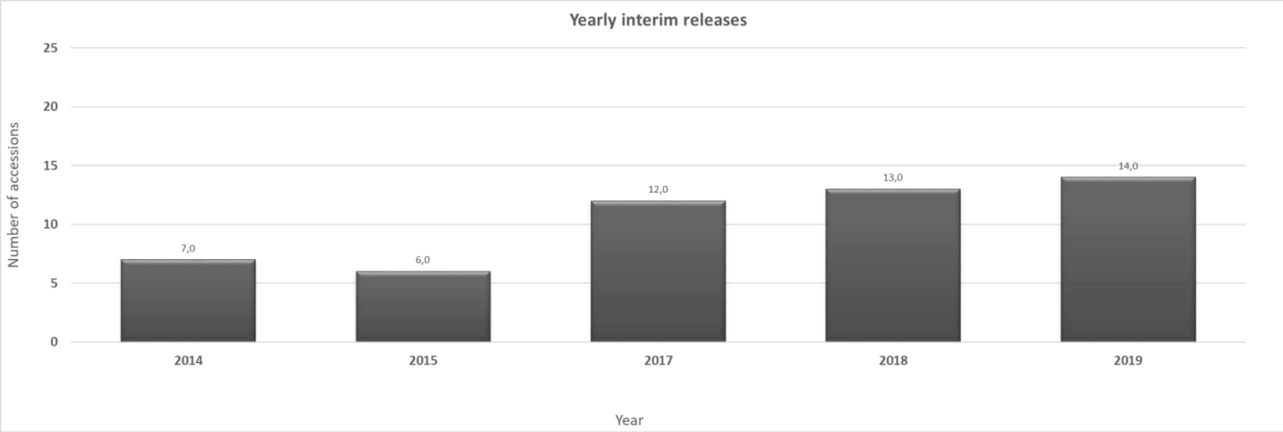

Figure 6.10.2. Yearly interim releases from 2014 to 2019

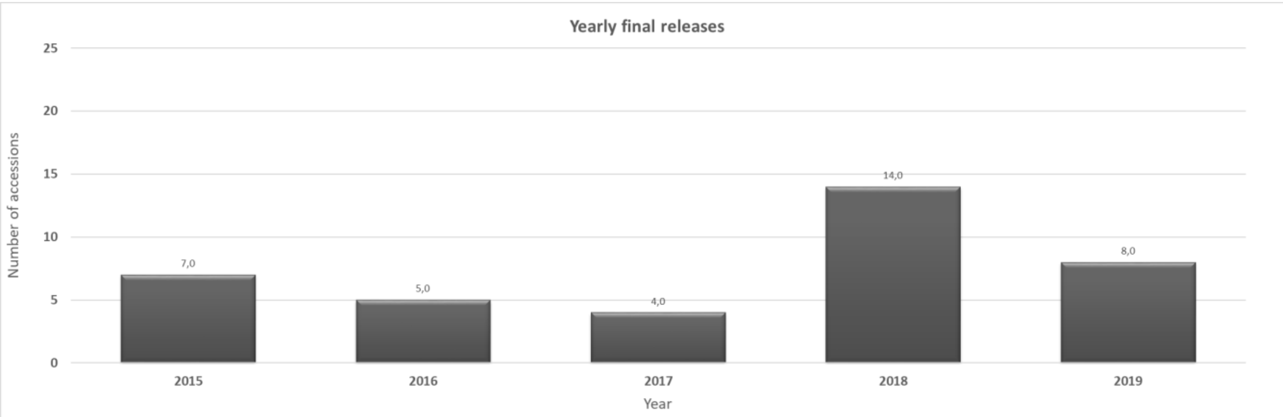

Figure 6.10.3. Yearly final releases from 2015 to 2019

Accessions in pipeline

The progress on the total of 54 accessions currently in the pipeline is summarized in Figure 6.10.4. The phases in the pipeline are summarised as follows:

- Phase 1: Introduction
- Phase 2: STG phase
- Phase 3: Successful STG micrografted to rootstock seedling
- Phase 4: Biological indexing phase
- Phase 5: Line tested virus-free and CTV pre-immunisation commences
- Phase 6: Interim release to CFB
- Phase 7: 12-month biological indexing
- Phase 8: Final release

Two accessions, introduced in 2015 and are still in the STG phase (Phase 2), while two selections from 2014 were successfully micrografted (Phase 3), and one selection is in the biological indexing phase (Phase 4). Additionally, accession 1917, which was introduced in 2012, was successful and is currently in the biological indexing phase (Phase 4). Progress on these older accessions is prioritised.

From the 2016 introductions, one accessions is still in the STG phase (Phase 2) with no STG success yet. The rest of the 2016 accessions, however, all proceeded to different phases in the pipeline, including one micro-grafted, two currently in the biological indexing phase (Phase 4 and 5) and three selections were released (Phase 6). Introductions in 2017 all went through except for two accessions. One accessions introduced in 2017 are now in the biological indexing phase and five accessions moved on to Phase 5. Six introductions from 2018 are currently in the biological indexing phase and STG are performed on six accessions as soon as new shoots are available. Three accessions have micro-grafted plants and are growing to have sufficient buds for biological indexing. Six accessions received in 2019 already went through STG and are growing to have enough buds for biological indexing. Thirteen accessions are in the STG pipeline. At least 20 accessions will be ready for release in 2020, if all confirmation tests in biological indexing are negative.

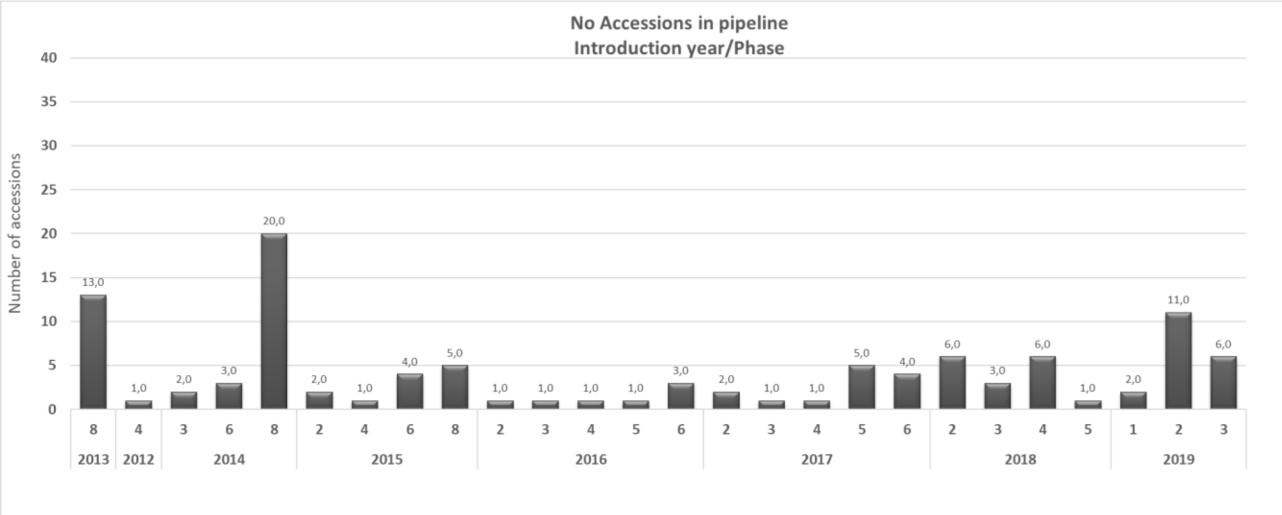

Figure 6.10.4. Number of accessions currently in the pipeline. The introduction year and number of samples in the different STG phases are indicated.

Figure 6.10.5 indicates the average time that the accessions still in the pipeline have been in the system. The expected- and long-term average time for PEQ completion is 2.5 yrs and 3 yrs, respectively. The lagging accessions receive priority and various interventions are already made to speed-up release.

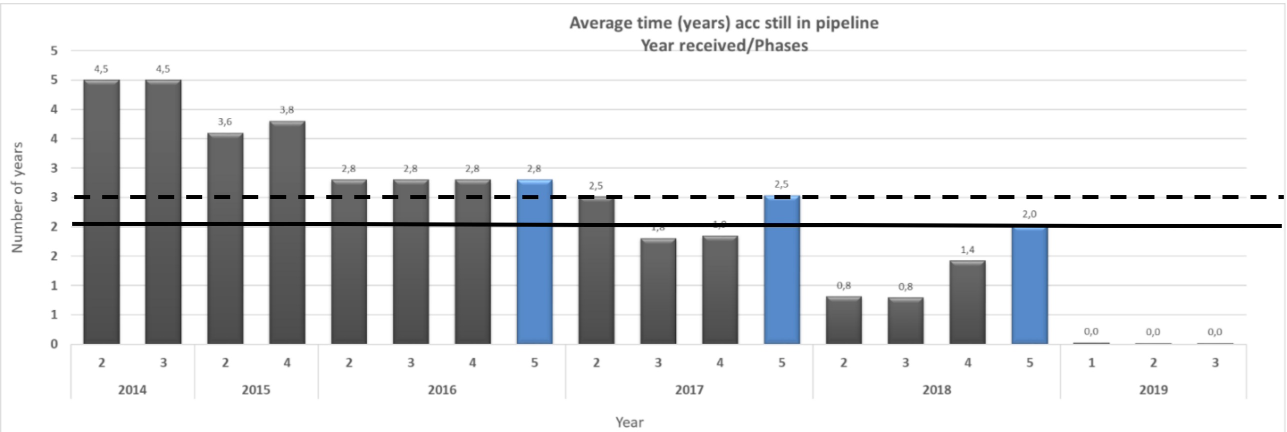

Figure 6.10.5. The average time of accessions in the pipeline indicating year of introduction and the phase. The expected- and long-term average time for PEQ completion are indicated with a solid line and dashed line, respectively. Blue bars indicate a decline in time per phase.

Figure 6.10.6 shows the average time of accessions from introduction to interim release for accessions introduced in 2014-2017. The dashed line indicates the long-term average period to release accessions from PEQ. Previously, thirty-eight accessions were used to compile a baseline average of 3 years. In the past, twelve accessions took longer to reach the interim release phase and represent 30% of the accessions from this period. From the graph (Figure 6.10.6), it is clear that accessions introduced in 2017 proceeded quicker to the interim release phase (Figure 6.10.6). A clear improvement in average release times are shown.

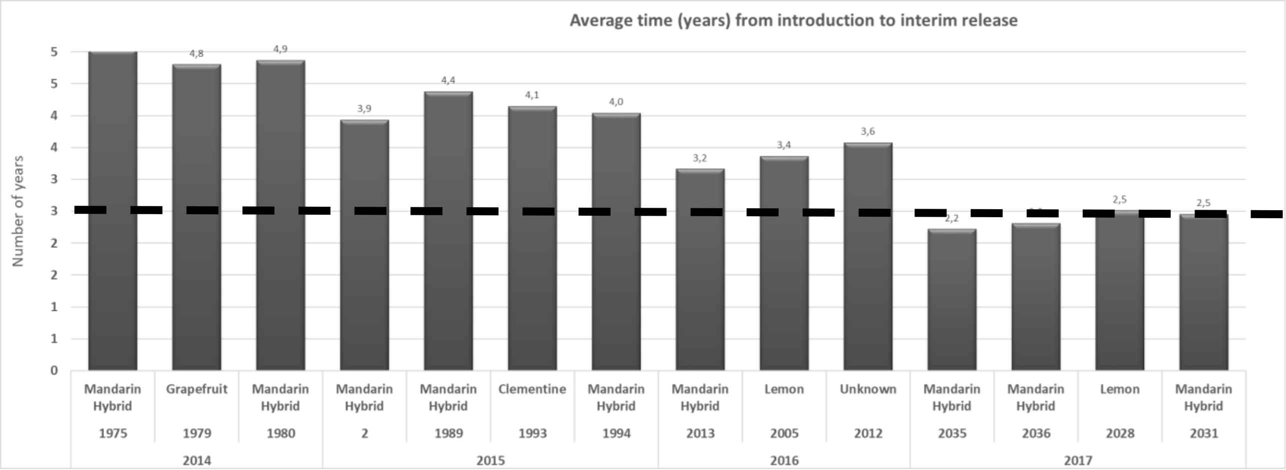

Figure 6.10.6. Average time (years) from introduction to interim release

Number of STGs required establishing a nurse plant

STG is not always successful at the first attempt. The number of STGs done on accessions that were released in 2019 varied as shown in Figure 6.10.7. Some accessions required less than 10 attempts, while for others, up to 67 STGs were performed. The success rate is unpredictable. We implemented the bigger cut during STG, but it is not a bulletproof solution. It depends more on compatibility with the rootstock and on biology.

Currently, three older accessions were identified for which nurse-plants are not yet established. These are 1973, 1991 and 1996. These selections are prioritised. From the previous report, there were six problematic accessions and progress was made in this regard. The average number of STGs per cultivar is summarised in Figure 6.10.8. On average, in our experience, mandarin and grapefruit varieties generally need more STGs before success.

The average time from introduction to first STG attempt is presented in Figure 6.10.9. An improvement in this time is seen from 2017 with a significant improvement visible in 2019 and 2020.

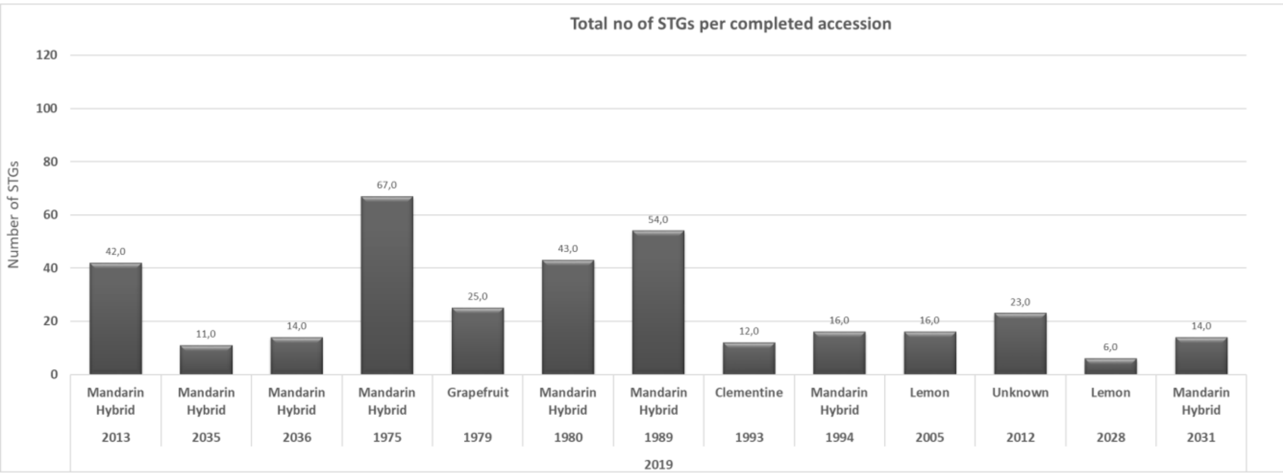

Figure 6.10.7. Number of STGs per completed accession.

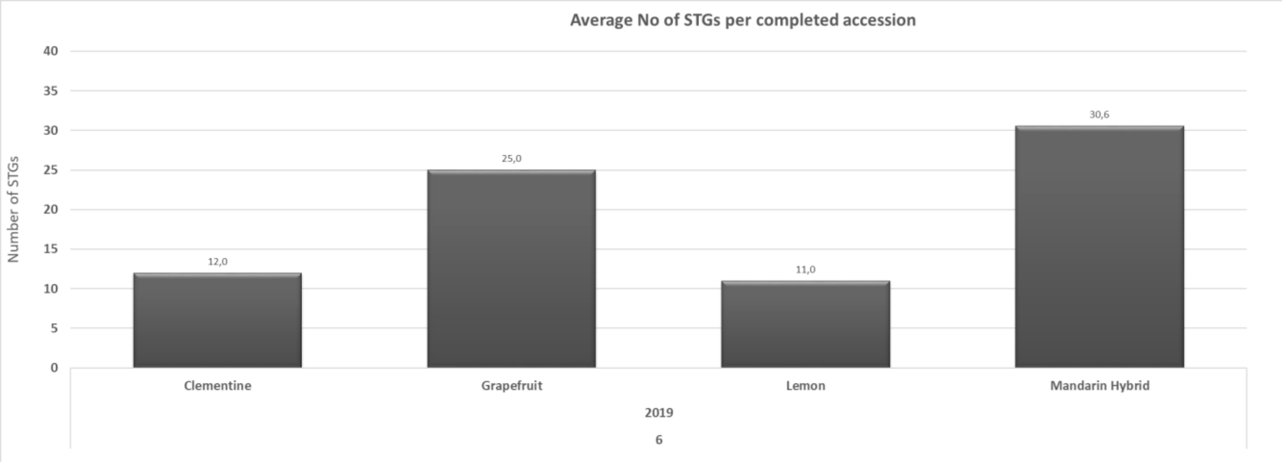

Figure 6.10.8. Average number of STGs per completed accession

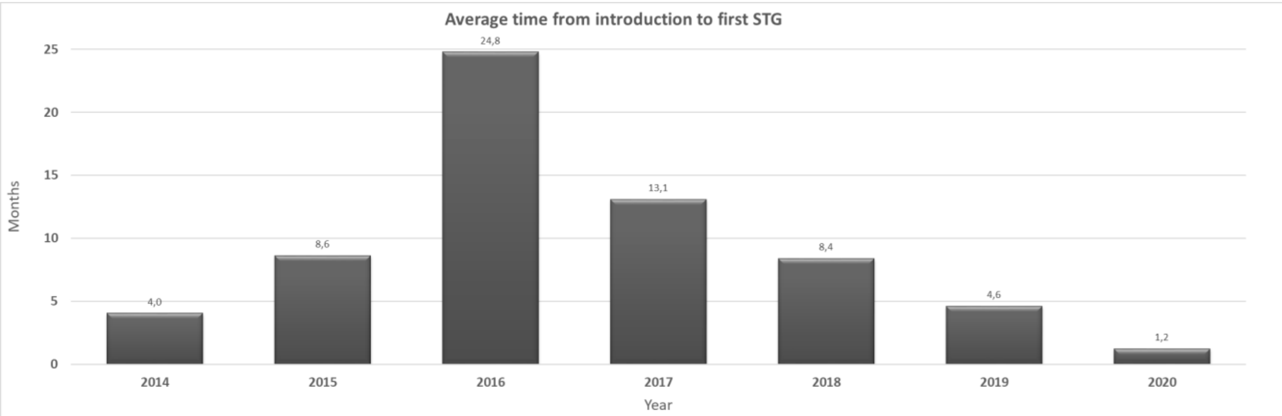

Figure 6.10.9. Average time from introduction to first STG

2. Maintenance of a virus-free nucleus block of citrus cultivars and germplasm

Follow-up Phytophthora tests were done on soil in glasshouses and tunnels in March and October 2019. Necessary treatment was applied to pots in glasshouse 2 and 3; eradication of *Phytophthora* from pots is ongoing.

Currently the ARC nucleus block contains 493 cultivars. Mistakes during repotting/relabelling in the past, have led to some mix-ups, which became apparent during trueness-to-type (TtT) evaluation of exported, field or CFB Evaluation Block trees. Citrus type verifications are done annually with assistance of Johan Joubert from CRI, with photo records for each selection. In total 138 selections have been confirmed as the expected citrus type in 2018 and 2019.

We are currently aware of 10 selections of which trees are not TtT. Six selections were sent for DNA fingerprinting, which is a useful diagnostic tool for mandarin and rootstock hybrids, but unfortunately not for grapefruit, lemons, oranges and Clementines. Two selections were re-introduced from CFB to correct the mistakes in the ARC nucleus block.

TtT verification records are compared with those for the same accessions in CRI nucleus block, as well as the CFB Evaluation Block. Based on supply dates to CRI nucleus block and CFB, and the TtT status of the lines at CRI and CFB, we suspect mistakes occurred between 2001 and 2007, and again between 2007 and 2017. This investigation is ongoing and the unknown lines will also be included in the CFB Evaluation Block for possible later identification.

3. Introduction of cultivars to the CIS Citrus Foundation Block following *Citrus tristeza virus* pre-immunisation

During this period, 14 imported (Figure 6.10.2 and Figure 6.10.3) and 3 local selections were interim released after biological indexing and molecular verifications. Seven virus-free selections were sent to agents to use in trials.

4. CIS diagnostic support for CRI-Nelspruit, including validation of diagnostic tests and improvement of diagnostic protocols

The following duplicate testing was done for CRI:

Pathogen	**Number of accessions-Q1**	**Number of accessions-Q2**	**Number of accessions-Q3**	**Number of accessions-Q4**
CTV-Pre-immunisations	15	6	6	-
CTV-indexed	13	8	8	-
CTLV	21	8	7	-
Greening	17	7	6	-
Viroids	13	8	7	-

The following tests were done for final releases from ARC:

Pathogen	**Number of accessions-Q1**	**Number of accessions-Q2**	**Number of accessions-Q3**	**Number of accessions-Q4**
CPsV	4	4	10	-
Liberibacter's	4	4	4	-
Visual inspection for Impietratura	4	4	10	-
CiVA	-	-	10	-

The following tests were done for interim releases from ARC:

Pathogen	**Number of accessions-Q1**	**Number of accessions-Q2**	**Number of accessions-Q3**	**Number of accessions-Q4**
CTV-Pre-immunisations	-	-	9	9
CTV-indexed	-	9	5	-
CTLV	-	10	4	4
Greening	-	10	4	-
Viroids	-	10	4	-
CPsV	-	10	4	-

5. Technical CIS support through participation on CISAC and CIS-Pathology committees

- Jooste, AEC. Participated in the 19th annual CISAC meeting, CFB, Uitenhage, 18 July 2019
- Jooste, AEC. Participated in the Citrus Foundation Block facility Evaluations on 17 July 2019.
- Jooste, AEC. Participated in the Citrus Post Entry Quarantine Workshop in Stellenbosch, 12 September 2019
- Jooste, AEC., Hlalele, N and Theledi, Z. Participated in two annual Citrus Pathology meetings in Nelspruit, 5 June 2019 and 5 November 2019
- CIS technical support (Ms Hlalele and Ms Theledi) assisted with preparation for the above meetings.

We are committed to improve on performance of PEQ processes and every introduction are treated with high importance. ARC-TSC, PEQ laboratory, thank Citrus Research International for the financial, technical and diagnostic support.

7 INTERNATIONAL VISITS

7.1 Report on the kick-off meeting of the FF-IPM project (H2020), Volos, Greece, 16-20 September 2019

Aruna Manrakhan (CRI)

Background

Citrus Research International (CRI) is one among 21 beneficiaries in the EU Horizon 2020 project entitled *In-silico* boosted pest prevention and off-season focused IPM against new and emerging fruit flies (Project acronym FF-IPM). The FF-IPM project targets three fruit fly (FF) pest species that are considered imminent threats to European horticulture: *Ceratitis capitata* (Mediterranean fruit fly), *Bactrocera dorsalis* (Oriental fruit fly) and *Bactrocera zonata* (peach fruit fly). The project aims to develop prevention, detection and IPM approaches for these fruit fly pests. The prevention tools which will be developed and tested during the course of this project are the use of E-Nose to track FF infested fruit and rapid molecular identification tools to identify intercepted FF in consignments. The detection tools which will be developed and tested in the project include the use of e-trapping for early detection of FF and detection strategies based on spatial modelling. OFF and ON season IPM approaches using different control tools will be tested.

CRI is involved in two work packages (WP) within the project (WP 3 and WP 5). WP 3 aims at developing and enhancing tools and methods for FF prevention. WP 5 aims at developing methods and strategies for FF establishment. As part of these two WPs, CRI will be testing early detection tools for *B. dorsalis* in the northern parts of South Africa. E-traps baited with methyl eugenol lures will be evaluated on *B. dorsalis*. A system of methyl eugenol baited e-traps designed by a decision-support-system tool which is based on CLIMEX modelling simulating population growth using historical and forecast weather data will be tested for early detection of *B. dorsalis*.

A kick off meeting of the FF-IPM project for all beneficiaries was held in Volos, Greece, between 16 and 20 September 2019 in order to discuss the roll out of the project. The first day of the kick off meeting was an open event which provided a stakeholder's perspective of the fruit fly problem (See Annex 1: FF-IPM meeting agenda). On day two, the different WPs were introduced and all the beneficiaries were also briefed on project management, tasks under the different WPs, communication and dissemination. Thereafter fine tuning workshops were held for the different WPs where beneficiaries had an open discussion on the roll out of the tasks under each WP.

The aim of this report is to provide (A) information gathered during the open event of the FF-IPM meeting when the different stakeholders were invited to present their perspectives of the FF problem and (B) relevant contacts for potential future collaboration on other citrus pests and diseases.

A. Key points on key talks in the open event of the FF-IPM kick off meeting

<u>1. European regulations and state implementation, Christos Arampatzis, Phytosanitary Authorities of Greece</u>

- Fruit flies are important threats for the EU due to high number of interceptions of fruit flies (fruit flies constituting almost 1/3 of all intercepted pests).
- Pest survey cards have been developed for surveillance of specific fruit fly pests (*B. dorsalis*, a joint *C. rosa* and *C. quilicii* pest survey card). Pest survey cards include information on the biology of the pest, its EU regulatory status and survey guidelines.

<u>2. The European Food Safety Authority involvement in fruit fly invasion, G. Stancanelli**,** EFSA</u>

- In the European Union, risk assessment and risk management are separated.
- EFSA receives questions from EU commission, EU parliament and member states. These questions are then evaluated, assessed and EFSA advises.

- EFSA has a plant health panel (PLH) which is now in its 5th term (2018-2021).
- The objectives of the PLH are to provide high quality, independent and scientific advice to EU risk managers and to contribute to development of science-based approaches for phytosanitary pest risk assessment
- EFSA was tasked by EC DG Sante to categorize about 130 insect pests including non-European Tephritidae.
- There are challenges in the categorization of non-European Tephritidae due to large number of species, taxonomical disagreements, lack of information for some of the species.
- **The deadline for categorization of non-European Tephritidae by EFSA is November 2019.**
- EFSA has established a tool kit for pest surveys. The tool kit consists of pest survey cards, survey guidelines (guidelines for 3 pilot organisms including *Phyllosticta citricarpa* are available) and statistical tools to design surveys (RIBESS+) for pest freedom and pest prevalence (SAMPELATOR)
- EFSA also carries out media and literature monitoring which is captured on a platform entitled MEDISYS.

<u>3. Detection of major fruit fly species in Europe – cases in Austria and Italy, Alois Egartner and Andrea Sciarretta, AGES and University of Molise</u>

- Fruit fly trapping has been conducted in Vienna and other sites in other provinces in Austria
- More than 1100 Medfly adults were captured in traps placed in Vienna between 2010 and 2018.
- Medfly infestation was recorded in peach, apricot and pear in Vienna.
- *B. dorsalis* (7 specimens in 2018) and *B. zonata* (1 specimen in 2018) were also captured in traps in Vienna.
- *B. dorsalis* was detected in Campania region in Italy in 2018.

<u>4. Ongoing fruit fly invasion events in Middle East and national responses, David Nestel and David Opatowski, ARO and PPI of Israel</u>

- Fruit fly species that have recently invaded Israel are *Dacus ciliatus* and *Bactrocera zonata*.
- *Bactrocera zonata* is currently in Tel Aviv in Israel and under official control.
- A systems approach for mitigation of *B. zonata* has been put in place in Israel and this consists of monitoring of the males using methyl eugenol baited traps, bait application, orchard inspection, packhouse practices such as sorting and grading, inspection of packed consignments.
- Until today, no *B. zonata* has been found in commercial citrus fruit in Israel.
- There is a potential for establishment of *B. zonata* in a few areas in southern Europe based on modelling outputs from the CLIMEX model.

<u>5. Spanish growers' association perspectives on the control of the Mediterranean fruit fly, Nicolas Juste, ANECOOP</u>

- Citrus is grown over 165 000 ha in the Valencia community
- All farms are mapped and categorized according to citrus variety and other crop characteristics in a Geographical Information System
- A network of 2 019 traps are deployed across the orchards.
- These traps are serviced weekly by six field inspectors on 21 trapping routes.
- Medfly is controlled using SIT (300 million sterile male flies released per week over 140 000 ha).
- Bait stations and mass traps are additionally deployed in orchards of early varieties of citrus, stone fruit, fig, pomegranate, persimmon and table grapes.
- Co-ordinated bait spraying is also organized and the trigger for these sprays is 0.5 flies per trap per day (3.5 flies per trap per week). Aerial and ground based sprays are conducted. The bait applied for ground based sprays is a mixture of lambda-cyhalothrin and protein hydrolysate.
- A monitoring network for surveillance of non-European fruit flies has also been deployed (50% of the costs financed by European Union).
- Contingency plans have been put in place for HLB and *B. dorsalis*.

B. Relevant contacts for potential future collaboration on other citrus pests and diseases

1. Dr Mattia Crivelli, PCA Technologies s.r.l. (m.crivelli@pcatechnologies.com) will be involved in developing an electronic nose for detection of FF infested fruit in WP3 of the FF-IPM project. I spoke to Dr Crivelli on the use of this technology for detection of HLB in plants. He said that he would be interested in collaborating with CRI in the development of this method.
2. Dr Helene Delatte, CIRAD (helene.delatte@cirad.fr) is currently working on the molecular analysis of species in the *Trioza erytreae* complex. She would be interested to receive *T. erytreae* specimens from South Africa for inclusion in her analysis. CIRAD would be a good contact point as well for identification of *Diaphorina* and *Trioza* species.

Acknowledgments

My participation at the kick-off meeting of the FF-IPM project was funded under CRI Project 1261 (FF-IPM).

Annex 1: Agenda of the kick off meeting of the FF-IPM project

Kick off meeting of the FF-IPM project
16 – 20 September 2019
Volos
"FF-IPM"
"*In-silico* boosted, pest prevention and off-season focused IPM against new and emerging fruit flies ('OFF-Season' FF-IPM)"

Agenda

Sunday 15 September 2019	
Arrival – Hotel Xenia Portaria	
Monday 16 September 2019 – Hotel Xenia Portaria *Open Event – A stakeholders perspective on the fruit fly problem*	
08:30 – 09:00	Registration
09:00 – 09:15	Welcome remarks – UTH Officials (Rector, the Dean of the School of Agricultural Sciences, the Chair of the Department of Agriculture, Crop Production and Rural Environment
	Chair: **Panos Milonas** and **Ana Larcher Carvalho**
09:15 – 09:30	Fruit fly invasion at regional level (Europe and the Mediterranean) **F. Petter,** EPPO
09:30 – 09:45	The European Food Safety Authority involvement in fruit fly invasion, **G. Stancanelli,** EFSA
09:45 – 10:15	European regulations and state implementation – **Christos Arampatzis and Bruno Faraglia,** Phytosanitary Authorities of Greece and Italy
10:15 – 10:45	Detection of major fruit fly species in Europe – cases in Austria and Italy **Alois Egartner and Andrea Sciarretta,** AGES and University of Molise
10:45 – 11:00	Discussion
11:00 – 11:30	Coffee break

11:00 – 11:15	Current activities and future perspective regarding fruit fly population monitoring in northern Greece **Antonis Ifoulis,** Regional Plant Protection Authority of North Greece
11:15 – 11:30	The fruit fly problem from the European fruit production industry **Kostas Pavlopoulos,** ALME
11:30 – 11:45	Ongoing fruit fly invasion events in Middle East and national responses **David Nestel and David Opatowski,** ARO and PPI of Israel
11:45 – 12:00	National and growers' organizations response to *Bactrocera dorsalis* invasion events in South Africa **Aruna Manrakhan and Minette Karsten,** CRI and SU
12:00 – 12:15	Spanish growers' association perspectives on the control of the Mediterranean fruit fly **Nicolas Juste,** ANECOOP
12:15 – 12:45	Response to fruit fly invasion in Australia **Antony Clarke,** Queensland University of Technology
12:45 – 13:00	Discussion
13:00 – 15:00	Lunch break
	Chair: **Helene Delatte** and **Josep Jaques Miret**
15:00 – 15:30	Fruit fly taxonomy and invasion events **Marc de Meyer,** Royal Museum of Central Africa
15:30 – 16:00	Predicting the distribution and range expansion of major fruit fly species **Darren Kriticos,** CSIRO
16:00 – 16:30	Adoption and implementation of IPM against fruit flies **Slawomir Lux,** in Silico ipm
16:30 – 17:00	Coffee Break
17:00 – 17:15	In-silico boosted, pest prevention and off-season focused IPM against new and emerging fruit flies – the FF-IPM project **Nikos Papadopoulos,** University of Thessaly
17:15 – 18:00	Discussion

Tuesday 17 September 2019 – Hotel Xenia Portaria *Kick-off Meeting*		
08:00 – 08:30	Gathering of participants	
08:30 – 09:00	Welcome remarks – Agenda - Introduction	UTH & PO
09:00 – 09:30	The FF-IPM project – overview, expectations, challenges	UTH
09:30 – 10:00	Introduction of partners and their role in the FF-IPM project	All
10:00 – 10:30	Coffee break	
10:30 – 11:00	**George Predoiu –** European Community - The FF-IPM project officer	
11:00 – 11:20	**WP1: Project management –** Nikos Papadopoulos and Georgia Pachlitzanakis • Administrative and financial issues, Scientific and Data management • Interactions and integration, communication tools • Risk management • Exploitation committee and advisory board • Quality procedures and reporting, • Next meeting planning	
11:20 – 11:40	**WP2: Extension of the biological knowledge of the target FF species –** Nikos Papadopoulos Objectives, tasks, deliverables, expected results, milestones, approaches, preliminary results	

11:40 – 12:00	**WP3: Development and enhancement of tools and methods for FF prevention –** Marc De Meyer Objectives, tasks, deliverables, expected results, milestones, approaches, preliminary results
12:00 – 12:20	**WP4 - Development and enhancement of tools and methods for FF prevention and OFF- and early season management –** Josep Jaques Objectives, tasks, deliverables, expected results, milestones, approaches, preliminary results
12:20 – 12:30	Discussion
12:30 – 14:00	Lunch break
14:00 – 14:20	**WP5 - Methods & strategies for prevention of FF establishment and range expansion in Europe –** Darren Kriticos Objectives, tasks, deliverables, expected results, milestones, approaches, preliminary results
14:20 – 14:40	**WP6 - Enhancement of methods & strategies for OFF- & ON-Season FF management –** Slawomir Lux Objectives, tasks, deliverables, expected results, milestones, approaches, preliminary results
14:40 – 15:00	**WP7 - Sustainability – development of infrastructure and establishment of business-based innovative services for decision-support in FF prevention and management –** Helene Delatte Objectives, tasks, deliverables, expected results, milestones, approaches, preliminary results
15:00 – 15:20	**WP8 - Communication and dissemination –** Filippos Karamanlis Objectives, tasks, deliverables, expected results, milestones, approaches, preliminary results
15:20 – 15:40	**Ethics – procedures, verification assurance**
15:40 – 16:00	Discussion
16:00 – 16:30	Coffee break
17:00 – 18:00	General discussion
20:00 – 22:00	Dinner – UTH will host the social event
Wednesday 18 September 2019 - Hotel Xenia Portaria *Fine tuning workshop (data harmonization and activities coordination)*	
08:30 – 09:00	Welcome Coffee
09:00 – 12:30	WP3 and WP5 activities planning and coordination
12:30 – 14:00	Lunch break
14:00 – 17:00	WP4 and WP6 activities planning and coordination
17:00 – 18:00	WP7 and WP8 activities planning and coordination
18:00 – 19:00	General Discussion
20:00 – 22:00	Dinner
Thursday 19 September 2019 *Fine tuning workshop (data harmonization and activities coordination)*	
08:30 – 09:00	Welcome Coffee
09:00 – 17:00	WP2 data collection, curing and archiving
Friday 20 September 2019 *Excursion*	
08:00 –	Departure to Meteora

10:00 – 14:00	Visit to 3 monasteries
14:00 – 16:00	Lunch – Trikala
16:00 –	Departure to Volos
Saturday 21 September 2019 *Departure*	

8 EXTENSION / VOORLIGTING

By Hannes Bester, M.C. Pretorius, Wayne Mommsen, Keith Lesar, Catherine Savage, David Groenewald (CRI), Andrew Mbedzi and Melton Mulaudzi (CGA-GDC)

8.1 VOORLIGTINGOORSIG

8.1.1 Die 2019 Seisoen

Die finale volumes wat gedurende 2019 uitgevoer is, was bykans tien miljoen kartonne laer as die oorspronklike skatting aan die begin van die seisoen, en net oor die agt miljoen kartonne laer as in 2018. Die uitvoervolumes op sagtesitrus en suurlemoene was ietwat op teenoor die vorige jaar, maar die volumes op pomelo's, nawels en Valencias was beduidend laer. Terwyl die droogte sy tol in sekere areas geëis het, het swak kleur, baie letsels en vruggrootte bygedra tot laer uitpakpersentasies. Die seisoen is deurgaans as baie moeilik beskryf.

Vruggehalte was oor die algemeen nie goed nie. Die vrugte het gesukkel om op te kleur, net soos in 2018, met sure wat vinnig geval het in sekere areas. Dit kon toegeskryf word aan verskeie faktore, hoofsaaklik klimaatsfaktore. Uitpakke was swak a.g.v swak kleur, baie letsels en klein vruggroottes. Letsels, bederf en valskodlingmot was die drie hoofredes vir afkeurings deur PPECB. In sekere areas het van die nawels droog voorgekom, met dik skille en lae sappersentasie. Witluis en blaaspootjie was in feitlik alle areas 'n groot probleem. Dis duidelik dat die swartvlek spuitprogramme en die geweldige druk vanaf die markte op die gebruik van chemie die beheer van hierdie plae toenemend moeiliker maak.

Terugvoer uit die mark was dat die bederf hoog was, veral op Satsumas, suurlemoene en nawels. Vrugte het in baie gevalle pap in die mark aangekom. Vrugkleur was ŉ groot probleem en a.g.v baie letsels is te veel klas twee vrugte in verhouding tot klas een uitgevoer, wat groot druk op die prys geplaas het. Die algemene siening in die mark was dat die gehalte van vrugte uit Suid-Afrika swak was.

Daar was ook vroeg in die seisoen reeds druk op veral die Durban-hawe met klagtes van trokke wat baie lank moes wag om af te laai. Daarmee saam het heelwat afkeurings op vrugte na Korea, a.g.v witluis, verdere druk op die logistieke stelsel geplaas, asook stakings wat later in die hawens voorgekom het.

In sekere produksiegebiede het die droogte krisis-afmetings aangeneem, veral in die noordelike gebiede van die land. Hoewel goeie reëns laat in die seisoen in verskeie dele van die land uitgesak het, was dit in die noordelike streke te laat om ŉ verdere afname in uitvoervolumes vir 2020 te voorkom.

Produsente het die seisoen as baie moeilik beskryf, met baie uitdagings wat oorkom moes word: swak kleur, swak vruggehalte, bederf, markte wat by tye onder druk was, probleme met stakings in die hawens, afkeurings a.g.v witluis, en vele meer. Ten spyte van al die uitdagings is daar steeds groot aanplantings wat plaasvind, wat toon dat die produsente optimisties bly oor die toekoms van die sitrusbedryf.

8.1.2 Die 2020 Seisoen

Die eerste skattings dui op ŉ rekord uitvoer-oes van 143,3 miljoen kartonne, hoofsaaklik a.g.v jong boorde wat hoër produksies lewer, asook nuwe boorde wat in produksie kom. Goeie reëns het wyd oor die produksie-streke geval en die ergste droogte is gebreek, ten minste vir hierdie seisoen. Terugvoer uit die streke is dat die oes baie goed lyk wat volumes, vruggrootte en voorkoms betref. Produsente is opgewonde oor pryse vir die eerste suurlemoene wat reeds op die water is.

Daar heers egter groot onsekerheid oor die nadelige uitwerking wat die COVID-19 pandemie hierdie seisoen op operasionele aktiwiteite, soos oes, verpakking, beskikbaarheid van verpakkingsmateriaal, en logistiek sal hê. Daar is op die oomblik 'n baie goeie aanvraag na sitrus regoor die wêreld, veral na suurlemoene, met gepaardgaande goeie pryse wat behaal word. Alle moontlike maatreëls op alle vlakke in die sitrusbedryf word in plek gestel om die uitvoere so glad moontlik te laat verloop. Indien dit geslaagd is, kan uistekende pryse op plaasvlak verkry word a.g.v die groot vraag na sitrus en die swak waarde van die Rand. Dis bemoedigend om te sien hoe positief die produsente op hierdie stadium is, ten spyte van die groot uitdagings wat die bedryf vanjaar in die gesig staar.

Alles moontlik word gedoen om boord-inspeksies en fitosanitêre inspeksies na spesiale markte so glad moontlik te laat verloop. Die nuwe eCertification stelsel wat hierdie seisoen geïmplementeer word, behoort baie tot die suksesvolle uitreiking van fitosanitêre sertifikate by te dra, en om hierdie proses vir produsente en uitvoerders baie te vergemaklik. Pakhuise het nog verdere uitdagings as gevolg van MRL vereistes in spesiale markte en vraagtekens word geplaas oor die vermoë om kwaliteit sitrusvrugte in die toekoms uit te voer a.g.v druk uit die mark om minder chemie te gebruik.

8.1.3 **CRI Postharvest Technical Forum**

Gedurende die tweede kwartaal het drie verskillende uitvoerders klagtes van oorsee ontvang oor kartonne wat inmekaar gesak het en paletvragte wat onstabiel en beskadig is. Al die gevalle is ondersoek. Die verslae met die foto's wat ontvang is, het duidelik getoon dat die probleme veroorsaak is deur paletvragte wat nie na behore op padmotorvoertuie gestabiliseer en vasgemaak was nie, asook rowwe hantering. Die uitvoerders het dit met die betrokke instansies opgeneem en die probleme is opgelos.

Die CGA se logistieke personeel het 'n stelsel geïmplementeer waarvolgens CRI-PTF op hoogte gehou word van verpakkingsverwante probleme wat in Durban ondervind word. Die probleme is dan met die spesifieke pakhuise opgeneem en reggestel. Die grootste probleme is palettiserings-protokolle wat nie gevolg word nie, asook rowwe hantering in die hawe.

CRI-PTF is deur 'n uitvoermaatskappy genader wat probleme ondervind het met kartonne wat inmekaar gesak het. Volgens die inligting wat bekom is, was dit duidelik dat die probleme veroorsaak is deur swak gehalte kartonne wat vervaardig en opgemaak is deur 'n kartonvervaardiger wat nie by die Packaging Working Group geakkrediteer is nie, palettiserings protokolle wat nie gevolg is nie en kartonne wat nat geword het. Die bevindings is aan die uitvoermaatskappy deurgegee en hulle het dit met die betrokke produsent opgeneem.

CRI is deur twee verskillende uitvoer-organisasies genader wat ernstige probleme ondervind het met onderskeidelik massa kratte (bulk bins) en E15D kartonne wat inmekaar gesak het. Wat die massa kratte betref, het CRI in samewerking met 'n Sanas geakkrediteerde laboratorium 'n volledige ondersoek gedoen en bepaal dat die kratte nie aan die aanbevole spesifikasies voldoen het nie. Wat die E15D kartonne betref kon bepaal word wie die kartonne vervaardig het en toetse is gedoen om te bepaal of die kartonne aan die spesifikasie voldoen. Die uitslae is met die uitvoer-organisasie bespreek.

Een van die groot uitvoerders van pomelo's na Japan het gedurende die laaste gedeelte van die 2019 seisoen probleme ondervind met 600 x 400mm teleskopiese kartonne wat inmekaar gesak het, asook "cubing" van hulle vrugte. Op hulle versoek is daar in samewerking met twee van hul kartonverskaffers verskeie ondersoeke gedoen. Daar is bevind dat die buitestuk van hul reeks teleskopiese kartonne nie die regte hoogte gehad het nie en dus nie tot die stapelsterkte bygedra het nie, en dat daar ook probleme met die groottegradering van sekere tellings was. 'n Eenparige besluit is geneem om proewe met versterkte binnestukke te doen. Die besluit is geneem dat proewe met die eerste pomelo's na Japan in 2020 gedoen sal word.

Een van die "UK Multiples" het aan die einde van 2019 te kenne gegee dat Suid-Afrika se E15D kartonne nie op standaard is nie en in 'n baie swak toestand op die oorsese mark aangekom het. Hulle was van mening dat kartonne afkomstig van Peru van beter gehalte is en dat hulle dit oorweeg om die SA kartonvervaardigers te verplig om die spesifikasies van die kartonne wat hulle van Peru ontvang, te volg. In samewerking met die spesifieke groep en een van Suid-Afrika se uitvoerorganisasies is die hele aangeleentheid ondersoek. Kartonne van Peru is ontvang en stapelsterkte (BCT) toetse wat by Sappi se laboratorium gedoen is, het getoon dat die BCT waardes baie goed is. As deel van die ondersoek is die BCT waardes van al die E15D kartonne se akkreditasie toetse van 2018 en 2019 nagegaan. Die papierkombinasies wat gebruik word speel ‘n baie groot rol. Een van die groot kartonvervaardigers se papierkombinasies was deurgaans aansienlik ligter as dié van die ander vervaardigers. Die ondersoek het verder getoon dat sekere pakhuise ook nie die aanbevole palettiserings protokolle gevolg het nie. Baie probleme met onstabiele palette is ook veroorsaak deur uiters rowwe hantering. Die gevolgtrekkings uit die ondersoek is dat geen probleme met Suid-Afrikaanse kartonne en onstabiele palette ervaar sal word nie as kartonne met die regte papierkombinasie vervaardig sal word, die Verpakkingswerkgroep se palettiserings protokolle gevolg word, en rowwe hantering gestaak word.

Die grootste vervaardiger van “Linerboard” en “Fluting” is in die proses om ŉ nuwe verbeterde “Linerboard” met ŉ laer basiese massa te ontwikkel. Sodra hierdie produk beskikbaar is, sal daar kartonne vervaardig word en laboratorium toetse gedoen word. Die toetsprogram is met een van die geakkrediteerde kartonvervaardigers bespreek en hulle is bereid om die proewe te doen.

Verskaffing van kartonne deur “vervaardigers” wat nie geakkrediteer is nie bly ŉ probleem. Hulle het nie masjiene om riffelbord te vervaardig nie en juis daarom kan hulle nie geakkrediteer word nie. Gedurende die tweede kwartaal het dit weer aan die lig gekom dat een van die instansies kartonne aan ŉ produsent verskaf het wat hoegenaamd nie aan die spesifikasies voldoen het nie. Dit het aanleiding gegee tot ŉ Snykant artikel wat uitgestuur is, waarin die hele akkreditasie stelsel weer verduidelik is en produsente weer versoek is om slegs kartonne wat deur geakkrediteerde kartonvervaardigers vervaardig word, te gebruik.

ŉ Ondersoek is gedoen met “NanoProtect” se behandeling van uitvoerkartonne met hulle “Nano Coating”, wat na hulle mening die kartonne waterdig sou maak en die stapelsterkte van kartonne aansienlik sou verhoog sodat ligter papierkombinasies gebruik kon word en sodoende die prys van die kartonne sou verlaag. A15C kartonne met ligter papierkombinasies is gebruik om die buitekant van die binne- en buitestukke met die Nano Coating te bedek, waarna stapelsterkte toetse gedoen is. Die besparing van 71c per karton op die ligter papierkombinasie is verreken teen die R3.75 wat dit gekos het om die karton te behandel, wat beteken dat ‘n karton dus R3.04 addisioneel sou kos. Verder was die stapelsterkte (BCT) van die ligter karton 374kgf, in plaas van die minimum vereiste van 600kgf. Hierdie negatiewe resultate is aan “NanoProtect” gestuur en daar is geen verdere terugvoer ontvang nie.

Ibhayi Global Packaging tesame met ŉ toonaangewende Landbou Koöperasie in die Oos-Kaap het CRI-PTF genader met die versoek om hulle riffelbord-papier “Box on Pallet” massa krat te gebruik. Hulle idee was om die bestaande massa kratte (bulk bins) met hulle “Box on Pallet” te vervang. Die ontwerp alleen dui daarop dat dit hoegenaamd nie vir sitrus geskik sal wees nie. Om die twee grootste tekortkominge te noem: Daar is geen versterking in die vier hoeke nie en ook geen ventilasie-gate nie. Ten spyte daarvan het hulle nogtans onderneem om die kratte na ŉ sitruspakhuis in die Oos-Kaap te neem vir voorlopige toetse. Geen verdere inligting is tot hede ontvang nie.

CRI-PTF is deur ŉ maatskappy, wat nog nie vantevore by sitrus betrokke was nie, genader met ‘n nuwe prototipe massa krat (bulk bin) ontwerp, met die voorneme om die massa kratte aan sitrusprodusente te verkoop. Voorbeelde van hierdie massa kratte is tydens die CRI Na-oes werkswinkels by die Eiland en Loskopdam uitgestal. Dis aan hulle verduidelik dat daar ŉ proses is wat gevolg moet word alvorens die kratte op ŉ kommersiële basis gebruik kan word. Voorlopige toetse is gedurende Maart by ŉ pakhuis in die Marble Hall omgewing gedoen. Kratte met ŉ binnemaat hoogte van 870mm (bestaande J50B) is met suurlemoene gevul. Soos wat verwag is het die kratte al in die pakhuis inmekaar gesak. Die vervaardigers was uit die aard van die saak teleurgesteld en het onderneem om weer na die ontwerp en hulle materiaal te gaan kyk.

Na ŉ baie voorlopige ondersoek met OptiFlo Freezer Spacers is ŉ versoek aan hulle gerig om die bestaande “spacers” laer te maak en ook die kante toe te maak sodat koue lug vertikaal deur die paletvragte geforseer kan word. ŉ Aangepaste “spacer” is beskikbaar gestel vir meer volledige toetse. Die “spacer” is bo-op ŉ standaard 1210 x 1010mm sitruspalet geplaas en A15C kartonne, gevul met suurlemoene, is daarop geplaas. Dit het baie gou duidelik geword dat die aangepaste “spacer” se effektiewe lengte- en breedte-afmetings te klein is. Die hoeke van die kartonne het aan al vier kante oorgehang. Na die sesde laag op die palet gestapel is, is die kartonne op die onderste laag erg deur die “spacers” beskadig. Die proef is gestaak en ‘n volledige verslag is aan hulle gestuur. Daar is onderneem om weer van voor af na ‘n ontwerp vir uitvoersitrus te kyk.

CRI-PTF is deur SafeLoad genader, wat in die proses is om plastiese palette te ontwikkel. Dis aan hulle verduidelik dat CRI vir etlike jare al besig is met proewe met plastiese palette van ander vervaardigers en dat die palette, wanneer sterk genoeg, heeltemal te duur, of wanneer die prys aanvaarbaar is, doodeenvoudig net nie sterk genoeg is nie. Hulle is egter besig om na ŉ totale nuwe ontwerp te kyk en het onderneem om, wanneer hulle die eerste prototipe gereed het, weer met CRI in verbinding te tree.

Tydens die jaarlikse na-seisoen vergadering met Sappi einde Augustus, is kommer uitgespreek oor die drastiese afname in bestellings vir “Kraftpride” en “Ultraflute”, asook baie bestellings wat gekanselleer is. Dis aan hulle verduidelik dat die 2019 seisoen se uitvoere 10 miljoen kartonne laer was as die oorspronklike skatting, waarna hulle meer begrip getoon het. Sappi het ingestem dat hulle gedurende 2020 sal voortgaan om binne die akkreditasie-stelsel die kartonne op hulle kostes te toets. Versoeke van produsente/pakhuise om kartonne te toets het baie toegeneem. Gelukkig kon die laboratorium alles hanteer en die verslae is aan die produsente/pakhuise gestuur.

“OPP Residues” is op organiese vrugte in Europa gevind. Dit het aanleiding gegee tot ŉ ondersoek om te bepaal wat die oorsprong van die kontaminasie is. Hiervoor is ŉ OPP taakspan onder leiding van PPECB gestig. Waarborge is van die papiervervaardigers en die geakkrediteerde kartonvervaardigers ontvang wat bevestig het dat daar geen SOPP in die papier of die kartonne is nie en die bewyse is aan PPECB en ander rolspelers deurgegee. SOPP vir die beheer van swamgroei word wel op dennehout palette gebruik, maar daar is nog geen bewyse dat “SOPP residues” wat op vrugte gevind is, afkomstig van die palette is nie. Intussen word daar intensiewe navorsing gedoen om suksesvolle plaasvervangers vir SOPP te vind. In die paletspesifikasie word gestipuleer dat dennehout-palette met ŉ 6% SOPP oplossing behandel moet word om swamgroei te verhoed. Die spesifikasie sal gewysig moet word en daar word tans intensiewe navorsing gedoen om ŉ suksesvolle en aanvaarbare alternatief te kry.

Die Pakmateriaal Spesifikasies en Palettiserings Protokolle dokument vir 2020 is opgedateer en versprei. In samewerking met die Sappi Technology Centre personeel is die 2020 skedule vir die toets van die geakkrediteerde kartonvervaardigers se kartonne opgestel en versprei. Daar is ook voorsiening gemaak vir meer monsters wat deur pakhuise gestuur, of deur CRI personeel getrek, gaan word. Laboratorium toetse sal gedurende weke 11 tot 33 gedoen word. Tydens ŉ spesiale vergadering by die Sappi Technology Centre is egter besluit om eers alle toetse gedurende die “lockdown” periode te staak.

8.1.4 **Produksiestreke**

Suidelike produksiestreke:
In die suidelike produksiestreke het die 2019 seisoen laat begin, maar vroeg tot ŉ einde gekom. Uitvoervolumes in die Sondagsrivier was beduidend hoër as die 2018 seisoen, hoofsaaklik a.g.v die groot suurlemoen- en sagtesitrus-aanplantings, en sommige produsente is besig met verdere uitbreidings. Volumes in die Wes-Kaap was soortgelyk aan 2018 en ook hier is produsente besig met uitbreidings. Veral die Oos-Kaap is vanjaar erg deur die probleme in die hawens geraak en hopelik sal hierdie probleme nie in 2020 weer kop uitsteek nie. In die suidelike produksiestreke was die uitpakpersentasies laer a.g.v wind- en blaaspootjie-skade, en selfs skade a.g.v bladspringers. Die interne gehalte was oor die algemeen goed, maar letsels a.g.v wind en plae het ‘n impak op die uitpakke.

Goeie reën het laat in die jaar in groot dele van die suide uitgesak en beskikbaarheid van water behoort nie ŉ probleem in die nabye toekoms te wees nie. Hoewel 2019 in vele opsigte ŉ jaar met baie uitdagings was, was dit beslis nie ŉ ramp nie. Verskeie produsente is besig met aansienlike uitbreidings en heelwat pakhuise is besig om opgegradeer te word.

ʼn Werkswinkel is met verskeie kundiges en produsente gedurende Oktober in die Sondagsrivier gehou om die oorsaak van boomvrektes in die Oos-Kaap te probeer bepaal. Alles dui daarop dat die boomvrektes deur ʼn aantal komplekse faktore veroorsaak word, wat verder nagevors moet word.

In die suidelike produksiestreke lyk die oes vir 2020 baie belowend. Die uitwendige gehalte, vruggrootte en oeslading is oorwegend baie goed. Die beheer van valskodlingmot en vrugtevlieg, asook ander fitosanitêre plae en siektes word nougeset toegepas. Daar is egter wel geïsoleerde gevalle in die Gamtoosrivier-vallei waar fitosanitêre probleme in verwaarloosde boorde nie voldoende aangespreek word nie en ander produsente in die streek op risiko plaas. Hierdie boorde is in elk geval nie ekonomies winsgewende eenhede nie, en ingryping deur die betrokke owerhede is nodig om die Sitrusbedryf se marktoegang-program volhoubaar te beskerm. Die aangeleentheid sal verder aandag geniet. Daar is ʼn beduidende toename in die volumes van veral suurlemoene en sagtesitrus, veral die laat manderyne. Produsente is vol vertroue dat die logistieke probleme wat verlede seisoen ervaar is, vanjaar beter sal verloop.

Sentrale produksiestreke:
Die pakseisoen in die sentrale produksiestreek het in 2019 baie stadiger begin as wat beplan was weens laat reëns gedurende herfs, maar ook weens swak kleur. Oorbemesting van boorde blyk ook ʼn groot rol te speel in die laat opkleur van vrugte, in sekere gevalle was die stikstof-inhoud so hoog dat geen stikstof toediening vir die komende seisoen aanbeveel is nie. Die sentrale produksiestreek se finale oesvolumes was net soos meeste ander streke ook noemenswaardig minder as vorige jare. Daar was wel ʼn paar produsente wat bogemiddelde produksie op mandarin kultivars behaal het. Vrugkleur was die grootste enkele probleem tydens oes en het daartoe bygedra dat oorryp vrugte in die markte gelei het tot swak kwaliteit en hoë bederf.

Die vrugtevlieg vangste was baie hoër in die sentrale streke as in die verlede maar beheermaatreëls was opgeskerp. FCM druk was nie so hoog as wat verwag is nie, maar produsente volg nougeset die ‘systems approach’. Die kwaliteit van vrugte oor die algemeen was baie goed. Heelwat windskade het in van die areas voorgekom met bladspringer skade wat in die Groblersdal/Marble Hall area meer beduidend voorgekom het. Alternaria is in boorde met Madaryne aangemeld. Alhoewel die voorkoms laag was is dit ʼn bron van kommer indien klimaatstoestande meer gunstig gaan wees vir siekte-ontwikkeling of indien beheermaatreëls nie nougeset gevolg word nie. Boorde wat erg deur hael getref is gedurende die seisoen het groot uitdagings met suurvrot besmetting in die pakhuis ervaar. Daar was heelwat “vreemde” simptome op bome en vrugte die seisoen opgemerk wat soms onverklaarbaar was. Produsente gebruik middels wat aanbeveel word om boomgroei, vrugset, kleur ens. te verbeter sonder om die moontlike gevolge wat hierdie toedienings op kwaliteit en produksie gaan hê te verstaan.

Hierdie produksiestreek het voldoende watervoorraad om ʼn normale oes vir 2020 te set. Die blom was wel uitgerek en het tot vier sette in sekere gevalle voorgekom. Die aanvanklike aanduiding is dat ʼn goeie blom voorgekom het op al die kultivars. Die belangriheid om die FMS en FFMS nougeset na te volg, is by verskeie studiegroepe benadruk. Scout opleiding is laat in 2019 by beide Kwa-Zulu Natal studiegroepe aangebied.

Die Sentrale produksiestreek het tot einde van die eerste kwartaal van 2020 heelwat minder haelbuie vir die seisoen gehad in vergelyking met ander seisoene. In van die areas was daar wel skade vroeg in die seisoen, asook aan die einde van die somer. Die haelskade gaan nie ʼn noemenswaardige rol speel in die totale volumes wat verwag word nie. Die meeste areas het vroeër begin met hul vrugtevlieg-beheer, en boordsanitasie word ook baie meer noukeurig as deel van die FMS en FFMS bestuursstelsel toegepas. Laat blaaspootjie het in meeste van die areas voorgekom, asook witluisgetalle wat in sekere boorde begin toeneem het. Alhoewel FCM getalle steeds laag is, is daar tekens dat getalle begin toeneem en daarom word produsente aangemoedig om noukeurig te moniteer. Heelwat bladspringer-skade het op Valley Gold in die Marble Hall, asook in die Nelspruit area op sagtesitrus voorgekom. Suigmot-skade is op vroeë Satsumas in die Burgersfort area opgemerk, maar die skade was reeds gedoen en geen beheer was nodig nie. Scout-opleiding is vir die Onderberg en Nelspruit produsente aangebied. Produsente hoop om vroeg in die mark te wees, veral met suurlemoene waarvoor baie goeie pryse verwag word.

Noordelike produksiestreke:

In the Northern production region packing of citrus got off to a slow start in 2019, with some packhouses having to stop-start periodically while they waited for fruit to arrive. Fruit fly numbers in traps were a concern for many growers across the entire region. This indicated a very high infestation which forced growers to increase efforts to achieve control to below threshold values before harvest. A number of packhouses requested and received training for staff on FCM monitoring and identification as this was advised by DAFF to make sure staff were adequately trained to perform this vital function in the FMS. In general, the citrus packouts have been good in some areas and poor in other areas, however it seems the major challenge was to optimise returns for citrus fruit internationally.

In die noorde het verpakking van sitrus heelwat vinniger tot ŉ einde gekom met die kleiner oesvolume. Die voordeel was dat snoei en boordsanitasie op datum gedoen was en die bome kon goed rus voor die volgende seisoen. Die blom vir 2020 het sterk deurgekom en omtrent twee weke vroeër as verwag. Die insidense van Phytophthora, veral met jongbome moet beter aangespreek word. Selfs groot bome wat onder stremming is, is vatbaar vir sekondêre infeksies en die insidensie van bome wat sterf het effens toegeneem in sekere gebiede. Dit is baie duidelik dat strenger beheermaatreëls in kwekerye toegepas moet word om die risiko van Phytophthora so laag as moontlik te hou.

In die Noorde was die drie grootste probleme wat in 2019 aangespreek moes word die beskikbaarheid en kwaliteit van water, vrugkleur en plaagbeheer. Produsente het hul bes gedoen om die effek van die droogte in verskeie gebiede te versag. In Letsitele het produsente baie boorde uitgehaal, wat die aantal kartonne vir die gebied in 2020 sal afbring. Oor die algemeen was dit ŉ uitdaging met bome wat ŉ onegalige en laat blom gehad het. Groeistuwing op die verkeerde tyd kan waarskynlik impak hê op vrugkleur nader aan oes en dit was dus meer belangrik om bemesting reg toe te dien. CRI grondkundiges het met verskeie produsente in die Noorde tyd deurgebring oor hul besproeiing en bemestingpraktyke. In baie gevalle was daar onsekerheid oor effektiewe water skedulering, asook kunsmis toediening, omdat dit so moeilik was om te bepaal hoe groot die oes gaan wees.

ŉ FMS werkswinkel was in Musina en in Letsitele gehou op 13 en 14 November, waartydens opsies vir doeltreffende, area spesifieke strategieë aangespreek is. Die belangrikheid om FCM heel in die begin van die seisoen aan te spreek, reeds in Julie, saam met boord sanitasie, was duidelik uitgelig, asook die belangrikheid om opgeleide mense vir FCM monitering te gebruik. Daar is ŉ projek op die been gebring om te kyk na doeltreffende witluisbestuur. Die doel van die projek is om beter riglyne te gee vir biologiese bestuur van witluis, wat ŉ baie belangrike deel is van die witluis beheerprogram.

In die Noorde lyk dit of vrugkleur in 2020 vir die eerste keer in jare normaal vir die streek sal wees. Inteendeel, vrugte gaan moontlik twee weke vroeër uitgevoer kan word in sekere gebiede. Die impak van die droogte in Letsitele gaan gesien word op die laat Valencia-uitvoere, maar volgens vroeë aanduiding lyk dit of die vroeër- en middelseisoen variëteite se volumes neig om naastenby soortgelyk te wees aan 2019 se uitvoervolumes. Terugvoer van produsente is dat minder kwaliteitprobleme opgemerk word in boorde, soos kraakskil en vrugsplit. Plaagdruk word ook as laag beskou.

HLB bly hoog op die prioriteitslys en produsente in Letsitele is tydens die Maart studiegroepvergadering by die Junction aan die belangrikheid van monitering vir ACP herinner. ACP identifikasie-kaarte is uitgedeel vir nuwe scouts tydens die scout-opleiding in die Onderberg en in Nelspruit. In Noord-Zimbabwe word monitering vir ACP nog steeds noukeurig gedoen deur CGA lede.

8.1.5 **Postharvest extension**

Warm temperatures across the country resulted in a slow start to the season with many packhouses unable to start packing at their usual time, or forced to pack sporadically since the fruit had not coloured up sufficiently.

The usual green mould and latent pathogens have been a problem, but sour rot is by far the primary disease concern with many packhouses struggling to control it. With no suitable fungicide available, for especially the European markets, there is a strong request from the packhouses to develop and test a suitable product. The postharvest research programme is testing various registered actives for potential use against sour rot, as well as keeping up to date with any possible new actives. In addition to sour rot, there has also been a rise in reports of scale and associated sooty mould as well as unusually high levels of storage mould (saprophytic fungus on calyx end dead tissue) this season.

In terms of general disease management there is a strong drive towards chem-free options and organic actives. This has resulted in a plethora of alternatives being presented to packhouses that are not always safe or registered. Some of the products being marketed have undisclosed ingredients, but packhouses are eager to use them regardless. The focus has been to caution packhouses to wait until the products can be vetted and properly registered.
Many packhouses and exporters have concerns over the fungicides available and their restrictions, with particular concern over an adequate treatment for sour rot. Chemical companies are working on alternatives, but for the 2020 season there is no solution as of yet. Fruit being sent to the EU market is of greatest concern.

Towards the end of the 2019 season, green mould (*Penicillium digitatum*) has been the biggest cause of decay in the markets, particularly on lemons. In cases where packhouses have sent in swabs of the decay, loss of sensitivity to the fungicides has been detected. With proper sanitation during the off season, resistant populations should not survive so the problem should resolve for 2020, however there are several alternative fungicides available for green mould control if need be. Other issues of concern were sooty mould and storage mould on the fruit, as well as many complaints of high blemish standards by the markets.

Two new packhouse forums were started last year, namely the Citrusdal and Clanwilliam packhouse forum and the Nelspruit packhouse forum. All the packhouses present at the meetings were open to sharing their concerns and discussing solutions. Both forums plan to meet again in early 2020, with bi-monthly meetings until November. Sunday's River Valley Packhouse Forum is still going strong and meets regularly with high participation. Patensie (Gamtoos Valley) Packhouse Forum is smaller and meets less regularly but is also still working well.

The 2019 season was a challenging one with high levels of postharvest decay. Part of the cause of the decay has been attributed to delays, first in the beginning of the season with late and poor colouring fruit and then port delays. Despite every effort being made, it is unlikely that the port situation will be resolved in 2020, so focus is being centered on packing and exporting high quality fruit of optimum maturity. This message was being conveyed to the industry and was further stressed at the postharvest workshops in January and February 2020. The majority of decay was green mould, but there is a suspicion that much of the initial decay was sour rot that had been overgrown with the more aggressive green mould. This is impossible to ascertain positively since the majority of decay was seen in the form of claims overseas. Likewise, the extent of resistant green mould (*Penicillium digitatum*) populations is not certain, although there has been a rise in loss of sensitivity in samples tested by CRI's diagnostic center. Also, of concern is the level of pyrimethanil resistance in orchard samples. In terms of resistant green mould control, various alternative fungicides have been registered and are available should the industry need them. However, there is no viable alternative for sour rot control and many producers are concerned.

Various alternative products to increase shelf life are being advertised to the industry, mostly in the form of fruit coatings. CRI researchers are monitoring any potential products but as of yet, none are an alternative to the current fruit waxes for conventional packing, but may be a possibility for organic or chem-free packing. There is a definite need in the industry for more chem-free treatment options. Although imazalil is still available for the foreseeable future, the use of many postharvest actives remain unstable. The lowering of the 2,4-D MRL to 0,1 for China is forcing some packhouses to withdraw the active from their packhouse postharvest programmes entirely. This may result in fruit losing their calyx buttons, which in itself is a quality issue.

During the 2019 season, the threat of the loss of imazalil was very keenly felt. With the active azoxystrobin being registered for postharvest green mould control, which has shown good promise in trials, some packhouses have added it to their 2020 chemical programme. A huge draw to using the active is the high MRL for EU which allows the packhouses to achieve a third of the MRL, as required by some supermarkets, quite easily. At this point, CRI, as well as the chemical company supplying azoxystrobin, recommend staying with imazalil as the active of choice until more trial work is completed on azoxystrobin. Regardless, feedback from the packhouses using the new active will be instrumental in understanding more about its use in a commercial setting.

The majority of packhouses completed packing by September and were done much sooner than in 2018. The few packhouses still operating through October and November were doing so with minimal staff and packing for the local market only. With the high decay seen, packhouses have been critically examining their processes and looking to increase sanitation in the packhouse. With multiple FCM and fruit fly interceptions in the EU, orchard sanitation will have to be more diligent in the coming season and this will aid greatly in reducing postharvest decay.
The 2020 season was off to a very promising start with several packhouses packing early with lemons. Unfortunately, the coronavirus COVID-19 is severely taking a toll on management and costs. As an essential service, citrus packhouses remain open and committed to worker safety. Shortages on sanitisers and other health products such as masks and digital thermometers, as well as a general fear amongst the labourers are the biggest challenges. Packhouse managers are doing everything in their power to keep their staff healthy and informed.

8.1.6 **Research priorities**

Meetings were held during April and May 2019 in all the production regions with the respective technical committees of each of the study groups to discuss their research needs for the next cycle. Summaries of 2018 Annual Reports, as well as a list of all the current funded projects, were sent out to all growers a few weeks prior to these meetings for preparation and input by the growers and technical people in the areas. As a result, grower participation was significantly better than in the past. The research requests that were raised during the CRI Postharvest workshops by members of the Exporters Technical Panel and packhouse managers was also listed, as well as the few requests that came from the CGAGDC.

Probably the most burning issue in terms of citrus production and exports is the issue of residues. The requirements from the markets becomes increasingly stricter, demanding lower MRLs and fewer actives on the fruit. This is applicable to the use of pre- and postharvest chemicals. There is a definite swing towards a more eco-friendly approach for production of fresh produce. This, by implication, means that CRI needs to focus on a much softer approach with regards to IPM and disease management strategies.

The importance of keeping a dedicated research focus on market access issues and biosecurity was stressed by each and every study group. This includes mostly phytosanitary and sanitary issues like FCM, fruit fly, CBS, cold sterilization, exotic pests and diseases, and very importantly, the use of fewer actives and lowers residues.

Project proposals were submitted after portfolio strategic sessions took place beginning of June, followed by the process of vetting of the proposals by the various research committees. The feedback of research projects submitted to address the research needs, listed by the TTGs, were sent to each respective TTG during the first quarter of 2020.

8.1.7 **CRI Na-oes werkswinkels vir 2020**

Die beplanningsvergadering vir die 2020 CRI na-oes werkswinkels is gedurende November in Nelspruit gehou. Die beplanningskomitee vir die werkswinkels is saamgestel uit CRI Voorligting & Navorsing, pakhuisverteenwoordigers uit die grootste produksiestreke, verteenwoordigers van CGA, PPECB, ETP en ander kundiges uit die bedryf.

The six annual CRI Postharvest workshops were held in the main production regions during January and February and were once again a huge success with delegate numbers reaching over 1000. Good feedback was received from the delegates on a wide range of topics that were covered, with very good information provided from various industry role players such as PPECB, Fruit SA and grower and packhouse representatives from each of the regions. CRI staff across multiple departments gave excellent and informative presentations based on their fields of expertise.

8.1.8 **Biosekuriteit**

Die bedreiging wat HLB vir die bedryf inhou is ŉ groot bron van kommer vir die produsente. Tydens die navorsingsbehoefte-bepalings is dringende versoeke vanaf die studiegroepe gerig dat CRI alles moontlik moet doen om te verseker dat ACP en HLB nie in die land inkom nie. Een van die grootste bekommernisse is die feit dat daar ŉ groot moontlikheid is dat China sitrus in die buurlande wil vestig, en selfs gerugte dat daar reeds plantmateriaal van China na een van die buurlande ingebring is. Hierdie gerugte word verder ondersoek. Bactrocera dorsalis het weer in sekere areas kop uitgesteek en pogings om dit uit te wis gaan voort.

CRI voorligting het op 24 Julie 2019 in Letsitele en op 30 Julie 2019 in Malelane die HLB aksieplan aan die sitrusprodusente bekend gemaak. Inligting oor HLB siekte, die vektor ACP asook die status van die siekte en vektor in Afrika was aangebied. Die boodskap was duidelik gemaak oor die belangrikheid van monitering en samewerking. Die tweede dag van die werkswinkel het CRI die ACP monitering opleiding aangebied vir die sitrus scouts.

Tydens die Geïntegreerde Plaagbeheer & Siektebestuur werkswinkels wat gedurende Augustus en September aangebied is, is ŉ hele sessie aan biosekuriteit bestee waartydens die HLB aksieplan in diepte bespreek is, asook veranderinge aan die FMS & FFMS vir 2020. Direk na afloop van elke werkswinkel is pakhuis-opleiding oor die FMS & FFMS aan al die pakhuise aangebied.

8.1.9 **Geïntegreerde Plaag en Siektebestuur werkswinkels**

Die Geïntegreerde Plaag en Siektebestuur werkswinkels is in die verskillende produksiegebiede aangebied en die totale bywoning was 884 persone. Die werkswinkels is weer by die Eiland, Loskopdam, Nelspruit, Addo en Paarl aangebied. Tydens die eerste sessie is groot klem op biosekuriteit en marktoegang geplaas, veral op die “HLB Action Plan”. Sessies twee en drie het geïntegreerde plaagbestuur aangespreek en sessie vier siektebestuur. Die terugvoer was deurlopend besonder goed.

8.1.10 **11de Sitrusnavorsingsimposium**

Die reëlings vir volgende jaar se Sitrusnavorsingsimposium, wat van 23 tot 26 Augustus by Champagne Sports Resort aangebied word, vorder goed. Die groep hoofborge is reeds vasgemaak, asook die diensverskaffers. Gedurende Oktober is ŉ vergadering met die venue gehou om al die detail uit te klaar en die nodige reëlings in plek te kry.

Die uitbraak van die COVID-19 pandemie regoor die wêreld en die daaropvolgende verpligte inperkingsmaatreëls het egter die aanbieding van die CRI Sitrusnavorsingsimposium wat vir Augustus 2020 beplan word, in die weegskaal geplaas. Die onsekerhede rakende die uitwerking van die pandemie op die ekonomie, regeringsmaatreëls om die pandemie te bekamp, bywoning en verskeie ander faktore plaas druk op enige verdere beplanning en reëlings. Dit maak op hierdie stadium sin om die simposium uit te stel tot Augustus 2021.

Opsomming van aktiwiteite vir die periode April – Junie 2019

Datum	**Studiegroep/Aktiwiteit**	**Onderwerpe/Aksies**	**Betrokkenes/ Sprekers**
1 Apr – 30 June 19	Admin	Updating postharvest production guidelines	Catherine Savage
1 Apr 19	Innovation Hub Pta.	Verpakking-navorsings prioriteite	Stuart Esterhuyzen Dawid Groenewald
1 – 5 Apr 19	LAC bootvaart	LAC bootvaart	MC Pretorius
2 Apr 19	Johannesburg: Sappi Plusnet	Borgskappe vir simposium	Hannes Bester Liezl vd Linde Dawid Groenewald

2 Apr 19	Karino Citrus Co-op	Packhouse visit	Catherine Savage Keith Lesar
3 Apr 19	Johannesburg: BASF	Borgskappe vir simposium	Hannes Bester Liezl vd Linde
3 Apr 19	Letsitele Groep91	Discussion: FCM control strategy in winter months	Wayne Mommsen Sean Moore Richard Schulze Henk Van Rooyen
4 Apr 19	Innovation Hub Pta.	Nano Coating proef beplanning	Lindi Strydom Dawid Groenewald
4 Apr 19	Johannesburg: Villa	Bemestingskomitee vergadering	Hannes Bester Pieter Raath
8-9 Apr 19	Nelspruit	Divisional Managers meeting	Hannes Bester Vaughan Hattingh Tim Grout Paul Fourie Solomon Gebeyehu Jon Pinker
9 Apr 19	Midknight Marble Hall Study group	Presentations	MC Pretorius Catherine Savage
10 Apr 19	Rosle	Orchard and Packhouse visit	MC Pretorius Catherine Savage
15 Apr 19	Letsitele DNB Packers Bosveld Sitrus	PalletPlast plastiese palet en OptiFlo Freezer Spacer proewe	Dawid Groenewald
16 Apr 19	Letsitele Houers Bosveld Sitrus	Beplanning van proewe vir 2019 en evaluering van "mulching" proef	Wimpie Mostert Frikkie van Wyk Dawid Groenewald
16 - 17 Apr 19	Brits farm & packhouse visits	Slade, SVS, Pauly, and vd Merwe Packhouse technical visit and interaction with developing growers	Wayne Mommsen Catherine Savage Andrew Mbedzi
16 – 17 Apr	Groblersdal	Produsentbesoeke – Probleem boordbesoeke: Dewagendrift, Hertzog broers, W Hartman	MC Pretorius
24 Apr 19	Sappi Technology Centre	Vergadering met Sappi Senior personeel. Akkreditasie toetse en ontwikkeling van nuwe ligter papier	Senior Sappi personeel Dawid Groenewald
24 – 25 Apr	Karino en Croc Valley	Pakhuisbesoek, bespreking en boordbesoeke	MC Pretorius James Warrington
26 Apr 19	Tomahawk	Packhouse visit	Catherine Savage Keith Lesar
29 Apr 19	Fort Beaufort	Katrivier navorsings-prioriteits vergadering	Hannes Bester
29 Apr 19	Karino/ Croc Valley	Bespreking en boordbesoeke	MC Pretorius Pieter Raath James Warrington
29 Apr – 03 May 19	Waterberg and Groblersdal Marblehall areas wax instruction	Bloomport, AJ de Villiers, Culmpine, Nordi, Potgietersrus packhouse, Zebediela, Schoenbee,	Catherine Savage

	Courtesy and packhouse visits	MHC, Katlego citrus, Frans Smit Trust, WJ Kruger, Salamber, Vaalbos Trust, Willinda, ZBR, Rosle, Maritz, Croc Valley - Wa	
30 Apr 19	Addo	Sondagsrivier navorsingsprioriteits vergadering	Hannes Bester
30 Apr 19	Onderberg - GFC	Produsent besoeke	MC Pretorius
30 Apr 19	Waterberge	Farm visits	Wayne Mommsen
2 Mei 19	Patensie	Patensie navorsings-prioriteits vergadering	Hannes Bester
2 Mei 19	Sappi Technology Centre	Toets van kartonne wat met Nano Coating behandel is	Dawid Groenewald
6 Mei 19	Swellendam	Swellendam navorsings-prioriteits vergadering	Hannes Bester
6 – 10 May 19	Nelspruit area	Lourenza, Ryton, Twycross, Croc Valley, Joubert and Sons, Karino packhouse visits	Catherine Savage Keith Lesar
7 Mei 19	Ashton	Breederivier navorsings-prioriteits vergadering	Hannes Bester
	Paarl	Paarl/Stellenbosch/Swartland navorsingsprioriteits vergadering	Hannes Bester Pieter Raath
8 Mei 19	Citrusdal	Citrusdal navorsings-prioriteits vergadering	Hannes Bester Pieter Raath
9 Mei 19	Kakamas	Benede-Oranjerivier navorsingsprioriteits vergadering	Hannes Bester
9 Mei 19	Nelspruit	Navorsingsprioriteits bespreking Disease management	MC Pretorius J v Niekerk
10 Mei 19	Harswater	Vaalharts navorsings-prioriteits vergadering	Hannes Bester
11 – 15 Mei 19	Kloppenheim - Machadodorp	Nematologie Vereeniging simposium	MC Pretorius Elaine Basson
12 – 16 May 19	Paarl, Citrusdal, and Clanwilliam	F1, Imibala, Stellenpak packhouse visits Time with Johan de Beer. Noordhoek, Sitrus Select 1 & 2, ALG, Goodehoop, Mouton, Silwer Pak, Patrysberg Verpakking, Bergendal packhouse visits. Time with John Sinclair	Catherine Savage Keith Lesar
13 Mei 19	Brits	Brits navorsingsprioriteits vergadering	Hannes Bester Wayne Mommsen
	Pretoria	Australia ambassade	Hannes Bester
	Naboomspruit	Waterberg navorsings-prioriteits vergadering	Hannes Bester Wayne Mommsen
13 Mei 19	Innovation Hub Pta	Vergadering oor verskeie verpakkings aangeleenthede	Hannes Bester Wayne Mommsen Dawid Groenewald

14 Mei 19	Schoeman Boerdery en Schoonbee Landgoed	OptiFlo Freezer Spacer Proef en NNZ Pallet Netting vergaderings	Dawid Groenewald Christo de Jonge Danie Pieters
14 Mei 19	Messina	Tshipise/Weipe/Suid-Zimbabwe navorsings-prioriteits vergadering	Hannes Bester Wayne Mommsen Pieter Raath
15 Mei 19	Letsitele	Letsitele navorsings-prioriteits vergadering	Hannes Bester Wayne Mommsen Pieter Raath
	Hoedspruit	Hoedspruit navorsings-prioriteits vergadering	Hannes Bester Wayne Mommsen Pieter Raath
16 Mei 19	Ohrigstad	Ohrigstad navorsings-prioriteits vergadering	Hannes Bester MC Pretorius
	Burgersfort	Burgersfort navorsings-prioriteits vergadering	Hannes Bester MC Pretorius
17 Mei 19	Groblersdal	Groblersdal navorsings-prioriteits vergadering	Hannes Bester MC Pretorius
17 Mei 19	Letsitele Mahela and Komati	Collect Spray record data for Thrips and Mealybug	Wayne Mommsen Eddie Vorster Dezi Fourie
20 Mei 19	Malelane	Onderberg/Swaziland/ Pongola navorsings-prioriteits vergadering	Hannes Bester MC Pretorius Catherine Savage
	Nelspruit	Nelspruit navorsingsprioriteits vergadering	Hannes Bester MC Pretorius Catherine Savage
	Millers: Johann vd Vyver & Charl Kotze	IPM & DM workshops borgskap	Hannes Bester MC Pretorius
21 Mei 19	Nkwalini	Nkwalini navorsingsprioriteits vergadering	Hannes Bester MC Pretorius Liezl vd Linde
	Umhlanga: Afribrand	Simposium reëlings	Hannes Bester MC Pretorius Liezl vd Linde
22 Mei 19	Umhlanga: Arysta	Simposium borgskap	Hannes Bester MC Pretorius Liezl vd Linde
	Richmond: Mike Woodburn	Boordbesoeke	Hannes Bester
	Ixopo	Southern-KZN navorsingsprioriteits vergadering	Hannes Bester
23 Mei 19	Brits	Sitrus Studiegroep: RiverBioScience main Sponsor	Wayne Mommsen Danie JV Vuuren Mariana Le Roux
28 – 29 Mei 19	Ohrigstad	Produsentbesoeke en bemestings gesprekke	MC Pretorius Pieter Raath
29 Mei 19	Lesitele: Letaba Estates	HLB Discussion leading up to Greening Workshop	Wayne Mommsen Harry Grove Andries Van Wyngaardt
3-5 Jun 19	Nelspruit	Hortologie, Biosecurity, DM & IPM Portfolio's Strategiese sessies	Hannes Bester MC Pretorius Wayne Mommsen

			Catherine Savage
6 Jun 19	Nelspruit	Divisional Managers meeting	Hannes Bester Vaughan Hattingh Tim Grout Paul Fourie Solomon Gebeyehu Jon Pinker
7 June 19	Tshipise & Weipe Packhouse staff	Present FCM monitoring and identification training	Wayne Mommsen
12 Jun 19	Johannesburg: Villa	Simposium borgskap	Hannes Bester Liezl vd Linde
13 Jun 19	Karino	Riverside, Hectorspruit Packhouse, Hectorspruit Chem Free, Vergenoeg, Whiskey packhouse visits	Catherine Savage
8 – 17 Jun 19	Argentinië	Agrentynse sitrussimposium aanbieding oor grondgedraagde siektes en boordbesoeke	MC Pretorius Paul Fourie Paul Cronje
19 Jun 19	Sappi Technology Centre	Toets van Swellenfruit se kartonne	Dawid Groenewald
25 Jun 19	Insectec Tzaneen	Present Scout Training	Wayne Mommsen Hannah Otto Wiliana Scheepers Angelique Potgieter
26 – 27 Junie 19	Groblersdal/ Marble Hall	Produsent besoeke – probleem oplossing en besprekings: Rosle, Schoonbee, Piet Sitrus	MC Pretorius
27 Jun 19	Sappi Technology Centre	Ontleding van Villa se kartonne vir 2x10l MAX-In Zn	Dawid Groenewald
27 Jun 19	Excelsior Packhouse staff	Present FCM monitoring and identification training	Wayne Mommsen
28 Jun 19	OR Tambo Lughawe	Vergadering met Hannes Bester.	Hannes Bester Dawid Groenewald

Opsomming van aktiwiteite vir die periode Julie – September 2019

Datum	Studiegroep/Aktiwiteit	Onderwerpe/Aksies	Betrokkenes/ Sprekers
28 Jun – 13 Jul 19	Australia	Besoek produksie-eenhede saam met Nutrano	Hannes Bester
16 Jul 19	Johannesburg: CRI-BOD meeting	Agenda	Hannes Bester
	Johannesburg: SYNC'D	Simposium reëlings	Hannes Bester Liezl vd Linde
17 Jul 19	Johannesburg: FNB Bayer	Simposium Borgskappe	Hannes Bester Liezl vd Linde
3 Jul 19	Syngenta National technical conference – Mopani KNP	Aanbieding en bespreking oor IPM en Disease uitdagings asook CRI se rol in die industrie	MC Pretorius

9 – 12 Jul 19	CoastInds Umhlanga Ridge Conference	Entomology Society Conference	Wayne Mommsen
9 Jul 19	Houers, DNB Packers & Bosveld Sitrus	Massa Krat lugvloei, Hegvel toetse.	Wimpie Mostert Frikkie van Wyk Jacques Nel Dawid Groenewald.
10 Jul 19	Houers	Meer koste-effektiewe kartonne	Wimpie Mostert Frikkie van Wyk Dawid Groenewald
	Nelspruit	Biosekuriteits risiko bespreking met Privaat konsultante en beleggers oor aanplantings in Eswatini asook moontlike nuwe insekbeheer produkte	MC Pretorius Kallie JV Vuuren Leon Doyer
11 Jul	Karino	Veld proef bespreking – Nematodes en Phytophthora - Syngenta	MC Pretorius Syngenta Span
12 Jul 19	Sappi Technology Centre	Probleme met kartonne vir akkreditasie toetse	Dawid Groenewald STC Laboratorium Personeel
16 – 17 Jul	CIS - Uitenhage	CIS: 1. Evaluasie/ Inspeksie 2. Fasiliteit oorhandigings funksie	MC Pretorius CIS personeel
18 Jul 19	Letaba Estates	Beplanning vir Biosekuriteit Werkswinkel	Wayne Mommsen Harry Grove
18 Jul 19	Schoeman Boerdery	Werk op massa kratte	Christo de Jonge Frikkie van Wyk Dawid Groenewald
18 Jul 19	MSK Group packhouses	Packhouse visits - Oorsprong, Uitsig, Malelane	Catherine Savage
22 Jul 19	Vergadering: Komati Sitrus Lestsitele	FMC proewe bespreking: Exirel & Coragen	Wayne Mommsen Desire Fourie Pierre Smit Andries Fourie Jacques Bredenkamp
23 – 26 Jul 19	E. Cape Packhouses	Packhouse visits - Panzi, SunCape, Good Hope, Sunny Bank, SRCC meeting and 10 packlines (Hermitage x3; Summerville x1; Kirkwood x4; Ingqweba x2), PSB meeting and 3 packlines (Blou & Wit Dak packhouses)	Catherine Savage
24 Jul 19	Letaba Estates	Biosekuriteit Werkswinkel	Aruna Manrakhan MC Pretorius Glynnis Cook Wayne Kirkman Wayne Mommsen
25 Jul 19	Patensie	Patensie region packhouse forum meeting	Catherine Savage
25 Jul 19	Letaba Estates	ACP monitering opleiding	Aruna Manrakhan MC Pretorius

			Wayne Mommsen
30 Jul 19	Sappi Technology Centre	Toets van kartonne wat deur pakhuise gestuur is	Dawid Groenewald STC Laboratorium Personeel
30 Jul 19	Malelane	Biosekuriteit Werkswinkel	Aruna Manrakhan MC Pretorius Glynnis Cook Solomon Gebeyehu Wayne Mommsen Catherine Savage
30 Jul 19	Malelane	ACP monitering opleiding	Aruna Manrakhan MC Pretorius Wayne Mommsen
31 Jul – 2 Aug 19	Nelspruit	Voorligtingsbeplanning	Hannes Bester Wayne Mommsen MC Pretorius Liezl vd Linde Catherine Savage
6 Aug 19	Letsitele Constantia	Studiegroep: Terugvoer oor HLB werkswinkel	Wayne Mommsen
8 Aug 19	Houers Rabek Palette, Tzaneen	Ontwerp van Freezer Spacers SOPP op palette	Wimpie Mostert Frikkie van Wyk Dawid Groenewald Allen Campbell Frikkie van Wyk Dawid Groenewald
8 Aug 19	Brits	CBS Navorsing Beplanning	Bheki Mabunda Providence Moyo Wayne Mommsen
	Nelspruit – Croc Valley	1. Pakhuis besoek 2. CRI – Citrogold Israel besoekers	MC Pretorius Patrick Adlam MC Pretorius Tim Grout Bryan Offer
12 Aug 19	Exact Pakhuis, Letsitele Bosveld Sitrus	Probleme met kartonne by Exact en evalueer “Mulching” proef by Bosveld	Dawid Groenewald
12 Aug 19	Kakamas: produsente besoeke	Neil Rossouw Jacques De Wet Nina Steenkamp Kobus van Zyl Rossouw Basson Hannes Buys	Hannes Bester
13 Aug 19	Kakamas: produsente besoeke	Karsten Bdy Junior Aggenbach Johan van Aarde	Hannes Bester
13 Aug 19	Sappi Technology Centre	Toets van Exact se kartonne	Dawid Groenewald STC Laboratorium Personeel
14 Aug 19	Kakamas: produsent besoeke	Groenheuwel Zwartbooisberg	Hannes Bester
16 Aug 19	Letsitele	DNB Packers FMS Bespreeking	Wayne Mommsen Jacques Nel

20 -23 Aug 19	Zambia	FMC Plaagbeheer Werkswinkel	Wayne Mommsen Sean Moore MC Pretorius Wayne Kirkman
20 Aug 19	Sappi Technology Centre	Jaarlikse Na-seisoen vergaderiing	Sappi Senior Personeel Dawid Groenewald
20 Aug 19	Patensie: Studiegroep	Agenda	Hannes Bester
20 - 21 Aug 19	Zimbabwe	Packhouse visits - Nottingham estates	Catherine Savage Wilma du Plooy Marco van Straaten
22 Aug 19	Innovation Hub	Akkreditasie toets probleme	André Cuturi Bentley Daniel Dawid Groenewald
26 – 29 Aug 19	E Cape packhouses	Packhouse visits - PSB follow up, Unifruitti 4 lines, SRCC (4 lines), Ponders End, SRCC (2 packhouses - 4 lines)	Catherine Savage
27-28 Aug 19	Letsitele: Limpopo 1 IPM & DM werkswinkel		Hannes Bester Wayne Mommsen MC Pretorius Liezl vd Linde Elma Carstens Sean Moore Aruna Manrakhan Solomon Gebeyehu Jan van Niekerk Providence Moyo Elaine Basson
29-30 Aug 19	Loskopdam: Limpopo 2 IPM & DM werkswinkel	Agenda	Hannes Bester MC Pretorius Liezl vd Linde Elma Carstens Sean Moore Aruna Manrakhan Solomon Gebeyehu Jan van Niekerk Providence Moyo Elaine Basson
29 Aug 19	E Cape - Sunday's River	Packhouse forum Meeting	Catherine Savage
30 Aug 19	Innovation Hub	"Safeload Plastic Packaging"	Jaco Geldenhuys Dawid Groenewald.
29-30 Aug	Limpopo Sitrus Dendron & Pont Drift	Plaasbesoeke	Wayne Mommsen Pietman Pieterse Zane Langman
3-4 Sept 19	Nelspruit: Limpopo 3 IPM & DM werkswinkel	Agenda	Hannes Bester MC Pretorius Liezl vd Linde Elma Carstens Sean Moore Aruna Manrakhan

			Solomon Gebeyehu Jan van Niekerk Providence Moyo Elaine Basson Wayne Mommsen Catherine Savage
10-11 Sept 19	Addo: Oos-Kaap IPM & DM werkswinkel	Agenda	Hannes Bester Wayne Kirkman MC Pretorius Liezl vd Linde Elma Carstens Sean Moore Aruna Manrakhan Solomon Gebeyehu Jan van Niekerk Providence Moyo Elaine Basson Karin Nel
11 Sept 19	Nelspruit: Larten	Scout opleiding	Wayne Mommsen Andrew Cooper
12-13 Sept 19	Paarl: Wes-Kaap IPM & DM werkswinkel	Agenda	Wayne Kirkman MC Pretorius Liezl vd Linde Elma Carstens Sean Moore Aruna Manrakhan Solomon Gebeyehu Jan van Niekerk Providence Moyo Elaine Basson Karin Nel
16 Sept 19	Stellenbosch	Lean van Biljon Pieter Raath Steve Turner	Hannes Bester
17 – 20 Sept	Stellenbosch	Aanbieding oor CIS plantmateriaal and Soilborne diseases by Annual Soilborne Disease Interest Group Meeting US – Prof Adele McCloed	MC Pretorius Adele McCloed Sandra Lamprecht
17 – 19 Sept 19	W Cape - Citrusdal	Packhouse forum meeting	Catherine Savage Wilma du Plooy
18 Sept 19	Stellenbosch: Cultivar Evaluation Committee meeting	Agenda	Hannes Bester Tim Grout Paul Cronje Johan Joubert Werner Swiegers
19 Sept 19	Johannesburg: IPM Research Committee meeting	Agenda	Hannes Bester Tim Grout Sean Moore Solomon Gebeyehu
19 Sept 19	Letsitele : Mahela Vergadering	Witluis bestuur en Navorsing geleentheid	Wayne Mommsen Eddie Vorster Carl Fourie

			Dewald Kamffer
20 Sept 19	Johannesburg: DM Research Committee meeting	Agenda	Hannes Bester Tim Grout Paul Fourie Jan van Niekerk Solomon Gebeyehu
23 Sept 19	Innovation Hub	OPP op Toedraai papier en finalisering van meer koste-effektiewe karton proef. 2019 Seisoen nabetragting.	Wimpie Mostert Dawid Groenewald
	Groblersdal	Dewagendrift Boerdery – Pakhuis besoek – hoë bederf probleme bespreek	MC Pretorius Wilma Du Plooy
26 Sept 19	Reemoon: Gerard Meyer & Pierneef Smit	Borgskappe vir Simposium en Na-oes werkswinkels	Hannes Bester
	Nelspruit	OroAgri bekendstellings besoek – nuwe personeel en produkte	MC Pretorius Tim Grout Johan J/V Rensburg

Opsomming van aktiwiteite vir die periode Oktober – Desember 2019

Datum	**Studiegroep/Aktiwiteit**	**Onderwerpe/Aksies**	**Betrokkenes/ Sprekers**
1 Okt 19	Brits/Rustenburg: Voorligting/Navorsing samewerking	CBS navorsing projek op die been te bring	Providence Moyo Bheki Mabunda Wayne Mommsen
2 Okt 19	Nelspruit	Suurlemoen boord terugsterwing en onderstam probleem	MC Pretorius Johan Joubert
2-4 Okt 19	Besoek CSR	Reëlings vir symposium in Aug 2010	Hannes Bester Liezl vd Linde Dawid Groenewald Catherine Savage Wayne Mommsen MC Pretorius
7 Okt 19	AgroFresh: Arri De Kock Wehan Groenewald Nathalie Gocha	Bespreek betrokkenheid by die sitrusbedryf	Hannes Bester
8 Okt 19	Letsitele Constantia Studiegroep	Area FCM terugvoer en besprekings	Wayne Mommsen
9-10 Okt 19	Sondagsriviervallei	Werkswinkel oor boomvrektes in Oos-Kaap	Hannes Bester MC Pretorius Jan v Niekerk Paul Fourie Paul Cronje Pieter Raath Johan Joubert
9 Okt 19	Hoedspruit Pakhuisforum	Agenda	Wayne Mommsen Catherine Savage
10 Okt 19	Letsitele Pakhuisforum	Agenda	Wayne Mommsen Catherine Savage
14 Okt 19	IPM workshop Patensie	Citrus IPM en data ontleeding	Sean Moore Wayne Mommsen

15 Okt 19	Scout Opleiding Patensie	Kursus aangebied	Wayne Mommsen
	Stellenbosch: Cooling Working Group meeting	Agenda	Hannes Bester Dawid Groenewald Catherine Savage Tarl Berry Paul Cronje
	Acorn: Michael Prinsloo	Oesskattings-tegnologie	Hannes Bester
	Jaco Theron	Toekomstige betrokkenheid	Hannes Bester
16 Okt 19	N.W Studiegroep Mooinooi	Kultivars; Phytophthora en aalwurm bestuur	MC Pretorius Johan Joubert Wayne Mommsen
	Stellenbosch: ETP meeting	Agenda	Hannes Bester Dawid Groenewald Catherine Savage Tarl Berry Paul Cronje
	Oro Agri: Johan Janse van Rensburg	Navorsing en borgskappe	Hannes Bester
17 Okt 19	MPact: Niel Hugo	Karton akkreditasie	Hannes Bester
18 Okt 19	Innovation Hub	Everest Packaging Akkreditasie	Dawid Groenewald Brian Segger Raymond Lund
22 Okt 19	Philagro: Jakkie Stander	Oesmanipulasie	Hannes Bester
	Nelspruit : Packhouse Forum meeting	Agenda	Catherine Savage MC Pretorius Wilma du Plooy
	Sappi Technology Centre	Toets van massa kratte	Dawid Groenewald
23 Okt 19	Stellenbosch: FMS Working Group meeting	FMS changes	Hannes Bester Vaughan Hattingh Paul Cronje Sean Moore Elma Carstens
	Burgersfort : Witluis bestuur projek	Naranja Nethuis en Oopland witluis bestuur	Wayne Mommsen Sean Moore Martin Hill
28-31 Okt 19	Natal Nkwaleni en Ixopo Studiegroep	Studiegroep vergaderings/IPM & DM werkswinkel terugvoer/Scout opleiding/Postharvest feedback/Packhouse visit	Wayne Mommsen Catherine Savage MC Pretorius Johan Joubert
4 Nov 19	Nelspruit: Voorligtingbeplannings-vergadering	CRI Symposium-beplanning	Liezl van der Linde Hannes Bester MC Pretorius Catherine Savage
	Aerobotics: James Ueckermann	Boordbestuur-tegnologie	Hannes Bester
	Postharvest Research Strategic meeting	Agenda	Hannes Bester Catherine Savage Wilma du Plooy Jan v Niekerk Paul Fourie Lindo Mamba

5 Nov 19	Nelspruit: CRI Na-oes werkswinkels beplanningsvergadering	Agenda	Hannes Bester MC Pretorius Dawid Groenewald Catherine Savage Wilma du Plooy Jan v Niekerk Wayne Mommsen Liezl vd Linde
6 Nov 19	Polokwane: FFE Boeredag	Agenda	Hannes Bester Johan Joubert Dawid Groenewald Wayne Mommsen
7 Nov 19	Schoeman Boerdery	100ste bestaansjaar van Schoeman Boerdery se funksie	Dawid Groenewald
	Tulima Packhouse	Post-season packhouse visit	Catherine Savage
12 Nov 19	Johannesburg: CRI Board meeting	Agenda	Hannes Bester Vaughan Hattingh Tim Grout Jon Pinker
	QC meeting – Patensie sitrus (Madelaine Ludwig)	2019 season and challenges	Catherine Savage
13 Nov 19	Citrus Coordinating Meeting	Agenda	Catherine Savage
	QC meeting – SRCC (Christo Theron)	2019 retention sample results	Catherine Savage
	Noorde : FMS Werkswinkel Musina	FMS/FCM bestuur	Sean Moore Wayne Mommsen
14 Nov 19	Noorde FMS Werkswinkel Letsitele	FMS/FCM bestuur	Sean Moore Wayne Mommsen
	Nelspruit	DAFF Vergroeningsvergadering	MC Pretorius Vaughan Hatting Elma Carstens
15 Nov 19	Nelspruit	BAC meeting	MC Pretorius
19 Nov 19	Nelspruit	HLB & Greening training	MC Pretorius Solomon Gebeyehu Aruna Manrakhan
20 Nov 19	DALRRD Roadshow Letsitele	Market feedback and Special market Requirements	Wayne Mommsen
	Sappi Technology Centre	Bespreking en toets van FFE se kartonne	Marlene Calitz Dawid Groenewald
	Stellenbosch: Navorsingsprioriteite	Bespreek formaat en detail van terugvoer aan produsente	Hannes Bester Paul Cronje Pieter Raath Jan v Niekerk
21 Nov 19	Worcester: APL: Roché Kenny & Stefan Boshoff	Karton-ontwerpe en ontwikkelingswerk	Hannes Bester
	Hoedspruit Studiegroep	DALRRD Roadshow en QMS terugvoer MRL terugvoer	Wayne Mommsen
	Onderberg studiegroep	Agenda – Greening monitering, FMS en FFMS	MC Pretorius Pieter Raath

		bespreking; Bemestings tekorte	
22 Nov 19	Prins Albert: André Groenewald	Sitrus-ontwikkeling	Hannes Bester
25 Nov 19	Letsilele Studiegroep Tegniesekommitee vergadering	FCM bestuur bespreking	Wayne Mommsen Henk Van Rooyen Dezi Fourie Harry Grove Brian Trollip Gerhard Vorster Carl Fourie Lezel Beetge
26 Nov 19	Hoedspruit : Technidex workshop	Technidex workshop – products and services	Catherine Savage
28 Nov 19	PE: Navorsingsprioriteite	Bespreek formaat en detail van terugvoer aan produsente	Hannes Bester Sean Moore
	Plaasbesoek	Oesmanipulasie, Chimera en Boom topwerke	Paul Cronje Wayne Mommsen
4 Des 19	Sappi Technology Centre	Samestelling van verslag oor FFE kartonne	Dawid Groenewald
3 – 5 Dec 19	Plaasbesoeke Letsitele en Hoedspruit	Grondkundiges besoek plase, kyk na profielgate en bespreek bemesting en waterskedulering	Wayne Mommsen Pieter Raath Vivian White
10 Des 19	Nelspruit	FF IPM Stakeholder workshop	MC Pretorius Aruna Manrakhan Elma Carstens DALDARD

Opsomming van aktiwiteite vir periode Januarie – Maart 2020

Datum	**Studiegroep/Aktiwiteit**	**Onderwerpe/Aksies**	**Betrokkenes/ Sprekers**
3 Jan 20	Boordbesoeke	Hoedspruit Witluis Studie	Wayne Mommsen
6 Jan 20	Boordbesoeke	Burgersfort Witluis Studie	Wayne Mommsen
16 Jan 20	IPM Extension	Sitrus IPM en Scout Voorlegging aan Farm Trace	Wayne Mommsen
21 Jan 20	Voorligting Beplannings vergadering. Nelspruit	Agenda	Hannes Bester MC Pretorius Wayne Mommsen Liezl vd Linde Catherine Savage Dawid Groenewald
22-24 Jan 20	Dullstroom: CRI Management Committee meeting	Agenda	Vaughan Hattingh Jon Pinker Sean Moore Paul Fourie Hannes Bester Jan v Niekerk Paul Cronje Christine S-Grove Wayne Mommsen

			MC Pretorius
28-29 Jan 20	Eiland: Limpopo 1 CRI Na-oes werkswinkel	Agenda	Hannes Bester Wayne Mommsen Liezl vd Linde Catherine Savage Dawid Groenewald Wilma Du Plooy Lindo Mamba Sean Moore Paul Cronje Tarl Berry
30-31 Jan 20	Loskopdam: Limpopo 2 CRI Na-oes werkswinkel	Agenda	MC Pretorius Hannes Bester Catherine Savage Dawid Groenewald Wilma Du Plooy Lindo Mamba Sean Moore Paul Cronje Tarl Berry
3-4 Feb 20	Nelspruit: Mpumalanga CRI Na-oes werkswinkel	Agenda	MC Pretorius Hannes Bester Catherine Savage Dawid Groenewald Wilma Du Plooy Lindo Mamba Sean Moore Paul Cronje Tarl Berry
4 Feb 20	Letsitele Studiegroep	Constansia Boeresaal	Wayne Mommsen
6-7 Feb 20	Durban: KZN & Swaziland CRI Na-oes werkswinkel	Agenda	MC Pretorius Hannes Bester Catherine Savage Dawid Groenewald Wilma Du Plooy Lindo Mamba Sean Moore Paul Cronje Tarl Berry
11-12 Feb 20	Jeffreysbaai: Oos-Kaap CRI Na-oes werkswinkel	Agenda	Hannes Bester Catherine Savage Dawid Groenewald Wilma Du Plooy Lindo Mamba Wayne Mommsen Paul Cronje Tarl Berry
11 Feb 20	Nelspruit	Karino: Kultivar blok en Croc Valley probleem-boorde besoek	MC Pretorius Johan Joubert
13 Feb 20	Pretoria	Greening Stakeholder DAFF meeting	MC Pretorius Paul Fourie Solomon Gebeyehu

13-14 Feb 20	Paarl: Wes-Kaap CRI Na-oes werkswinkel	Agenda	Hannes Bester Catherine Savage Dawid Groenewald Wilma Du Plooy Lindo Mamba Wayne Mommsen Paul Cronje Tarl Berry
14 Feb 20	Pretoria	Universiteit Pretoria TUT – data ontleding	MC Pretorius Prof Nico Labuschagne Dr Wilma Augustyn
17 Feb 20	Brits	Heuer's Kwekery: Phytophthora-probleme Produsent met vroeë Nova kultivar besoek Phytophthora probleemboorde besoek	MC Pretorius Johan Joubert
19 Feb 20	Boordbesoeke	Hoedspruit Witluis-studie	Wayne Mommsen
20 Feb 20	Boordbesoeke	Burgersfort Witluis-studie	Wayne Mommsen
20 Feb 20	Johannesburg	Onderhoude vir Kwekery-adviseur pos	Paul Fourie Jacolene Meyer Hannes Bester
25 Feb 20	Innovation Hub, Pretoria	Vergaderings met Corruseal i.v.m. hulle nuwe struktuur en Safeload - nuwe ontwerp plastiese palet	Johan Nel Jaco Geldenhuys Dawid Groenewald
25-26 Feb 20	Nelspruit	Performance Reviews	Hannes Bester Catherine Savage MC Pretorius Liezl vd Linde Wayne Mommsen Dawid Groenewald
27 Feb 20	Nelspruit	Symposium Organising Committee meeting	Hannes Bester MC Pretorius Wayne Mommsen Dawid Groenewald Liezl vd Linde Catherine Savage
3 Mar 20	Letsitele Studiegroep	Agenda	Wayne Mommsen
4 Mar 20	Sappi Technology Centre	Tesco/Peru cartons BCT test results	Dawid Groenewald
5 Mar 20	Innovation Hub, Pretoria	Vergadering met Bidvest Afcom – Strapping en "Carton Erecting Machines	Dawid Groenewald
5 Mar 20	Onderberg	Boordbesoeke	MC Pretorius
5 Mar 20	Puricare: Dr Derek Askew	Waterbehandelings	Hannes Bester
9 Mar 20	Waterberge: Produsente-besoeke	Abé De Villiers Bdy: Abé De Villiers Bufland Bdy: Dewald Eksteen	Hannes Bester Wayne Mommsen Pieter Raath Vivian White

10 Mar 20	Letsitele: Produsente-besoeke	Letaba Estates: Harry Grové Groep 91: Henk van Rooyen & Albie Christie Landman Bdy: EC Landman	Hannes Bester Wayne Mommsen Pieter Raath Vivian White
11 Mar 20	Stellenbosch	ICA meeting	Catherine Savage
11 Mar 20	Tshipise: Produsente-besoeke	Alicedale: Peter Nicholson & Kelvin Smit & Dawie Smuts	Hannes Bester Wayne Mommsen Pieter Raath Vivian White
11 Mar 20	Nelspruit	Wenkem: Produk bekendstelling Phytophthora boord probleem besoek	MC Pretorius James Warrington
11 Mar 20	Citrusdal	Citrusdal and Clanwilliam Packhouse Forum Meeting	Catherine Savage
12 Mar 20	Ashton	Decay meeting / Courtesy Packhouse visit - UniPack	Catherine Savage
13 Mar 20	Nelspruit	Karino boordbesoeke scout opleiding beplanning/reëlings	MC Pretorius Hannes Breedt
16 Mar 20	Patensie	Volg fitosanitêre risiko in verwaarloosde boorde op: Phillip Dempsey	Hannes Bester
16 Mar 20	Nelspruit	Croc Valley Packhouse visit	Catherine Savage
17 Mar 20	Onderberg	Scout opleiding	MC Pretorius Wayne Mommsen
17 Mar 20	Nelspruit	Nelspruit Packhouse Forum Meeting	Catherine Savage Keith Lesar Wilma du Plooy
18 Mar 20	Pakhuis in Mable Hall omgewing	Proef met nuwe ontwerp massa krat	Dawid Groenewald
18 Mar 20	Nelspruit	Scout Opleiding	MC Pretorius Wayne Mommsen
19 Mar 20	Boordbesoeke	Hoedspruit Witluis-studie	Wayne Mommsen
20 Mar 20	Boordbesoek	Burgersfort Witluis-studie	Wayne Mommsen
23 Mar 20	Sappi Technology Centre	Spesiale vergadering i.v.m. laboratorium toetse tydens "lockdown" periode	Dawid Groenewald

8.2 OTHER MEANS OF TECHNOLOGY TRANSFER

8.2.1 **SA Fruit Journal** by Tim G Grout (CRI)

Table 8.2.1.1. SA Fruit Journal articles in 2019-20 besides Extension Briefs.

Issue		Pages	Title	Author/s
Jun/Jul	18(3)	62-63	CRI Post-harvest Workshops: 2019	Catherine Savage

		58-59	CRI Training Workshops for Citrus Scouts	Wayne Mommsen
Oct/Nov	18(5)	60-61	Apply your fungicides correctly for the control of citrus black spot	Providence Moyo, Bheki D.X. Mabunda, Jan van Niekerk and Paul H. Fourie
		62-64	Investigating the spread of the Asian Citrus Psyllid, Diaphorina citri, south west of Morogoro in Tanzania	A. Manrakhan, C. Love, P. Lyimo, R. Clase, G. Cook, M. Mwatawala, D. J. Nel and T. G. Grout
Feb/Mar	20(1)	67-71	The Influence of Non-Permanent Netting on Foliar Spray Deposition, Insect Pest Prevalence and Production of 'Nadorcott' Mandarin (Citrus reticulata)	Ockert P.J. Stander, J. North, J.M. Van Niekerk, T. Van Wyk, C. Love, and M.J. Gilbert
		72-79	Irrigation of Citrus Trees: A Practical Approach	Pieter Raath, Chris Malan and Teunis Vahrmeijer
		80-82	Do smaller navel-ends in Navel oranges mean lower pest levels?	Sean Moore, Wayne Kirkman, Stephan Verreynne and Giverson Mupambi
Apr/May	20(2)	54-55	Monkey beetles attack citrus fruit	Sean Moore, Mellissa Peyper, Riaan Stals and Rikus du Preez
		62-65	2020 CRI Postharvest Workshops	Anonymous

8.2.2 **CRI website** by Tim G Grout

Numbers of unique visitors and numbers of visits were fewer in 2019/20 than in 2018/19 (Table 8.2.2.1), even though the bandwidth increased with the Production Guidelines being uploaded in the public part of the website.

Table 8.2.2.1. Visits and page requests on www.cri.co.za since April 2019.

Month	**Unique visitors**	**Number of visits**	**Pages**	**Hits**	**Bandwidth**
Total 2018/9	49 164	91 539	274 366	1 155 139	30.26 GB
Apr 2019	3 765	6 635	24 198	107 048	2.82 GB
May 2019	3 517	6 851	20 467	101 107	2.95 GB
Jun 2019	3 263	6 270	15 992	87 770	2.47 GB
Jul 2019	3 962	6 916	22 210	126 344	4.00 GB
Aug 2019	3 301	6 941	22 577	116 926	3.75 GB
Sep 2019	3 082	6 159	17 246	106 157	3.72 GB
Oct 2019	2 873	5 632	15 809	117 405	3.83 GB
Nov 2019	3 017	5 627	18 835	99 807	3.04 GB
Dec 2019	2 669	5 063	16 939	69 959	1.83 GB
Jan 2020	3 893	7 423	29 655	166 539	4.49 GB
Feb 2020	3 755	8 535	27 215	179 765	5.41 GB
Mar 2020	2 900	7 033	18 265	120 607	3.48 GB
Total 2019/20	39 997	79 085	249 408	1 399 434	42.0 GB

8.2.3 **CRInet** by Tim G Grout

CRInet provides a good opportunity for growers to share opinions or ask questions on any technical citrus topic but it is mostly being used for dissemination of information from CRI or CGA. The number of messages sent each year fluctuate with the topic being discussed but over the last nine years have averaged 57 per annum. In December 2019, Yahoo Groups changed their policy about having past messages on their website so now messages can only be received via email, but attachments are permitted. There are currently 538 CRInet members.

8.2.4. **Cutting Edge** by Tim G Grout (CRI)

Table 8.2.4.1. Cutting Edge issues during 2019-20.

No.	Title	Month	Author/s
266	Imazalil EU MRL Update	Apr	Paul Hardman
267	Inferior Quality Packaging Materials	Apr	Dawid Groenewald
268	Mealybug alert	Apr	Sean Moore, Vaughan Hattingh, Elma Carstens and Wilma du Plooy
269	EU Flies	May	Vaughan Hattingh, Elma Carstens, Aruna Manrakhan and Sean Moore
270	Citrus Scab notice	May	Jan van Niekerk, Providence Moyo, Elma Carstens, MC Pretorius and Vaughan Hattingh
271	Chlorine use in packhouses	May	Catherine Savage, Keith Lesar and Wilma du Plooy
272	Citrus Scab management	May	Jan van Niekerk, Providence Moyo, Elma Carstens and MC Pretorius
273	Bulk bin packaging	Jun	Tarl Berry, Dawid Groenewald, Paul Cronje and Mitchell Brooke
274	Fruit Fly inspections for EU	Jul	Vaughan Hattingh, Aruna Manrakhan, Sean Moore and Paul Hardman
275	Bulk bins update	Jul	Tarl Berry, Dawid Groenewald, Paul Cronje and Mitchell Brooke
276	Fruit fly identification	Aug	Aruna Manrakhan, Tim Grout and Peter Stephen
277	Citrus FFMS	Aug	Aruna Manrakhan, Elma Carstens, Tim Grout, Sean Moore and Vaughan Hattingh
278	EU Chlorpyrifos MRLs	Aug	Paul Hardman
279	Consumer Assurance Update	Aug	Paul Hardman
280	Changes in FF recommendations	Oct	Aruna Manrakhan and Sean Moore
281	Improvement on FF control 2019-2020	Oct	Aruna Manrakhan and Vaughan Hattingh
282	FMS compliance improvement	Oct	Sean Moore and Vaughan Hattingh
283	Consumer Assurance Update	Nov	Paul Hardman
284	FMS changes	Dec	Sean Moore, Vaughan Hattingh, Elma Carstens, Paul Hardman and Paul Cronje

285	Effective management of mealybug for exports	Dec	Sean Moore, Vaughan Hattingh and Elma Carstens
286	Cellular temperature	Dec	Tarl Berry, Paul Cronje, Paul Hardman, Werner van Rooyen, Bernard Henning, Elma Carstens and Vaughan Hattingh
287	Guide for identification of insect pests	Feb	Sean Moore, Aruna Manrakhan and Peter Stephen
288	Changes in CRI production guidelines	Feb	Aruna Manrakhan and Sean Moore
289	Consumer Assurance Update	Mar	Paul Hardman
290	Export Citrus Post-harvest Treatment Declarations and Labelling	Mar	Paul Hardman
291	Product use in FMS	Mar	Sean Moore
292	Changes in trap threshold for oriental fruit fly	Mar	Aruna Manrakhan, Elma Carstens and Vaughan Hattingh
293	Supporting guidance for packhouse managers	Mar	Wima du Plooy

9 PUBLICATIONS IN 2019

9.1 Refereed Publications (or ISI ranked journals)

Boersma, N., Boardman, L., Gilbert, M., Terblanche, J.S. 2019. Cold treatment enhances low-temperature flight performance in false codling moth, Thaumatotibia leucotreta (Lepidoptera: Tortricidae). Agricultural and Forest Entomology 21(2): 243-251.

Cook, G., W. Kirkman, R. Clase, C. Steyn, E. Basson, P.H. Fourie, S.D. Moore, T.G. Grout, E. Carstens and V. Hattingh. 2019. Orchid fleck virus associated with the first case of citrus leprosis-N in South Africa. Eur. J. Plant Pathol. 155: 1373–1379.

Goddard, M., M.P. Hill & S.D. Moore. 2019. An analysis of the fruit-sucking and fruit-piercing moth complex in citrus orchards in South Africa. African Entomology 27(1): 1-9.

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.

Grout, T.G. and K.C. Stoltz. 2020. Carbon Dioxide Fumigation to Shorten Cold Disinfestation Treatments for *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) in Citrus Fruit. J. Econ. Entomol. 113 (1): 144-151.

Guarnaccia, V., Gehrmann, T. Silva-Junior, G.J. Fourie, P.H. Haridas, S. Vu, D. Spatafora, J. Martin, F.M. Robert, V. Grigoriev, I.V. Groenewald, J.Z. and Crous P.W. 2019. Phyllosticta citricarpa and sister species of global importance to Citrus. Mol Plant Pathol. 20(12):1619-1635.

Hatting, Justin L., Moore, Sean D. and Malan, Antoinette P. 2019. Microbial control of phytophagous invertebrate pests in South Africa: Current status and future prospects. Journal of Invertebrate Pathology 165: 54-66.

Lado J, E. Alós, M. Manzi, PJR. Cronje, A. Gómez-Cadenas, MJ. Rodrigo and L .Zacarías (2019) Light Regulation of Carotenoid Biosynthesis in the Peel of Mandarin and Sweet Orange Fruits. Front. Plant Sci. 10:1288.

Lado, J., P.J.R. Cronje, M.J Rodrigo and L. Zacarias. 2019. Citrus. pp. 377-398. In: Postharvest physiological disorders in fruits and vegetables. Eds. S. Tonetto de Freitas and S. Pareek. CRC Press, Taylor & Francis Group, Florida.

Moore, S. and Jukes, M. 2019. Advances in microbial control in IPM: entomopathogenic viruses. In: Kogan, M. and Heinrichs, E. A. (ed.), Integrated management of insect pests: Current and future developments, Burleigh Dodds Science Publishing, Cambridge, UK (ISBN: 978 1 78676 260 3).

Stander, O.P.J., J. North, J.M. van Niekerk, T. van Wyk, C. Love and M.J. Gilbert. 2019. Influence of Nonpermanent Netting on Foliar Spray Deposition, Insect Pest Prevalence, and Production of ‘Nadorcott’ Mandarin (Citrus reticulata). Hortscience 54(4):667–675.

Talon, M., Caruso, M., Gmitter, F.G.Jr. 2020. The Genus Citrus. 538 pp. Woodhead Publishing, Elsevier.

Urbaneja A., T.G. Grout, S. Gravena, F. Wu, Y. Cen, and P.A. Stansly. 2020. Citrus pests in a global world. Chapter 16 pp. 333-348 in M. Talon, M. Caruso, F.G. Gmitter Jr. (editors) The Genus Citrus. 538 pp. Woodhead Publishing, Elsevier.

Van Niekerk, J.M., E. Basson, C. Olivier, G.-L. Carelse, V. Guarnaccia. 2019. Chlorine and mefenoxam sensitivity of Phytophthora nicotianae and Phytopthora citrophthora from South African citrus nurseries. Phytopathologia Mediterranea 58(3): 629-638.

Weldon, C. W., Mnguni, S., Demares, F., DuRand, E., Malod, K., Manrakhan, A. and Nicolson, S. W. (2019). Adult diet of a tephritid fruit fly does not compensate for impact of a poor larval diet on stress resistance. Journal of Experimental Biology 222: jeb192534. DOI: 10.1242/jeb.192534

Wu, W., Beretta, C., Cronje, P., Hellweg, S., Defraeye, T. 2019. Environmental trade-offs in fresh-fruit cold chains by combining virtual cold chains with life cycle assessment. Applied Energy 254: 113596. 11 pages.

Wu, W., P. Cronjé, P. Verboven, T. Defraeye. 2019. Unveiling how ventilated packaging design and cold chain scenarios affect the cooling kinetics and fruit quality for each single citrus fruit in an entire pallet. Food Packaging and Shelf Life 21: 100369 (13 pages)